UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE ODONTOLOGÍA
DEPARTAMENTO DE ESTOMATOLOGÍA III

TESIS DOCTORAL

Cambios en los tejidos blandos y duros tras la reconstrucción de la cresta ósea y tras el uso de implantes dentales con diferente geometría

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Ignacio Sanz Martín

DIRECTOR

Mariano Sanz Alonso

Madrid, 2018

UNIVERSIDAD COMPLUTENSE DE MADRID

Facultad de Odontología

Departamento de Estomatología III

CAMBIOS EN LOS TEJIDOS BLANDOS Y DUROS TRAS LA RECONSTRUCCIÓN DE LA CRESTA ÓSEA Y TRAS EL USO DE IMPLANTES DENTALES CON DIFERENTE GEOMETRÍA.

Tesis Doctoral

Ignacio Sanz Martín

Director

Prof. Mariano Sanz Alonso

Madrid, Abril 2017

AGRADECIMIENTOS

Este proyecto no hubiese llegado a buen puerto sin la ayuda de tantos compañeros que han contribuido con su trabajo y esfuerzo desinteresado. Ha sido para mí un honor y un placer colaborar con todos los que han hecho de esta Tesis una gran experiencia investigadora y humana.

En primer lugar me gustaría empezar por agradecer al Profesor Mariano Sanz Alonso, director de esta Tesis, la oportunidad que me brindó hace ya más de 5 años para unirme al equipo de la UCM y poder participar y aprender en los proyectos de investigación. Además, por supuesto, de su disponibilidad y ayuda constante para sacar estas investigaciones adelante. Por todo lo que he aprendido. Por su ánimo, apoyo, comprensión y paciencia en cada etapa de este camino, además de su capacidad para sacar lo mejor de cada situación y vicisitud.

En segundo lugar me gustaría reconocer el trabajo de Fabio Vignoletti y Javier Nuñez. Ambos han estado involucrados en el diseño de los modelos experimentales, la realización de las cirugías y el análisis de los resultados. Independientemente de su fantástica capacidad investigadora, ha sido un placer para mí compartir este camino juntos. Por haberme tratado siempre como a un hermano pequeño con su cariño, consejos y apoyo. Gracias!

A Fernando Muñoz por su fantástico trabajo con la histología, por su voluntad por ayudarme con las medidas histológicas y la comprensión de las mismas. Por habernos recibido tantas veces en Lugo con cariño y hospitalidad y por su ayuda desinteresada para sacar esta Tesis adelante.

A María Permuy, por su constante disponibilidad y por su amable ayuda. Por responder fuera de horas y en domingos y por todo el trabajo realizado con las mediciones de volumen del Micro CT.

A Stephan Schmaelzle de Swissmeda, por haber hecho lo imposible durante los últimos meses por sacar la metodología de superposición adelante. Por su paciencia y buen hacer. Por haberse adaptado a las limitaciones de tiempo que teníamos y por su arduo trabajo fuera de su horarios y más allá de sus responsabilidades.

A todo el equipo del centro experimental del Gómez Ulla. Por su trabajo y esfuerzo, en particular a Paula García, Pablo Arias y Alejandro Coca.

A Javier Sanz-Esporrín por su dedicación en los proyectos y su ayuda con el Generalized Linear Model. También a todos los compañeros de la UCM que han colaborado en las cirugías experimentales. Muy especialmente a Nicola Discepoli, Fernando Luengo, Luca Ferrantino, Riccardo Di Raimondo y a Juan Bollaín.

A Ludovica Fierravanti y Jaime Alcaraz por el apoyo con las mediciones de los cambios del contorno del reborde alveolar.

A Alberto Ortiz-Vigón por su amistad y apoyo. En especial es estas últimas etapas en las que ha sido mi bastón de apoyo para lidiar con la burocracia y poderme centrar en escribir.

A Ignacio Sanz Sánchez, por su amistad incondicional y cariño. Por lo que siempre aprendo con él y por la guía que he hallado en su trabajo de Tesis.

Me gustaría además agradecer a mis padres su apoyo y comprensión todos estos años. Sin su ayuda nunca hubiese podido haber vivido todas estas fantásticas experiencias de las que tanto me he enriquecido. Gracias!

Además, a mis hermanos, por su cariño y por siempre estar ahí cuando los he necesitado. En especial a Jaime que ha sido un gran apoyo en la recta final de esta Tesis.

Para terminar, me gustaría agradecer a Angie, por haberme apoyado en todo este proceso y por haber comprendido siempre las ausencias y los momentos en los que he tenido que aparcar nuestra relación por sacar este proyecto adelante.

A todos, gracias de corazón!

PREFACIO

La presente tesis doctoral está basada en los tres siguientes artículos:

Artículo 1.

Hard and soft tissue integration of immediate and delayed implants with a modified coronal macro-design. Histologic, Micro CT and volumetric soft tissue changes from a pre-clinical in-vivo study. Sanz-Martin I, Vignoletti F, Nuñez J, Permuy M, Muñoz F, Sanz-Esporrín J, Fierravanti L, Shapira L, Sanz M. *Journal of Clinical Periodontology*. *Accepted for publication*.

Artículo 2.

Hard and soft tissue volume analysis of immediate and delayed implants with different cervical design. A novel methodological approach using superimposed Micro-CT and STL images. Sanz-Martin I, Vignoletti F, Nuñez J, Permuy M, Muñoz F, Sanz M. Clinical Oral Implants Research. *Submitted for publication*.

Artículo 3.

Contour changes after guided bone regeneration of large non-contained mandibular buccal bone defects using deproteinized bovine bone mineral and a porcine-derived collagen membrane. An experimental in vivo investigation. Sanz-Martin I, Ferrantino L, Vignoletti F, Nuñez J, Baldini N, Duvina M, Sanz M. *Clinical Oral Investigations. Submitted for publication*.

ÍNDICE DE CONTENIDOS

I. RESUMEN

Antecedentes.

La reabsorción de la cresta ósea después de la extracción dentaria provoca un colapso del reborde alveolar, que dependiendo de su grado, puede conllevar la necesidad de procedimientos regenerativos o del uso de implantes de diámetro reducido o diseño cervical modificado. Actualmente, el éxito en las restauraciones sobre implantes no se mide únicamente por la supervivencia del implante y la ausencia de complicaciones, sino también por la integración con los tejidos adyacentes. En este contexto, el tejido blando peri-implantario es de vital importancia en la consecución de resultados predecibles a largo plazo. A pesar de su contrastada importancia, el conocimiento de su comportamiento y cicatrización, el impacto del enfoque quirúrgico (inmediato o diferido) así como su interacción con el tejido óseo es exiguo. Estas limitaciones derivan, fundamentalmente, de las carencias inherentes a la metodología utilizada hasta la fecha.

Objetivos.

Los objetivos de esta serie de trabajos han sido i) analizar la integración y comportamiento del tejido duro y blando al usar implantes con un diseño cervical triangular comparados con implantes cilíndricos convencionales colocados en crestas cicatrizadas y alveolos post-extracción (Estudio 1), ii) estudiar la diferencia relativa en el volumen de tejido blando y duro aplicando una metodología novedosa basada en la superposición de imágenes de Micro CT y STL (Estudio II) y iii) Estudiar el comportamiento del tejido blando en procedimientos de regeneración ósea horizontal mediante el uso de sustitutos de tejido óseo y membranas reabsorbibles (Estudio III).

Material y Métodos. Resultados.

Estudio 1. Para el primer objetivo se utilizó un modelo preclínico de perro Beagle en el que se testaron implantes con un diseño cervical triangular (Test/T) e implantes

cilíndricos convencionales (Control/C). Se extrajeron el primer y segundo premolar además de la raíz mesial del primer molar. Ocho semanas después se colocaron implantes T y C de manera aleatoria en las crestas cicatrizadas de PM2 y M1 así como implantes inmediatos en los alveolos de la raíz mesial de PM3 y PM4. El procedimiento se repitió 8 semanas después en el lado contralateral y el sacrificio tuvo lugar 4 semanas después aportando resultados a 4 y 12 semanas. Se tomaron impresiones de silicona antes de la extracción y antes del sacrificio. Las muestras se procesaron para análisis histológico y análisis mediante Micro CT. Los resultados demostraron valores histomorfométricos verticales similares aunque el componente horizontal de la cresta resultó ser mayor para los implantes T. El porcentaje de contacto hueso-implante (BIC), los contornos del tejido bucal así como los volumen de componentes de la muestra fueron similares

Estudio 2. Basado en las muestras a 12 semanas de cicatrización de la investigación anteriormente expuesta se desarrolló una metodología capaz de superponer, usando referencias comunes, las imágenes DICOM obtenidas mediante el Micro CT con las imágenes provenientes del escáner óptico de los modelos que aportaban información de los tejidos blandos. Se analizaron tres volúmenes de interés; el volumen de hueso en vestibular del implante (B-BV), el volumen de tejido blando por encima del hombro del implante (SC-STV) y el volumen de tejido blando por debajo del hombro del implante (EC-STV). Se halló que i) la metodología utilizada fue capaz de aportar información en cuanto al volumen de tejido blando y duro, ii) el volumen de hueso fue mayor en los implantes T aunque el diseño del implante no afectó al volumen de tejido blando. iii) El modo de cicatrización (inmediato o diferido) afectó el comportamiento e interacción entre volumen de tejido blando y óseo.

Estudio 3. Para responder al tercer objetivo se desarrolló un modelo experimental en el perro Beagle donde, tras las extracciones, se crearon tres defectos en caja por

hemimandíbula. Tras 3 meses de cicatrización se realizó la re-entrada con el objetivo de regenerar los defectos creados. Las terapias que se aleatorizaron a cada defecto fueron: xenoinjerto óseo embebido en una matriz de colágeno (BRG), membrana de colágeno porcino nativa (MBG) y la combinación de ambas terapias (CBG). Se tomaron impresiones de silicona antes de las extracciones (T1), antes de la terapia regenerativa (T2) y 3 meses después de la misma (T3). La superposición de los escáneres ópticos de los modelos de escayola y el análisis del contorno del proceso alveolar reveló que la extracción produjo un colapso horizontal y vertical significativo. La anatomía del defecto tuvo un impacto claro sobre el aumento conseguido. El aumento de tejido fue superior para los grupos BRG y CBG, sin diferencias entre ambos. La anchura del proceso alveolar después de la terapia regenerativa no alcanzó los valores anteriores a la extracción dentaria.

Conclusiones.

Tras el análisis de los estudios puede concluirse que los implantes con diseño cervical triangular obtuvieron mayor anchura de cresta y volumen de hueso en vestibular, sin diferencias en cuanto a las medidas histomorfométricas verticales de tejido blando y óseo. El diseño del implante no afectó al contorno del tejido blando ni al volumen del mismo. El protocolo quirúrgico tuvo un impacto evidente en el comportamiento e interacción del tejido blando y duro. En lo que se refiere a técnicas regenerativas óseas, la utilización de sustitutos óseos con o sin membranas consiguió mejores resultados en el aumento del reborde sin alcanzar los valores iniciales previos a la extracción.

PALABRAS CLAVE: Implantes, macro-diseño de implantes, histología, micro CT, estudios experimentales, análisis de volumen, volumen óseo, volumen tejido blando, regeneración ósea, sustitutos óseos, membranas reabsorbibles.

II. INTRODUCCIÓN

El uso de implantes dentales para la reconstrucción de pacientes parcial y totalmente edéntulos se ha popularizado en las últimas décadas debido a los parámetros de supervivencia reportados en la literatura científica (Buser et al., 2012, Gotfredsen, 2012, Ostman et al., 2012). En el pasado la investigación en este campo se centró en los fenómenos relacionados con la osteointegración. Los resultados de estas investigaciones han conseguido una mejor comprensión de los mecanismos de interacción hueso-implante y de los factores que influyen en la biología de la cicatrización ósea, así como aquellos que influyen en la predictibilidad de los resultados clínicos. El desarrollo de nuevas superficies de implantes ha supuesto un claro avance en este aspecto, la introducción de superficies rugosas ha conseguido acortar los tiempos de carga y hacer más predecibles tratamientos como la carga inmediata y los implantes cortos que anteriormente generaban un mayor índice de fracasos (Lang and Jepsen, 2009, Wennerberg and Albrektsson, 2010).

El aumento en la predictibilidad de la integración ha conseguido mejorar los resultados en cuanto a la supervivencia de los implante sin embargo, la supervivencia del implante no asegura el éxito del mismo. Las complicaciones biológicas en forma de enfermedades peri-implantarias han demostrado tener un alto grado de prevalencia (Derks and Tomasi, 2015). De igual manera, la supervivencia del implante no asegura un resultado estético aceptable en el cual los tejidos peri-implantarios se encuentren en armonía con lo los tejidos adyacentes.

Las diferencias en la precepción del resultado estético son inherentes a su naturaleza subjetiva y a la demanda del paciente. A pesar de ello, existen una serie de parámetros estéticos bien definidos que se han utilizado para valorar la apariencia de las restauraciones sobre implantes en zonas de compromiso estético (Benic et al., 2012).

Es evidente que dentro de estos parámetros se encuentra la apariencia del tejido blando peri-implantario. Con el objetivo de analizar de manera objetiva su influencia en el resultado estético se han propuesto numerosos índices (Furhauser et al., 2005, Jemt, 1997). Entre los parámetros más importantes se encuentran el contorno del proceso alveolar y el color del tejido blando al compararlo con los dientes adyacentes (Belser et al., 2009).

La importancia del tejido óseo subyacente en el soporte de la mucosa peri-implantaria ha sido descrito de manera extensa en la literatura (Buser et al., 2004). Los deficiencias óseas del proceso alveolar tiene un impacto negativo en el aspecto de la mucosa peri-implantaria y en la consecución de resultados estéticos. El origen de estos defectos se halla, de manera prioritaria en los cambios fisiológicos y dimensionales que ocurren tras la extracción dentaria.

1. Cambios dimensionales después de la extracción dentaria

Estudios en humanos han concluido que la extracción dentaria origina una pérdida substancial de los tejidos blandos y duros. Pietrkovski y Massler (Pietrokovski & Massler, 1967) concluyeron, en un estudio observacional de pacientes que habían recibido extracciones dentarias, que la reducción en la anchura de la cresta era mayor en la parte bucal al compararla con la lingual o palatina en todos los individuos analizados. Como resultado de estos cambios el centro de la cresta migraba hacia palatino en el maxilar superior y hacia lingual en el maxilar inferior.

Estos hallazgos fueron confirmados en investigaciones clínicas que estudiaron los cambios en el reborde alveolar durante un período de 12 meses tras la extracción dentaria. Se realizaron mediciones clínicas y en modelos de escayola después de la extracción y tras 3, 6 y 12 meses. Tres meses después de la extracción se produjo una reducción de un 30% de la anchura buco-lingual, mientras que 12 meses después el proceso alveolar había perdido la mitad de las dimensiones buco-linguales (Schropp et al., 2003).

Cambios en el tejido óseo

Investigaciones experimentales han estudiado de manera sistemática la cicatrización del alveolo post-extracción en premolares de perros beagle (Araujo and Lindhe, 2005, Cardaropoli et al., 2005).

Los resultados de las mismas han concluido que tras la extracción existe un colapso de la tabla ósea vestibular causada por reabsorción del hueso fasciculado alveolar. La composición cortical de este hueso que tapiza las pareced del alveolo y sirve de anclaje a las fibras de Sharpey lo hace directamente dependiente del ligamento periodontal. La extracción dentaria, con la consiguiente eliminación de las estructuras dependientes del diente, entre ellas el ligamento periodontal, origina una reducción progresiva de la vascularización a la zona que se acompaña de una intensa actividad osteoclástica que provoca la reabsorción ósea (Araujo et al., 2005) . En aquellas tablas óseas que poseen un grosor reducido, la reabsorción del hueso fasciculado puedo suponer la desaparición de la práctica totalidad del hueso vestibular situado en la porción más coronal. Estos cambios en la cortical vestibular no se reproducen en la tabla lingual donde las variaciones observadas son menos patentes.

Estudios recientes en humanos han evaluado mediante análisis radiográfico tridimensional a 69 sujetos que habían recibido extracciones de premolares e incisivos concluyendo que la perdida más pronunciada ocurría en las porciones bucal y marginal dando una apariencia triangular a la cresta ósea (Misawa et al., 2016).

Los procesos biológicos que ocurren en el interior del alveolo después de la extracción se han estudiado en biopsias humanas en diferentes momentos después de la extracción. En las etapas iniciales el coágulo va madurando transformándose en un tejido de granulación con elevada presencia de macrófagos. Entre 4 y 8 semanas después de la extracción, se halla una importante proliferación celular y abundante tejido conectivo además de isletas de hueso neo-formado. Entre 8 y 12 semanas después, la maduración ósea continúa progresando hasta formar nuevo hueso con un patrón trabecular al final del tercer mes de cicatrización, con un mínimo componente osteoide o actividad osteoclástica (Evian et al., 1982, Trombelli et al., 2008).

Cambios en el tejido blando

A pesar de la información existente en la literatura científica referente a los cambios en la cresta ósea después de la extracción dentaria, existe un vacío en el conocimiento en cuanto al comportamiento del tejido blando adyacente al proceso alveolar tras la extracción dentaria. Recientemente Chappuis y cols. han aportado información al respecto al evaluar mediante análisis tridimensional la interpelación entre el tejido blando y el tejido óseo subyacente (Chappuis et al., 2017). El grosor del tejido blando antes de la extracción fue similar en pacientes con un biotipo grueso o fino con unas medias de 0.7 y 0.8mm. Ocho semanas después de la extracción se observó que los biotipos finos habían multiplicado el grosor del tejido blando por siete, mientras que en aquellos pacientes con un biotipo grueso el tejido bando permaneció estable y con cambios mínimos. Este engrosamiento espontáneo del tejido en biotipos finos permitió concluir que la reabsorción de la tabla vestibular en estos pacientes es reemplazada por tejido blando.

Factores que influyen en el grado de colapso

El grado de colapso del reborde alveolar depende de numerosos factores. Entre ellos la pieza a extraer o la posición de la pieza dentaria. El factor más estudiado ha sido el grosor de la tabla ósea vestibular tras la extracción (Braut et al., 2011, Januario et al., 2011, Vera et al., 2012).

Estudios clínicos que han evaluado el grosor de la tabla vestibular en maxilar anterior han revelado que el 87% de las mismas tenía un grosor menor o igual a 1mm y que tan solo un 3% tenía un grosor de 2mm (Huynh-Ba et al., 2010).

Parece existir una relación inversamente proporcional entre el grosor de la tabla y el grado de reabsorción que ocurre después de la extracción. La cicatrización de implantes colocados en alveolos post-extracción ha permitido evaluar el grado de colapso que ocurre en el proceso alveolar en función del grosor de la tabla concluyendo que alveolos con dimensiones buco-linguales de la tabla vestibular superiores a 1mm obtuvieron un menor grado colapso (Ferrus et al., 2010).

2. Relevancia del enfoque quirúrgico: Inmediato o diferido

La colocación de implantes en alveolos post-extracción no ha conseguido mitigar los cambios dimensionales que ocurren después de la extracción dentaria (de Sanctis et al., 2010).

Estudios experimentales han evaluado de manera sistemática la cicatrización de diferentes tipos de implantes colocados después de la extracción dentaria. Estos concluyeron que las dimensiones del alveolo influyeron en la magnitud de la pérdida ósea que se observó (Vignoletti et al., 2009a). Además los implantes inmediatos cicatrizaron con un componente mayor de porción epitelial al compararlos con los valores de los implantes colocados en crestas cicatrizadas (Vignoletti et al., 2009b).

Revisiones sistemáticas han concluido que la supervivencia de implantes colocados en alveolos post extracción es similar a la de implantes colocados en crestas cicatrizadas (Lang et al., 2012).

Sin embargo, no existe un consenso claro en la literatura científica en cuanto a los resultados estéticos de los implantes inmediatos. Mientras hay literatura que apoya su uso destacando las ventajas que otorgan la colocación de un provisional inmediato y la reducción del número de intervenciones (Cosyn et al., 2016). Existen ensayos clínicos que han concluido que los implantes inmediatos obtienen peores resultados estéticos al compararlos con los implantes diferidos (Tonetti et al., 2017).

A pesar de ello, la implantología inmediata sigue siendo un enfoque muy utilizado en el ámbito clínico por sus claras ventajas al conseguir reducir los tiempos quirúrgicos además de permitir la provisionalización inmediata en casos de existir suficiente estabilidad primaria.

La obtención de resultados estéticos con implantes inmediatos unitarios parece estar ligada al uso de protocolos estrictos que se basan en i) colocación del implante en una posición protéticamente guiada limitando de esta manera migraciones apicales del margen gingival (Evans and Chen, 2008) ii) relleno del gap con sustitutos óseos que reducen el grado de contracción de la tabla vestibular (Sanz et al., 2016) iii) utilización de injertos de tejido blando que ayudan a minimizan el colapso en la zona de transición del hombro del implante al margen gingival (Kan et al., 2009).

La combinación de todos estos factores hace de la implantología inmediata un tratamiento complejo con una alta demanda de experiencia clínica para el operador.

Es por ello que los implantes diferidos continúan siendo una opción importante en la práctica clínica ya que gozan de claras ventajas terapéuticas además de estar ampliamente avalados por la literatura científica.

Estudios experimentales han evaluado la cicatrización de diferentes tipos de implantes en crestas cicatrizadas. En estos se observó que la formación de la inserción mucosa en los implantes fue independiente del diseño de los mismos y del tipo de cicatrización (sumergida o transmucosa). El surco peri-implantario se continuaba con un epitelio largo de unión que terminaba a 1-1.5mm del tejido óseo donde se observaba una adhesión conectiva mediada por mucopolisacáridos y con fibras conectivas que presentaban una orientación perpendicular al pilar de cicatrización (Abrahamsson et al., 1996, Abrahamsson et al., 2001).

En cuanto a las posibles ventajas clínicas; los implantes colocados en crestas cicatrizadas permiten reducir las complicaciones derivadas de la colocación de implantes en alveolos post-extracción, donde es frecuente que el implante termine en una posición vestibular. De igual manera, posibilitan el desplazamiento del tejido blando crestal o palatino hacia vestibular restableciendo una anchura adecuada de mucosa queratinizada. Por último, en caso de deficiencias del reborde alveolar permiten una cicatrización sumergida limitando así las complicaciones derivadas de la exposición de la membrana y materiales de regeneración.

Es significativo señalar que existe limitada evidencia científica que haya comparado de manera directa los implantes inmediatos con implantes diferidos y haya estudiado de manera comprensiva su cicatrización. No sólo desde el punto de vista histológico donde sí se hallan comparaciones recientes (Passoni et al., 2016, Yi et al., 2016), si no desde el punto de vista del contorno de tejidos blandos, volumen de hueso y volumen de tejido blando.

3. Técnicas de aumento del reborde alveolar

La colocación de implantes en una posición protéticamente guiada necesita de unas dimensiones adecuadas del reborde alveolar. Por este motivo las técnicas regenerativas han cobrado importancia con el objetivo de permitir que pacientes con disponibilidad ósea reducida puedan recibir, de igual manera, implantes dentales.

Mientras que las técnicas de aumento horizontal gozan de una alta predictibilidad, la reconstrucción de defectos verticales ha demostrado ser más técnico-sensible y tener una mayor tasa de complicaciones (Rocchietta et al., 2008).

Dentro de las técnicas existentes para la reconstrucción de procesos alveolares atróficos destacan el uso de los bloques de hueso autólogo, la distracción osteogénica, las técnicas de expansión de la cresta y la regeneración tisular guiada.

La regeneración ósea guiada es, junto con los bloques de hueso autólogo, la técnica mejor descrita en la literatura científica. Las bases fisiológicas de esta técnica se fundamentan en el uso de membranas que permiten una cicatrización compartimentalizada de la herida ósea logrando que las células de estirpe osteogénica colonicen los defectos del reborde alveolar excluyendo así las células conectivas y epiteliales que poseen un mayor grado de diferenciación (Schenk et al., 1994).

Los resultados clínicos de esta técnica han sido evaluados recientemente en una revisión sistemática publicada por nuestro grupo en la que después de analizar los datos de 40 artículos científicos que valoraban el cambio en las dimensiones del proceso alveolar antes y después de técnicas de aumento horizontal, se concluyó que el uso de biomateriales y membranas era la opción más utilizada y con mejores resultados en el tratamiento de dehiscencias y fenestraciones (Sanz-Sanchez et al., 2015).

A pesar de la amplia evidencia clínica y preclínica existente en cuanto al comportamiento del tejido óseo después de procedimientos de regeneración ósea guiada existe un vacío en el conocimiento de los cambios que ocurren a nivel del tejido blando después de las técnicas de aumento. Sería pertinente, por lo tanto, conocer en qué medida son las técnicas de regeneración capaces de reconstruir el proceso alveolar y devolver el volumen inicial del proceso alveolar.

En cuanto a la predictibilidad de estas técnicas, es importante destacar que existen complicaciones asociadas a estas intervenciones que pueden comprometer el resultado de la regeneración (Fontana et al., 2011). Es por ello que los avances, en cuanto al diseño y materiales, se han centrado en implantes de menor tamaño y diámetro para reducir la morbilidad y el número de situaciones clínicas en las que es necesario realizar procedimientos de regeneración ósea.

4. Cambios en la geometría de los implantes

El uso de implantes de diámetro y tamaño reducido ha permitido simplificar los tratamientos con implantes limitando el número de intervenciones destinadas a la reconstrucción del proceso alveolar mediante elevaciones externas de seno, regeneraciones verticales u horizontales (Felice et al., 2014, Ioannidis et al., 2015, Pohl et al., 2017).

De igual manera, el uso de implantes estrechos ha sido también abogado en situaciones clínicas en las que el diente a restaurar tiene unas dimensiones reducidas como incisivos inferiores o laterales (Galindo-Moreno et al., 2012).

Los resultados de los implantes estrechos han sido contrastados en revisiones sistemáticas que han demostrado su éxito y fiabilidad (Klein et al., 2014).

Otra de las ventajas a destacar del uso de implantes de diámetro reducido es la de lograr preservar las dimensiones de la tabla vestibular. Al reducir el diámetro del implante se incrementa el tamaño de la cortical vestibular post-osteotomía en implantes diferidos y la distancia a la tabla vestibular en implantes inmediatos.

Con un objetivo similar, recientemente se ha introducido un nuevo diseño cervical de implantes con una morfología triangular de la porción más coronal. El objetivo de dicho diseño consiste, precisamente, en reducir la cantidad de titanio en vestibular del implante permitiendo así más espacio para tejido óseo e hipotéticamente un mayor grosor de la tabla vestibular y mayor estabilidad del tejido peri-implantario (*Figura 1*).

Figura 1. Implantes con diseño cervical triangular (izquierda) e implantes con diseño cilíndrico convencional.

5. Estabilidad del tejido peri-implantario

Actualmente, existe interés en la literatura científica por comprender los factores que influyen en la remodelación ósea tras la colocación de implantes dentales.

Estudios longitudinales han demostrado que el comienzo de la enfermedad peri-implantaria ocurre 3 años después de la carga de los implantes (Derks et al., 2016). Limitar la pérdida ósea inicial, evitando la exposición de la superficie rugosa del implante, puede ayudar a reducir la incidencia de complicaciones biológicas.

Varios factores han demostrado estar relacionados con la preservación del hueso crestal; entre los más citados se hallan la configuración plataforma-pilar y la localización del micrograp/cuello pulido con respecto a la cresta ósea (Schwarz et al., 2014). La reducción del número de conexiones y desconexiones del pilar protésico ha demostrado, de igual manera, limitar la pérdida ósea crestal a corto plazo (Molina et al., 2017).

A parte de los numerosos factores relacionados con el implante o componentes protésicos es importante clarificar el papel del tejido óseo en el mantenimiento a largo plazo de los niveles óseos, en la incidencia de complicaciones biológicas y en la consecución de resultados estéticos.

Relevancia del tejido óseo

La comprensión de la importancia del tejido óseo en los cambios dimensionales que ocurren a nivel del hombro del implante ha supuesto un punto de referencia para el logro de resultados harmónicos en pacientes con demanda estética. De hecho, las deficiencias óseas vestibulares se han ligado a resultados estéticos deficientes (Chen and Buser, 2014).

En un estudio clínico prospectivos que evaluó más de 3000 implantes dentales se midieron los cambios verticales en la cresta bucal en función al grosor de la misma. Los resultados indicaron que cuando el grosor de la cresta se aproximaba a valores cercanos a los 2mm la pérdida en altura se reducía de manera significativa (Spray et al., 2000).

Es por ello, que en situaciones clínicas con mínimo grosor de tabla se ha recomendado tradicionalmente procedimientos de regeneración ósea para limitar la reabsorción que pudiese ocurrir como consecuencia del trauma quirúrgico (Benic and Hammerle, 2014).

El tejido óseo regenerado ha demostrado ser estable y comportarse de manera similar al hueso nativo en periodos de evaluación mayores de 5 años (Benic et al., 2017, Benic et al., 2009).

A pesar de los datos positivos reportados en la literatura, el uso de técnicas regenerativas contempla el fracaso de las mismas por complicaciones asociadas como la dehiscencia de la herida, la exposición de la membrana o las infecciones post-operatorias (Annen et al., 2011).

Estudios prospectivos han evaluado a pacientes en los cuales, después de haber realizado técnicas de regeneración ósea guiada, existían dehiscencias residuales con exposición del titanio rugoso del implante. Después de evaluar a 16 pacientes con dehiscencias residuales en un período de 4 años concluyeron que los implantes con exposición de la superficie rugosa después de técnicas regenerativas presentaban un mayor sangrado al sondaje y estaban relacionados con deficiencias vestibulares de la mucosa peri-implantaria (Schwarz et al., 2012). Estas deficiencias de la mucosa vestibular exponían el metal del implante y afectaban a la apariencia de las restauraciones.

Relevancia del tejido blando

Es relevante advertir que el grosor del tejido se ha señalado, de igual manera, como un factor a tener en cuenta a la hora de evaluar cuánto remodelado óseo se produce después de realizar la cirugía de conexión del pilar (Cosyn et al., 2012).

Las dimensiones apico-coronales de la mucosa queratinizada (MQ) han recibido también notable atención. Existe cierta controversia en la literatura científica con respecto a la necesidad de realizar técnicas de aumento en pacientes con una banda de MQ reducida o inexistente. De igual manera está pendiente de clarificar la repercusión del la MQ en el mantenimiento de la salud peri-implantaria. Las últimas revisiones sistemáticas apuntan a que no existe suficiente evidencia que permita relacionar los niveles óseos peri-implantarios con la cantidad de MQ (Lin et al., 2013). Tampoco existe evidencia de que ésta sea un requisito para la osteointegración (Roccuzzo et al., 2015). En cambio, los implantes con una banda reducida de MQ pueden ser más propensos al acúmulo de placa lingual y sangrado al sondaje así como a la recesión de la mucosa peri-implantaria (Schrott et al., 2009).

Estudios longitudinales a 10 años recientemente publicados, han indicado que pacientes con falta de tejido queratinizado presentaban mayores niveles de inflamación incluso cuando estos pacientes presentan un buen nivel de higiene y estaban bien mantenidos (Roccuzzo et al., 2016).

De igual manera, la ausencia de tejido queratinizado parece incrementar las molestias al cepillado lo que puede dificultar una correcta higiene (Souza et al., 2016). Factores relacionados con la falta de tejido queratinizado como la falta de profundidad de vestíbulo también se han relacionado con mayores niveles de inflamación (Halperin-Sternfeld et al., 2016).

En lo que se refiere al grosor de la mucosa peri-implantaria; estudios experimentales clásicos en los que se colocaron implantes en la cresta cicatrizada de perros Beagle con un enfoque en dos fases, ha valorado el impacto del grosor del tejido blando en los cambios de los niveles óseos marginares. Tras seis meses de cicatrización se pudo comprobar, mediante análisis histológico, que las zonas en las que el tejido se había adelgazado quirúrgicamente sufrieron una pérdida de hueso para acomodar la anchura biológica. En las localizaciones donde se había mantenido el grosor los cambios a nivel del hueso marginal fueron mínimos (Berglundh and Lindhe, 1996). Recientemente, estudios clínicos han corroborado las hipótesis derivadas de los estudios experimentales. En un estudio clínico prospectivo se clasificó a los pacientes en función de la altura vertical del tejido después de realizar el despegamiento del colgajo bucal. Se establecieron entonces dos grupos, aquellos con un grosor de >2mm y aquellos con un grosor de ≤ 2mm . Este último grupo fue a su vez dividido en aquellos que recibieron un sustituto de tejido blando de origen alogénico y aquellos que no recibieron aumento. El estudio concluyó que cuando el grosor del tejido era inferior a 2mm se producía mayor pérdida ósea y que los tejidos aumentados se comportaban de manera similar a aquellos naturalmente gruesos (Puisys and Linkevicius, 2015).

La influencia del volumen de tejido blando en la estética

Investigaciones clínicas e in-vitro han sugerido que el grosor de la mucosa peri-implantaria en la zona de transición del hombro del implante al margen gingival tiene un impacto directo en el color del tejido y en la capacidad del mismo de camuflar los componentes protéticos (Bressan et al., 2011).

El grosor mínimo necesario para un resultado estético aceptable parece estar en 2mm (Jung et al., 2007). Las diferencias en el color de la mucosa peri-implantaria están directamente relacionadas con el material con el que se confecciona el pilar protésico. En grosores inferiores a 2mm, los pilares cerámicos ofrecen propiedades ópticas superiores al compararlos con los metálicos (Jung et al., 2008).

Por lo anteriormente mencionado el uso de injertos de tejido conectivo ha cobrado importancia para conseguir un aspecto similar a los tejidos adyacentes. Los injertos de tejido blando han sido usados durante las últimas cuatro décadas de manera rutinaria en el campo de la Periodoncia para diversas técnicas quirúrgicas (Zuhr et al., 2014a). Al mejorar las técnicas de manejo de tejido blando y su predictibilidad en dientes, éstas se han expandido paulatinamente al campo de la implantología utilizándose no sólo para aumentar el grosor del tejido peri-implantario sino también para minimizar el grado de colapso que ocurre en implantes inmediatos y para restaurar el contorno del reborde alveolar en implantes diferidos (De Bruyckere et al., 2015).

A pesar de la importancia emergente que se atribuye actualmente a los procedimientos de aumento de tejido blando, no existe una evidencia clara sobre la cantidad de grosor y volumen que se necesita para un éxito a largo plazo de los implantes. De igual manera, está pendiente de dilucidar cuál es la relación entre tejidos blandos gruesos y un hueso subyacente fino además de si un tejido grueso puede compensar la falta de hueso en el aspecto bucal de los implantes (Thoma et al., 2014).

Es por ello que, además de las técnicas convencionales establecidas en el ámbito de la investigación sobre implantes, son precisas nuevas técnicas capaces de analizar los componentes del tejido blando y duro de una manera tridimensional para poder comprender mejor el papel de cada uno y la interacción que existe entre ellos.

6. Análisis de los componentes del tejido peri-implantario

Análisis histológico

El análisis histológico nace como una herramienta fundamental para superar las limitaciones de las mediciones clínicas y radiográficas en los estudios experimentales. Su uso en el campo de la implantología y la terapia regenerativa ha permitido evaluar el comportamiento de materiales de regeneración y diferentes superficies de implantes aportando información a nivel celular de los procesos biológicos y permitiendo asesorar la capacidad de integración o potencial de regeneración de diferentes materiales.

En el campo de la implantología la técnica de inclusión en metacrilato descrita por Donath y Breuner (Donath and Breuner, 1982) ha permitido evaluar la interacción entre hueso y tejido blando con superficies metálicas. Esta se basa en el corte de secciones de unas 100 micras que son posteriormente desgastadas mediante discos de pulido que reducen su grosor hasta las 35-30 micras permitiendo analizar los componentes celulares y estructuras anatómicas con gran detalle y definición. Tradicionalmente, se han evaluado secciones buco-linguales que logran obtener un máximo de 2-3 cortes por implante (*Figura 2*).

Figura 2. Preparación histológica de un implante mediante la técnica de Donath & Breuner.

Con el objetivo de poder visualizar de manera correcta los diferentes tejidos se han aplicado numerosas tinciones. Entre ellas, quizás la más destacada en el campo de la implantología es la propuesta por Levai–Laczkó (Jeno and Geza, 1975).

Las mediciones realizadas en estos cortes están consideradas como el “gold standard” en la evaluación de nuevas superficies y diseños de implantes ya que permiten evaluar cualitativamente y cuantitativamente los resultados. Entre las medidas más importantes se encuentran la distancia entre el hombro del implante y el primer contacto hueso-implante, la distancia entre el implante y el punto más coronal de la cresta ósea o la anchura buco-lingual de la cresta a diferentes alturas. Estas mediciones permiten, de igual manera, la evaluación de los componentes de la anchura biológica. Es posible, por lo tanto, estudiar la extensión de la barrera epitelial así como el número de capas que la componen y la naturaleza de la adhesión conectiva y orientación de las fibras.

El análisis descriptivo de esta muestras permite además observar los diferentes procesos biológicos que acontecen durante el proceso de cicatrización.

A pesar de las ventajas anteriormente descritas se ha de tener cuenta que el análisis histológico es un procedimiento invasivo. El procesado del tejido destruye las muestras al precisar seccionarlas. Además aporta información bidimensional sobre una región muy localizada de la muestra lo que limita el análisis de las zonas periféricas del implante.

Es por lo tanto recomendable combinar estas mediciones bidimensionales con otros métodos que aporten información tridimensional de la totalidad de la muestra (Becker et al., 2015).

Micro CT

Durante los últimos años el uso de la micro-tomografía computerizada (Micro-CT) también conocida como "histología tridimensional" se ha extendido en el campo de la medicina y en particular en el de la biología ósea ya que permite un alto grado de definición de las estructuras óseas (*Figura 3*).

Figura 3. Reconstrucción tridimensional de un implante evaluado mediante Micro CT. Nótese la claridad de las estructuras anatómicas.

El uso del Micro-CT ha demostrado ser una alternativa válida para evaluar los cambios en el volumen óseos, así como para analizar la composición ósea de manera tridimensional (de Faria Vasconcelos et al., 2016). Esta tecnología posee el valor añadido de ser rápida, reproducible y ser capaz de conservar la muestra íntegra sin producir daños en su estructura (Bernhardt et al., 2004, Cuijpers et al., 2014).

El Micro-CT es capaz, mediante programas informáticos, de generar reconstrucciones tridimensionales de la estructura ósea humana (Kim and Henkin, 2015, Muller and Ruegsegger, 1997). Con este propósito se ha usado en el análisis del tejido óseo alrededor de implantes osteointegrados demostrando una correlación aceptable con las mediciones histomorfométricas (Park et al., 2005).

El micro CT permite además evaluar el porcentaje de contacto hueso implante (BIC) en los 360º de la muestra en contraposición a los cortes únicos de la histología convencional. En un artículo que evaluaba la fiabilidad de las mediciones de BIC en Micro CT comparándolas con histología clásica, se demostró una elevada variabilidad en las mediciones de las muestras histológicas (±35%) probando así las limitaciones de las mediciones en cortes histológicos únicos mediante la técnica de Donath. Se recomendó entonces utilizar un mínimo de tres cortes histológicos para poder asesorar mejor esta variable (Bernhardt et al., 2012).

El Micro CT permite la evaluación de volúmenes óseos en áreas de interés previamente definidas utilizando algoritmos matemáticos y programas informáticos específicamente diseñados que computan un elevado número de cortes (Neldam and Pinholt, 2014).

Este análisis puede, por lo tanto, aportar información relevante de parámetros que anteriormente han pasado desapercibidos por las limitaciones existentes en las técnicas histológicas tales como el volumen óseo vestibular, o la composición del mismo.

La limitación más destacada de esta técnica consiste en la falta de información fiable del tejido blando que imposibilita cualquier tipo de medida a este nivel.

Análisis del contorno de los tejidos blandos

La introducción de la tecnología digital en el campo de la implantología ha permitido el estudio en los cambios del contorno de los tejidos blandos después de procedimientos de aumento de tejidos blandos o duros (Jemt and Lekholm, 2003, Schneider et al., 2011).

Actualmente, el resultado de las reconstrucciones con implantes no se asesora únicamente mediante medidas clínicas sino con el uso de técnicas digitales que permiten asesorar tridimensionalmente de manera fiable los cambios en los tejidos (Benic et al., 2015).

La tecnología de análisis volumétrico de los tejidos blandos se basa en la obtención de archivos estereolitográficos (STL) que reportan información precisa sobre la anatomía de los tejidos blandos, las restauraciones sobre implantes y los dientes naturales. Los archivos STL describen la geometría de una superficie de manera tridimensional mediante un sistema de triangulación ampliamente usado en impresión tridimensional. Estos archivos se pueden obtener mediante la realización de un escáner intra-oral o mediante el escaneado del modelo de escayola realizado con un escáner de escritorio. Esto permite evaluar los cambios en la superficie de los modelos antes y después de diferentes intervenciones (Schneider et al., 2014)(*Figura 4*).

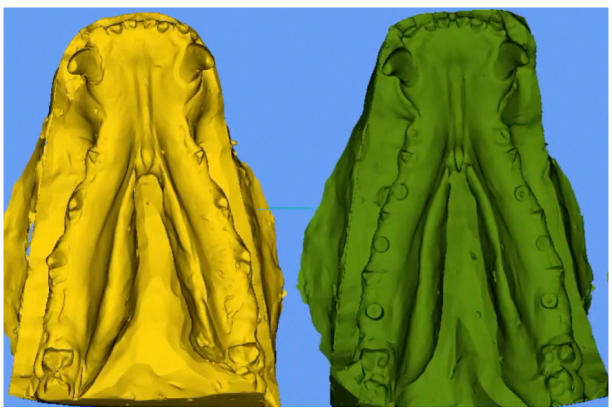


Figura 4. Imágenes tridimensionales de archivos STL provenientes del escaneado óptico de modelos de la mandíbula de un perro Beagle antes de la colocación de implantes (amarillo) y después de la colocación (verde)

Mediante el uso de programas informáticos específicos de análisis perfilométrico se superponen los modelos utilizando referencias comunes que no han cambiado en el tiempo transcurrido entre las dos intervenciones (Gonzalez-Martin et al., 2014).

La fiabilidad y reproducibilidad de este tipo de mediciones ha demostrado ser elevada en estudios in-vitro realizados para testar la metodología (Windisch et al., 2007).

La utilización de este tipo de tecnología se ha extendido a la evaluación del resultado de cubrimientos radiculares (Zuhr et al., 2014b), aumentos de tejido blando en crestas cicatrizadas (Zeltner et al., 2017) y estabilidad del tejido blando alrededor de implantes (Sanz Martin et al., 2016).

Superposición Micro CT-STL: Análisis de volumen de tejido óseo y blando

A pesar de las grandes ventajas que otorga la superposición de archivos STL para el estudio de los cambios dimensionales en el tejido blando esta técnica tiene ciertas limitaciones. La falta de información referente al tejido óseo impide dilucidar si los cambios observados en el contorno y volumen del tejido son producto de un aumento/reducción del tejido blando o de la cresta ósea. Es por ello que sería beneficioso poder sumar a esta tecnología información sobre la anatomía del reborde alveolar.

Tecnologías que permitiesen mediciones del volumen de tejido blando y duro supondrían un paso adelante para lograr una mejor comprensión de la interacción entre el tejido blando y el tejido óseo.

III. JUSTIFICACIÓN

El aumento de la predictibilidad en la osteointegración de implantes dentales ha llevado a clínicos e investigadores a evaluar nuevos diseños de implantes capaces de reducir el trauma quirúrgico y aumentar la cantidad de hueso en vestibular. Las hipotéticas ventajas de estas innovaciones y cambios han de evaluarse en ambientes controlados que permitan contrastar sus resultados y estudiar su fiabilidad.

El estudio de la estabilidad del tejido peri-implantario es clave para mantener los tejidos en salud y comprender la aparición de complicaciones. En este contexto, unas dimensiones adecuadas de tejido duro y blando han demostrado ser favorables para reducir los cambios fisiológicos que ocurren después de la conexión del pilar así como para la consecución de resultados estéticos satisfactorios.

A pesar de la importancia que se atribuye actualmente al tejido óseo y blando peri-implantario existen numerosas lagunas en el conocimiento de su comportamiento y mutua interacción. Entre ellas, la influencia de protocolos quirúrgicos diferidos e inmediatos, el impacto en los tejidos blandos de implantes con diseños destinados a preservar el hueso en vestibular o la relación entre tejidos blandos gruesos y un hueso subyacente fino y viceversa.

En referencia a los procedimientos destinados a regenerar deficiencias de la cresta ósea, es importante analizar el resultado de las técnicas regenerativos para conocer si el volumen conseguido a través del aumento es comparable con el inicial o si son precisas técnicas adicionales como el manejo de tejidos blando para restaurar el contorno del reborde alveolar.

IV. HIPÓTESIS

La hipótesis general de este trabajo es que el uso de implantes con un diseño cervical triangular, comparados con implantes cilíndricos convencionales, pueden aportar beneficios en las dimensiones de la cresta y estas a su vez pueden tener efectos positivos sobre los tejidos blandos. Además; la cicatrización de implantes colocados en alveolos post extracción presenta diferencias estructurales al compararlos con aquellos colocados en crestas cicatrizadas. En referencia al tercer trabajo; el uso de una terapia regenerativa combinada mediante el uso de sustitutos óseos y membranas proporciona mejores resultados en cuanto a aumento del reborde comparado que cada uno de los tratamientos por separado.

De manera concreta, se plantean las siguientes hipótesis específicas:

1. Los diseños cervicales triangulares pueden aportar un mayor ancho de cresta vestibular que contribuya a minimizar la pérdida ósea peri-implantaria, tener un mejor volumen de hueso vestibular y proporcionar un efecto favorable sobre el contorno de los tejidos blandos.

2. La superposición de imágenes con información del tejido óseo obtenidas mediante Micro CT con las imágenes provenientes del escaneado óptico de modelos de escayola (STL) que aportan información de los tejidos blandos pueden permitir analizar los volúmenes de tejido duro y blando y la interpelación que existe entre ellos ayudando a comprender el efecto de diferentes diseños cervicales de implantes y protocolos quirúrgicos.

3. El análisis de los resultados del tratamiento de defectos crónicos del reborde alveolar mediante técnicas de regeneración ósea guiada, obtiene mejores resultados al usar la combinación de membranas reabsorbibles y sustitutos óseos comparado con el uso de membranas y sustitutos óseos por separado.

V. OBJETIVOS

Objetivo general

Estudiar el comportamiento de un implante con un nuevo diseño cervical triangular colocado en crestas cicatrizadas y en alveolos post-extracción. De igual manera, analizar los resultados del tratamiento de defectos crónicos del reborde alveolar mediante el uso de diferentes alternativas terapéuticas.

Objetivos secundarios

1. Evaluar las diferencias entre protocolos de extracción inmediatos y diferidos y mejorar la comprensión sobre la influencia del protocolo quirúrgico en la cicatrización de los tejidos blandos y duros.

2. Analizar tridimensionalmente, mediante Micro CT, el volumen óseo vestibular y los componentes del mismo así como estudiar el contorno de los tejidos blandos antes y después de la colocación de implantes mediante superposición de archivos STL obtenidos del escaneado de modelos de escayola.

3. Desarrollar una metodología basada en la superposición de archivos DICOM obtenidos mediante Micro CT con archivos STL obtenidos mediante el escaneado óptico de modelos de escayola, capaz de aportar información en cuanto al volumen real de los tejidos peri-implantarios.

4. Mediante el uso de la metodología anteriormente descrita, estudiar la interacción entre el tejido óseo bucal y el tejido blando peri-implantario para poder comprender la relación que existe entre ellos así como la influencia de protocolos de cicatrización y diseños de implantes

5. Estudiar las diferencias en el contorno de los tejidos mandibulares antes de la creación de defectos del reborde alveolar y después de haber realizado técnicas regenerativas, con el objetivo de medir la capacidad de las mismas de reconstruir el proceso alveolar.

VI. MATERIAL Y MÉTODOS. RESULTADOS

La descripción detallada del material y métodos, así como los resultados de este trabajo de investigación han sido publicados como artículos científicos en tres publicaciones independientes con las siguientes referencias:

1. Hard and soft tissue integration of immediate and delayed implants with a modified coronal macro-design. Histologic, Micro CT and volumetric soft tissue changes from a pre-clinical in-vivo study. Sanz-Martin I, Vignoletti F, Nuñez J, Permuy M, Muñoz F, Sanz-Esporrín J, Fierravanti L, Shapira L, Sanz M. *Journal of Clinical Periodontology*. *Accepted for publication*.

2. Hard and soft tissue volume analysis of immediate and delayed implants with different cervical design. A novel methodological approach using superimposed Micro-CT and STL images. Sanz-Martin I, Vignoletti F, Nuñez J, Permuy M, Muñoz F, Sanz M. Clinical Oral Implants Research. *Submitted for publication*.

3. Contour changes after guided bone regeneration of large non-contained mandibular buccal bone defects using deproteinized bovine bone mineral and a porcine-derived collagen membrane. An experimental in vivo investigation. Sanz-Martin I, Ferrantino L, Vignoletti F, Nuñez J, Baldini N, Duvina M, Sanz M. *Clinical Oral Investigations. Submitted for publication*.

Artículo 1:

Hard and soft tissue integration of immediate and delayed implants with a modified coronal macro-design. Histologic, Micro CT and volumetric soft tissue changes from a pre-clinical in-vivo study. Sanz-Martin I, Vignoletti F, Nuñez J, Permuy M, Muñoz F, Sanz-Esporrín J, Fierravanti L, Shapira L, Sanz M. *Journal of Clinical Periodontology*. *Accepted for publication*.

Objetivo: Estudiar la cicatrización de los tejidos blandos y duros alrededor de implantes con una configuración triangular (Test/T) y cilíndrica (Control/C).

Material y métodos: Se colocaron implantes inmediatos y diferidos en 8 perros beagle. Los implantes T y C se aleatorizaron en el momento de la cirugía. Se analizaron las muestras 4 y 12 semanas después de la intervención quirúrgica mediante histología y Micro CT. De igual manera, se analizaron los cambios en el contorno de los tejidos blandos.

Resultados: El grosor de la cresta a nivel del hombro del implante fue similar para los implantes T y C. En las porciones más apicales el grupo T obtuvo mayores dimensiones horizontales de la cresta en implantes diferidos e inmediatos. No hubo diferencias significativas en las medidas histomorfométricas verticales de tejido blando y óseo. El análisis mediante Micro CT reveló menor volumen de Titanio y similar volumen de hueso. En análisis de los cambios del contorno del tejido no mostró diferencias entre implantes T y C.

Conclusiones: Los implantes triangulares obtuvieron un porcentaje similar de integración, volumen óseo y contorno del tejido blando aunque obtuvieron mayores dimensiones horizontales de la cresta.

Journal of Clinical Periodontology - PROOF

Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

Hard and soft tissue integration of immediate and delayed implants with a modified coronal macro-design. Histologic, Micro CT and volumetric soft tissue changes from a pre-clinical in-vivo study.

Journal:	*Journal of Clinical Periodontology*
Manuscript ID	CPE-12-16-6811
Manuscript Type:	Animal Experiment
Date Submitted by the Author:	n/a
Complete List of Authors:	Sanz Martin, Ignacio; Universidad Complutense, Periodontology Vignoletti, Fabio; Complutense University of Madrid, Núñez, Javier; Facultad de Odontologia, Universidad Complutense de Madrid, Master de Peridoncia; Permuy, Maria; University of Santiago de Compostela, Veterinary Clinical Sciences Muñoz, Fernando; University of Santiago de Compostela, Veterinary Clinical Sciences Sanz-Esporrín, Javier; Universidad Complutense, Periodontology Ferravanti, Ludovica; Facultad de Odontologia, Universidad Complutense de Madrid, Master de Peridoncia Shapira, Lior; Hebrew University and Hadassah Medical Center, Periodontology Sanz, Mariano; Universidad Complutense de Madrid, 3ETEP (Etiology and Therapy of Periodontal Diseases) Research Group
Topic:	Implantology
Keywords:	dental implants, implant macro-design, histology, animal model, immediate implant
Main Methodology:	Animal Model

Hard and soft tissue integration of immediate and delayed implants with a modified coronal macro-design. Histologic, Micro CT and volumetric soft tissue changes from a pre-clinical in-vivo study.

Sanz-Martin I [1], Vignoletti F [1], Nuñez J [1], Permuy M [2], Muñoz F [2], Sanz-Esporrín J [1], Ferravanti L [1], Shapira L [3], Sanz M [1].

[1] Section of Periodontology, Faculty of Odontology, University Complutense of Madrid, Madrid, Spain.

[2] Faculty of Veterinary Lugo. University of Santiago de Compostela. Lugo, Spain

[3] Department of Periodontology. Hebrew University - Hadassah Faculty of Dental Medicine, Israel.

Key words: “dental implants”, “animal model”, “Immediate implant”, “implant macro-design”, “histology”, “micro CT”, “volumetric analysis”.

Running title: Triangular versus cylindrical implants

Address for correspondence: Dr. Ignacio Sanz Martín

Facultad de Odontología

Universidad Complutense de Madrid

Plaza Ramón y Cajal s/n

28040 Madrid.

e-mail: ignaciosanzmartin@odon.ucm.es

Word Count Manuscript: 4740

Number of figures and tables: 4 figures, 3 tables.

ABSTRACT

AIM

To study the healing of peri-implant tissues around implants with a triangular coronal third (test) or cylindrical (control) when placed with delayed and immediate surgical protocols.

MATERIAL AND METHODS:

In eight beagle dogs, immediate implants were placed in the mesial sockets of the third and fourth premolars. Delayed implants were placed in healed sites at second premolar and first molar sites. Test and control implants were randomly assigned to both sites and the hard and soft tissue healing was evaluated with histology and Micro CT analysis at 4 weeks (T4, n=8) and 12 weeks (T12 n=8). The volumetric changes in the soft tissue contours were assessed by image analysis software from dental stone casts.

RESULTS:

Similar crestal resorptive changes occurred in both Test and Control implants. When measured at the implant shoulder level, the crestal bone width attained similar values in test and control implants. More apically (3 mm) test implants had significant more crestal width in delayed sites. Significant differences were also found in immediate sites at 2, 3, 4 and 5 mm. For vertical soft and hard tissue measurements, no significant differences were found between Test and Control. With Micro CT the evaluation of the buccal volume of interest (VOI) showed significantly less volume of implant component in T implants in all sites, although the bone volume was not significantly different between T/C. Soft tissue contours were similar around T/C implants.

CONCLUSION:

Triangular implants when compared with standard cylindrical implants showed similar percentage of osseointegration, buccal bone volume and soft tissue contours, although attaining significantly greater crestal bone width. No differences between studied implants were found in regards to soft tissue dimensions and crestal bone remodelling.

CLINICAL RELEVANCE

Scientific Rationale for Study: New implant macro-designs with a triangular neck have been recently introduced with the aim of promoting greater amounts of peri-implant bone. There is limited evidence on the healing of the hard and soft tissues around this new implant design.

Principal Findings: Crestal bone width was greater for triangular implants. No significant differences were found for the amount of vertical soft and hard tissues, buccal bone volume or buccal soft tissue contours.

Practical Implications: Triangular implants performed similarly in terms of hard and soft tissue integration in both the delayed and immediate implant surgical protocols, although attaining greater bone width dimension.

INTRODUCTION:

Implant therapy is currently considered an effective treatment for the functional and aesthetic rehabilitation of missing teeth, as evidence by long-term (more than 10-years) studies with different implant systems (Buser et al., 2012, Gotfredsen, 2012, Ostman et al., 2012). In spite of these high success rates, osseointegrated implants are susceptible to crestal bone level changes through physiological remodelling or due pathological processes, such as peri-implantitis (Laurell and Lundgren, 2011). It is currently believed that early bone loss might be a risk factor for the initiation of peri-implantitis (Schwarz et al., 2012) and, therefore, there is an increased interest in maintaining peri-implant bone levels, mainly the buccal bone, also due to the aesthetic implications of the possible concomitant loss of soft tissue volume (Merheb et al., 2014, Spray et al., 2000).

With this goal, different implant macro-designs have been experimentally evaluated reporting similar degree of hard tissue integration and mucosal attachment (Abrahamsson et al., 1996, de Sanctis et al., 2010, de Sanctis et al., 2009). There are however some factors that have shown to significantly reduce bone remodelling, such as a tight implant to abutment connection (Pessoa et al., 2016); a reduced number of abutment connections and disconnections (Molina et al., 2016); the distance between the bone crest to the implant to abutment connection (Alomrani et al., 2005) and the horizontal mismatching of the abutment to the implant platform (Schwarz et al., 2014). Similarly, the use of narrow implants has been advocated to increase peri-implant crestal bone thickness (Ioannidis et al., 2015, Galindo-Moreno et al., 2012). With a similar goal, a novel implant has been designed by making the coronal third of the implant triangular, thus increasing the space between the flat part of the triangle and the buccal wall, what in principle might achieve thicker bone after healing, thus promoting peri-implant tissue stability. These goals, however, have not been demonstrated experimentally.

In implant preclinical research, the healing of dental implants has been studied using mainly two-dimensional ground section histology, which allows for histometric and histo-morphometric analysis, what documents the healing dynamics of both hard and soft tissues (Berglundh et al., 2003, Berglundh et al., 2007). This histological protocol, however, only evaluates a narrow zone of 35-50 microns, what results in no more than three sections per sample, what clearly limits the information.

Micro-level computed tomography (Micro-CT) has been recently validated as an alterative to study the bone volumetric changes and the internal bone structure (de Faria Vasconcelos et al., 2016). Micro-CT provides a less invasive three-dimensional evaluation of the bone changes, what adds information on the biological events that occur at the periphery of the implant (Bernhardt et al., 2004, Cuijpers et al., 2014). The evaluation of the volume and stability of peri-implant soft tissues has also been difficult to measure reliably. The introduction of volumetric analysis based in STL image superimposition has allowed an accurate evaluation of tissue contour changes and thus, the impact that different implant designs or restorative interventions might have on the aesthetic outcomes (Schneider et al., 2011, Thoma et al., 2010).

This in-vivo pre-clinical investigation was therefore designed to evaluate the hard and soft tissue integration of this new implant design using histological analysis, Micro CT and volumetric analysis of soft tissue contour changes.

MATERIAL AND METHODS

This pre-clinical *in vivo* investigation was designed according to the modified ARRIVE guidelines (Vignoletti and Abrahamsson, 2012) using a randomised block, examiner-blind experimental design on eight adult beagle dogs with a weight ranging between

10 and 20 kg.

The study was carried out at the Experimental Surgical Centre of the Hospital ''Gomez-Ulla'' in Madrid, Spain.

Study Implants

Both test and control implants (MIS Implants Technologies Ltd., Bar-Lev Industrial Park, Israel) had an internal hexagonal connection, a diameter of 3.5 mm, an identical design in their apical half with a conical shape and self-cutting threads and were specially manufactured for this experimental investigation. Test implants were triangular in their coronal half, with a reduction in each of the three sides of the triangle of 0.4mm, which extended 3.9 mm below the implant shoulder. Control implants had a conventional cylindrical shape. Test and control implants of 10 mm in length were used at the delayed sites, while 11.5mm was used in immediate sites.

Surgical interventions and experimental model

Animals were sedated and under general anaesthesia with mechanical respiration throughout the surgery.

Intervention I: M1 and P2 were carefully hemisected, their exposed pulp was sealed with Calcium Hidroxide (Dycal. Dentsply. York, USA) and a glass-ionomer filling (Ketac. 3M ESPE. Berkshire, UK). Once the mesial roots were carefully extracted, the buccal and lingual wound margins were sutured with resorbable sutures (Vycryl 5-0. Ethicon. Sommerville, US).

Intervention II: Extraction sockets were left to heal for 2 months to provide healed sites for the delayed implants. These sites were accessed after rising buccal and lingual full-thickness flaps. The mesial roots of P3 and P4 were then extracted using a flapless protocol. The resulting extraction sockets served as the immediate implants sites (Fig. 1a).

In both sites, implants were placed using the drilling protocols recommended by the implant manufacturer. Immediately after the osteotomy preparation, allocation to test or control implants was done by opening sealed envelopes containing the randomization code. Both test and control implants were evenly distributed by location within the mandible and between healed sites and fresh extraction sockets (Fig. 1b). Well-trained periodontal specialists placed all the implants (FV, JN, IS, LS) being unaware of the randomization process and treatment allocation. When inserting the test implants care was taken to leave the flat side of the triangle facing the buccal aspect (Fig. 1c). In both delayed and immediate implants, the implant shoulder was placed levelled with the buccal bone crest. Healing abutments of 3 or 5 millimetres were then placed and the flaps were sutured, thus allowing a transmucosal healing. This experimental design provided two healed sites in PM2 and M1 (1T, 1C) and 2 immediate sites in PM3 and PM4 (1T, 1C) per dog hemi-mandible.

Intervention III: Following the experimental design and the sacrifice schedule, the same procedure was repeated on the other side of the mandible after 8 weeks of healing.

Biopsies and histological processing

Four weeks after Intervention III the 8 dogs were sacrificed with an overdose of sodium pentothal (40-60 mg/kg/i.v., Dolethal, Vetoquinol, France), thus providing 2 healing timelines: 4 and 12 weeks (T4 and T12). Specimens were prepared for ground sectioning, as described by Donath (Donath and Breuner, 1982).

Histological Analysis

The following landmarks were used for the histometrical measurements on the buccal and lingual side of the ground sections: implant shoulder (I); coronal level of the bone crest (BC); coronal level of bone to implant contact (BIC); peri-implant mucosa margin (PM) and apical border of the junctional epithelium (aJE).

The primary outcome were the horizontal changes in the buccal crest, the resulting crestal width (CW), which was recorded 1, 2, 3, 4 and 5 mm from the implant shoulder (Sup Fig 1). Vertical bone resorption and the dimensions of the peri-implant soft tissues were also recorded using the following linear measurements: I-BC, I-BIC, BC-BIC, PM-aJE and aJE-B.

Micro-CT analysis

All specimens were scanned before being sectioned using a high-resolution Micro-CT (Skyscan 1172, Bruker microCT NV, Kontich, Belgium). The X-ray source was set at 100Kv and 100μA with a voxel size of 12 μm and an Aluminium/Copper filter (Al/Cu). The scanning was performed over a 360° rotation acquiring images every 0.4°, which were later reconstructed using NRecon® software (Bruker microCT NV, Kontich, Belgium) and the algorithm described by Feldkamp (Feldkamp LA, 1984). Reconstructed images were evaluated with the Data Viewer® software (Bruker microCT NV, Kontich, Belgium) once the implant was perfectly aligned (Figs. 3 a-d).

Three different volumes of interest (VOI) were defined (Sup Fig. 2):

i) *Cylindrical VOI* using a region of 5 mm in diameter and 4mm in apico-coronal length at the central part of the implant, thus excluding the implant shoulder and the apical part. This VOI included the peri-implant tissues in all directions (distal, mesial, buccal and lingual).

ii) *Buccal VOI* using a region of 1.5mm from the mesial and distal aspect of the implant shoulder and 4mm towards the buccal aspect, thus selecting the coronal buccal aspect of the implant. This VOI divided the implant in two equal halves and extended 4mm apically from the implant shoulder

iii) *Buccal bone VOI* obtained by outlining manually the buccal alveolar crest from the buccal VOI. This VOI only included the bone component and the implant, thus allowing the evaluation of the percentage of "air" within the bone.

Data was analysed with the CTAn® software (Bruker microCT NV, Kontich, Belgium) using adaptive local threshold methods for segmenting the images and thus setting the best threshold parameters for the analysis of bone and metal. The percentage of bone and the ratio of bone volume to total volume (BV/TV), which corresponds to the bone density around the implant, were measured in a section of 20 pixels around the implant surface. In the same VOI, the degree of osseointegration was measured using the method described by Bruker (Bruker, 2015) in which the bone pixels in contact to those corresponding to the implant, were evaluated and the percentage of bone-implant contact (BIC) was calculated.

Using the same threshold settings, the quantity of bone, implant and "air" (includes the non-calcified tissues and marrow spaces) were evaluated in the buccal VOIs. In the buccal bone VOI, the percentage of the "air" within the bone provided an estimate of bone quality.

Image analysis

Impressions of the mandible were obtained before implant placement (BS) and at the time of sacrifice (FU) resulting in 8 pairs of models. Models were then optically scanned with a desktop 3D scanner (Zfx Evolution Scanner, Zimmer Dental. Bolzano, Italy) providing STL files, wich were assessed and matched with an image analysis software (Swissmeda Software, Swissmeda AG, Zürich, Switzerland) (Sup Figs. 4a, 4b)

A longitudinal slice dividing the implant mesio-distally into two equal parts was selected. Then a line coinciding with the axis of the implant was drawn creating a the transversal image of the sections. A screenshot of this image was then exported to an image processing software (ImageJ, National Institutes of Health. Maryland, USA) where the following linear measurements were performed by a blinded evaluator, previously calibrated (LF): (Figs. 4a,4b).

i) Horizontally the distance between the line coinciding with the axis of the implant and the buccal soft outline was measured at 0,2 4 and 6mm below the gingival margin (IMI) or alveolar ridge (DLI) at both time points. Differences between the two measurements were calculated by substracting BS and FU (Sanz Martin et al., 2016a, Sanz-Martin et al., 2016b).

ii) Vertically, the distance between two lines perpendicular to the axis of the implant assessed the changes in tissue height. The first line was coincident with the gingival margin of the tooth (IMI) or the crest (DLI) at BS and other line with the gingival margin of the implant at FU.

A more detailed description of the anethetic regimen, post operative care, biopsy handling, histologic processing and STL matching can be found in Annex 1.

Statistical analysis

Descriptive statistics (means, standard deviations) of continuous variables were analysed using a statistical software program (SPSS Version 20.0, IBM Corporation. New York, USA). The data was tested for normality by means of a Shapiro-Wilk test and found to be non-normal. The Mann-Whitney test was used analyse differences for continuous variables. Statistical significance was set at the alpha level of 0.05.

RESULTS

All animals healed uneventfully without significant complications. All implants showed clinical and histologic signs of osseointegration. During implant installation two vertical fractures occurred in two test implants, which were left to heal and were processed for histological evaluation.

Descriptive Histology

At 4 weeks of healing the supracrestal soft tissues around the shoulder of the implant were composed of an immature dense connective tissue (CT) with a marked cellular infiltration and vascularity. The junctional epithelium (JE) was well adhered to the abutment with varying apical extension, although mostly within the implant abutment and rarely reaching beyond the implant shoulder.

A moderate bone resorption was observed in both implant designs and surgical protocols depicting clear signs of remodelling and a marked osteoclastic activity in both buccal and lingual bone crests, although mainly around delayed implants (Sup Figs. 3a, 3b). In the areas adjacent to the implant surface, de novo bone formation was coupled with bone resorption.

In immediate implants, remnants of bundle bone were sometimes observed in the inner part of the socket wall, which frequently showed marked remodelling activity. The buccal gap was frequently filled partially with an osteoid like tissue (Sup Figs. 3c, 3d). Similar findings were observed in the delayed test implants where the chamber left from the triangular shape filled with newly formed bone.

At 12 weeks the supracrestal soft tissues were composed of a dense and mature CT and a JE with similar characteristics to the 4-week description. The CT was rich in elongated fibroblasts in the vicinity of the implant surface, although frequent inflammatory cells were identified infiltrating in the buccal connective tissue. This was particularly noticeable for implants in the P2 sites.

A moderate bone loss was observed at the buccal aspect in both implant types and with both surgical protocols. In the DLI, the first bone to implant contact (BIC) on the buccal aspect was located between 0.5mm to 1.5mm to the implant shoulder (Figs. 2a, 2b). In the IMI, a gap of various dimensions frequently occurred between the buccal socket walls and the implant surface. This marginal gap was less noticeable at 12 weeks compared to 4 weeks and it was filled with dense connective tissue for both test and control implants leaving part of the coronal implant surface exposed. This finding led to a more apical first BIC in the IMI when compared to the DLI (Figs. 2c, 2d). In both DLI and IMI bone remodelling was not only circumscribed to the alveolar crest but throughout the whole preparation, demonstrating remodelling processes persistent at 12 weeks in both the parent and new bone.

Histometric analysis (all values in mm)

1. *Horizontal Ridge alterations*

The results of crestal width measurements at T12 stratified by implant type and site are presented in Table 1.

In delayed implants at PM2 sites test and control implants presented similar values of CW at all height levels with none of the implants exhibiting measurable CW at the level of the implant shoulder (CW0). In M1 sites, crestal width values were similar for test and control implants at the more coronal height, while at 3, 4 and 5 mm below the implant shoulder CW values were higher the test group, being statistically significant at CW3.

In immediate implants at PM3 sites the CW0 and CW1 where similar in test and control implants. More apically at 2mm bellow the implant shoulder the values were 0.83mm and 0.41mm (T/C), CW3; 0.99mm and 0.47mm, CW4; 1.24 and 0.53mm and CW5; 1.23mm and 0.60mm, being these differences statistically significant at CW3, 4 and 5. In PM4 sites, the CW0 values were 0.47mm and 0mm (T/C), CW1; 1.49mm and 0.96mm, CW2; 1.22mm and 0.86mm, CW3; 1.22mm and 0.86mm, CW4; 0.84mm and 0.65 mm and CW5; 0.43mm and 0.67mm being these differences statistically significant at CW4 and 5.

2. *Vertical Ridge alterations*

Descriptive statistics of vertical hard tissue histometric measurements stratified by implant type, surgical approach and study timeline are depicted in Table 2. There were no significant differences between test and control implants for all the parameters analyzed.

The I-BC distances for the buccal and lingual aspects were minimal (0.2 mm approximately) in both delayed and immediate implant sites at 4 weeks of healing. At 12 weeks this distance increased although no significant differences were observed between test and controls. In respect to I-BIC values, in the DLI no difference was observed between test (0.67±0.40) and control implants (0.83±0.49) at four weeks in the buccal aspect. At 12 weeks these values increased to 1.24±0.72

and 1.08±0.88 for test and control implants respectively. In the IMI, at 4 weeks the I-BIC in the buccal aspect amounted to 1.55±1.21 for the test implants and 1.70±0.80 for the control implants whereas at 12 weeks these values slightly decreased to 1.54±0.89 and 1.18±0.47 for test and control implants, respectively.

3. Soft tissue dimensions

Descriptive statistics of soft tissue histometric measurements stratified by implant type; surgical approach and study timeline are listed in Table 2. There were no significant differences between test and control implants for all the parameters analysed.

In delayed implants the values of PM-aJE, for the test and control groups at 4 weeks were similar (2.07±0.25 and 2.17±0.63). These values remained stable at 12 weeks. The corresponding values for immediate implants at 4 weeks were 2.13±0.19 and 1.97±0.37, being also similar at 12 weeks.

In delayed implants the values of aJE-BIC at 4 weeks were 2.15±1.36 and 1.9±0.79 for test and control implants respectively with similar values at 12 weeks. In immediate implants these values were 2.97±1.01 and 2.96±1.08 for test and control implants at 4 weeks and remained stable at 12 weeks.

When pooling the data of test and control implant together and analysing the influence of the study timeline and surgical protocol on the hard and soft tissues, significant differences were observed (Sup Table 1). At 12 weeks, immediate implants when compared with delayed implants, presented higher values of I-BIC and BC-BIC in both the buccal and lingual aspects. No significant differences were observed in the soft tissue dimensions between these two surgical protocols.

Micro-CT results

The BIC results stratified by tooth site are shown in Table 3. Similar results were attained for both test and control implants with BIC % ranging from 46.63% to 51.63% in the DLI and from 49.38% to 57.25% in the IMI.

At T4 most of the osseointegration had been accomplished in both for both delayed and immediate implants test and control implants, respectively (BIC% DLI-T: 44.00 ± 7.7; DLI-C: 49.13 ± 11.5; IMI-T: 48.13 ± 14.1; IMI-C: 51.13 ± 9.9). At delayed sites at T12, the BIC was higher in test implants (56.5 ± 14.1) than in control implants (49.13 ± 11.4), although these differences were not statistically significant. At immediate sites, these BIC % were very similar (54.13 ± 11.4; 58.10 ± 10.7, respectively) (Sup table 2).

At delayed sites, the ratios of bone volume to tissue volume (BV/TV) at 12 weeks were significantly higher in the test when compared with the control group (60.38±7.41 and 51.00±7.43). The corresponding values at immediate sites were similar (60.38 ± 10.1 and 63.75 ± 8.3, respectively). The BV/TV rations stratified by tooth sites (Table 2) attained similar results for both test and control implants, ranging from 52 – 64 in both DLI and IMI.

Table 3 also depicts the percentages and volumes (in mm3) of bone, “air” and implant in the buccal VOI when stratified by tooth site. The total volume evaluated in all samples amounted to 152.75 mm^3. In the test group, a statistically significant lower percentage and volume of the implant component was found as compared to control. The other measured variables (“air” and bone) did not show statistically significant differences, with percentages of “air” ranging from 57.88% to 60% in the delayed sites and from 63.88% to 66.38% in the immediate sites. The percentages of bone ranged from 26.88% to 28.50% and from 20.38% to 21.25%, respectively.

Similarly, when a comparative analysis was carried out only at the buccal bone VOI, the volume of the implant component was significantly lower in the test group in all sites. The volume of "air" in the buccal bone, which included marrow spaces and non-calcified tissues, was similar when test and control implants were compared (Sup Table 3).

Soft tissue Volume analysis

Table 3 depicts the vertical and horizontal changes in the soft tissues stratified by implant site. In the delayed sites, a general trend of increased ridge width was observed at the level of the gingival margin (H0) and 2,4 and 6mm bellow it, independently of the implant design. In contrast, at immediate sites a generalized reduction was observed and this finding was similar in test and control groups. In regards to the vertical soft tissue changes, minor changes occurred in both immediate and delayed sites with no differences between implants (Figs. 4a, 4b).

DISCUSSION

The present investigation was designed to test a novel implant with a modified coronal third of the implant section, when placed with two different surgical protocols, the immediate and the delayed implant placement. Test and Control implants showed similar outcomes in the buccal bone crest width (CW) at the most coronal part of the crest (within 1 mm from the implant shoulder) in both surgical protocols. However, more apically (2, 3, 4 and 5 mm below the implant shoulder) significantly higher CW were attained in the test implants, being statistically significant for the delayed implants at CW3 and for immediate implants at CW 2, 3, 4 and 5. The secondary outcomes (vertical hard and soft tissue dimensions) did not show significant differences between control and test implants in both immediate and delayed sites. Similarly, the percentage of osseointegration was equivalent for both implant designs. At delayed sites, the ratios of bone volume to tissue volume (BV/TV) at 12 weeks were significantly higher in the test when compared with the control group (60.38±7.41 and 51.00±7.43). These statistically significant differences between the tested implant designs did not occur in the immediate implant sites. Test implants showed a statistically significant lower percentage of volume of the implant/titanium when compared to control implants. No further differences were encountered between test and control groups, both in buccal bone volume or soft tissue contours.

These histological outcomes in both immediate and delayed sites were in agreement with those reported using similar surgical protocols in a similar experimental model (De Santis et al., 2015, Favero et al., 2013). The significantly wider crestal values reported for the test implants with the immediate protocol indicate that the reduction in the diameter of the implant by the triangular sectioning was effective in providing a greater distance to the socket wall, which subsequently filled with bone when appropriate healing time was allowed. These findings are also in agreement with clinical studies on immediate implants reporting that the dimension of the horizontal gap influenced the ridge alterations, being the fill of the horizontal gap more pronounced when the horizontal diameter of the gap was bigger (Ferrus et al., 2010).

The fact that wider crest values were not attained in the most coronal part may be due to the healing times selected, being 12 weeks probably insufficient for complete healing. Another influencing factor might have been the abutments used, since they exceeded the horizontal diameter of the test implants in the three sides of the triangle. This external mismatching may have reduced the potential of the test implants to maintain the crestal bone. In immediate implants buccal bone resorption was not prevented, which is also in agreement with previous investigations both in experimental animals and in humans (Botticelli et al., 2004, Sanz et al., 2010).

In the secondary outcomes evaluated (vertical ridge alterations) similar results between test and control implants were attained, which is in contrast with the findings reported by (Caneva et al., 2012b) using implants of two different diameters (3.3 and 5mm) in immediate sites, reporting less vertical bone resorption for the narrow diameter implants. The implant designs used in this investigation were, however, not comparable, since the test implants had the triangular shape only in the most coronal part of the implant. Similarly, the differences in the mismatching of the healing abutments may have prevented higher I-BIC and I-BC dimensions, when compared to the cylindrical design, which had abutments matching their diameter.

The prototype test implants had a more pronounced reduction in the triangle when compared with the commercially available 3.3mm diameter implants sharing this design (0.4 vs 0.1mm). This increased reduction may have compromised the resistance of the implants. The two fractured implants integrated well and the hard or soft tissue findings did not differ from the rest of the test implants.

Regarding the BV/TV, the significant higher ratio found in the cylindrical VOI at 12 weeks in the delayed sites in the test group, corresponded to a higher percentage of bone like tissue. These significant differences, however, disappeared when only the buccal VOI was measured and the percentage of bone evaluated. This can be explained by the inclusion of the whole body of the implant in the BT/TV cylindrical VOI measurements, which may have added in the test implants the other two triangular areas, not facing the buccal bone which could have in turn led to greater space for new bone in-growth. These differences, however, were not found in the immediately placed implants, which resulted in similar BV/TV values when test and control implants were compared. Using this surgical protocol, the horizontal gap between the implant surface and the socket walls may dilute the possible differences due to the different implant macro-design. This gap depending on its dimension may need further time to properly fill with mineralized tissue (Vignoletti et al., 2009).

The quality of the osseointegration was evaluated my measuring the percentage of bone to implant contact (%BIC). Both, test and control implants showed similar percentages, thus showing that the differences in macro-design on the coronal third of the implant did not influence BIC values. Other factors that may impact the quality of the osseointegration, such as variations in surface micro-topography (Smeets et al., 2016, Wennerberg and Albrektsson, 2010) were equal in both implants. The analysis of BIC values by means of Micro CT has been reported as a reliable method to assess implant osseointegration (Neldam and Pinholt, 2014), with several reports proving a good correlation between BIC values obtained by Micro CT when compared with conventional histology (Neldam et al., 2015). The obtained results with BIC values ranging from 48-57% correlate well with other studies using Micro-CT (Mangano et al., 2013, Choi et al., 2015).

When only the buccal bone and implant component were included in the VOI, there was a similar percentage of air/soft tissue in test and control implants. Furthermore, the buccal outline of the alveolar crest was manually outlined, thus including only the bone and implant component. The results of this analysis showed that there was a similar percentage of air/soft tissue in test and control implants, therefore indicating a similar bone structure. The possible discrepancy between the histological results with significant differences in horizontal bone with and the lack of differences observed in bone volume between test and control could be explained by the increase in the area of analysis, which makes the likely differences less evident. Moreover, the buccal VOI extended mesially and distally to areas in which the gap left by the implant design was minimal or none.

When measuring the buccal volume of titanium, however, a significantly lower volume of titanium was found in the test group, these findings were expected and validate the coronal implant geometry of tested implants.

The evaluation of bone volume changes with Micro CT has been recently reported, concluding that this method allowed for reliable evaluation of crestal bone changes around dental implants (Beck-Broichsitter et al., 2015, de Barros et al., 2016, Khobragade et al., 2015). Moreover, this technology permits the evaluation of the bone around the whole circumference of the implant (Becker et al., 2016).

The analysis of soft tissue contours using matched STL data did also render similar results when comparing test and control implants, which indicates that the changes in the implant design did not influence the contour of the soft tissues. At immediate implants the reduction in both height and width was apparent in both implant groups. These findings are in agreement with other preclinical investigations using similar image technology around immediate implants (Caneva et al., 2012a). At delayed sites, in contrast, a gain in width was observed in both implant groups. This observation may be explained by the surgical protocol that allows a buccal displacement of the flap after implant placement.

CONCLUSIONS

The results from this study evaluating a novel implant design with a modified coronal third of the implant section demonstrated the attainment of thicker crestal bone when compared to standard cylindrical implants, mainly when these implants were placed in fresh extraction sockets. Vertical soft and hard tissue measurements, as well as soft tissue buccal contours were similar in both groups.

ACKNOWLEDGEMENTS

The authors acknowledge the help and support received from the staff at the Experimental Surgical Centre of the Hospital ''Gomez-Ulla''. Especially to Dr. Pablo Arias and Dr. Paula García. Also to Drs. Alejandro Coca, Juan Bollaín and Riccardo Di Raimondo for their contribution to the study.

Similarly, a high appreciation is expressed to Dr. Ilan Kallai and the MIS R&D team for the technical and scientific support provided.

CONFLICTS OF INTEREST

This study was partially funded by a research grant from MIS implants technologies (Bar-Lev Industrial Park, Israel). Dr. Ignacio Sanz Martín and Dr. Mariano Sanz Alonso have received lecture fees from MIS Implants. Dr. Shapira is a scientific advisor for MIS implants.

REFERENCES

Abrahamsson, I., Berglundh, T., Wennstrom, J. & Lindhe, J. 1996. The peri-implant hard and soft tissues at different implant systems. A comparative study in the dog. *Clin Oral Implants Res,* 7**,** 212-9.

Alomrani, A. N., Hermann, J. S., Jones, A. A., Buser, D., Schoolfield, J. & Cochran, D. L. 2005. The effect of a machined collar on coronal hard tissue around titanium implants: a radiographic study in the canine mandible. *Int J Oral Maxillofac Implants,* 20**,** 677-86.

Beck-Broichsitter, B. E., Garling, A., Koehne, T., Barvencik, F., Smeets, R., Mehl, C., Jeschke, A., Wiltfang, J. & Becker, S. T. 2015. 3D-tracking the regenerative potential of the mandible with micro-CTs. *Oral Maxillofac Surg,* 19**,** 29-35.

Becker, K., Klitzsch, I., Stauber, M. & Schwarz, F. 2016. Three-dimensional assessment of crestal bone levels at titanium implants with different abutment microstructures and insertion depths using micro-computed tomography. *Clin Oral Implants Res*.

Berglundh, T., Abrahamsson, I., Lang, N. P. & Lindhe, J. 2003. De novo alveolar bone formation adjacent to endosseous implants. *Clin Oral Implants Res,* 14**,** 251-62.

Berglundh, T., Abrahamsson, I., Welander, M., Lang, N. P. & Lindhe, J. 2007. Morphogenesis of the peri-implant mucosa: an experimental study in dogs. *Clin Oral Implants Res,* 18**,** 1-8.

Bernhardt, R., Scharnweber, D., Muller, B., Thurner, P., Schliephake, H., Wyss, P., Beckmann, F., Goebbels, J. & Worch, H. 2004. Comparison of microfocus- and synchrotron X-ray tomography for the analysis of osteointegration around Ti6Al4V implants. *Eur Cell Mater,* 7**,** 42-51; discussion 51.

Botticelli, D., Berglundh, T. & Lindhe, J. 2004. Hard-tissue alterations following immediate implant placement in extraction sites. *J Clin Periodontol,* 31**,** 820-8.

Bruker, A. 2015. Bruker-MicroCT (2015). Osteointegration. Analysis of bone around a metal implant (method note No. MN074).

Buser, D., Janner, S. F., Wittneben, J. G., Bragger, U., Ramseier, C. A. & Salvi, G. E. 2012. 10-year survival and success rates of 511 titanium implants with a sandblasted and acid-etched surface: a retrospective study in 303 partially edentulous patients. *Clin Implant Dent Relat Res,* 14**,** 839-51.

Caneva, M., Botticelli, D., Morelli, F., Cesaretti, G., Beolchini, M. & Lang, N. P. 2012a. Alveolar process preservation at implants installed immediately into extraction sockets using deproteinized bovine bone mineral - an experimental study in dogs. *Clin Oral Implants Res,* 23**,** 789-96.

Caneva, M., Botticelli, D., Rossi, F., Cardoso, L. C., Pantani, F. & Lang, N. P. 2012b. Influence of implants with different sizes and configurations installed immediately into extraction sockets on peri-implant hard and soft tissues: an experimental study in dogs. *Clin Oral Implants Res,* 23**,** 396-401.

Choi, J. Y., Moon, I. S., Yun, J. H., Park, K. H., Huh, J. K. & Lee, D. W. 2015. Effects of thread size in the implant neck area on peri-implant hard and soft tissues: an animal study. *Clin Oral Implants Res*.

Cuijpers, V. M., Jaroszewicz, J., Anil, S., Al Farraj Aldosari, A., Walboomers, X. F. & Jansen, J. A. 2014. Resolution, sensitivity, and in vivo application of high-resolution computed tomography for titanium-coated polymethyl methacrylate (PMMA) dental implants. *Clin Oral Implants Res,* 25**,** 359-65.

De Barros, R. R., Novaes, A. B., Jr., De Carvalho, J. P. & De Almeida, A. L. 2016. The effect of a flapless alveolar ridge preservation procedure with or without a xenograft on buccal bone crest remodeling compared by histomorphometric and microcomputed tomographic analysis. *Clin Oral Implants Res*.

De Faria Vasconcelos, K., Dos Santos Corpas, L., Da Silveira, B. M., Laperre, K., Padovan, L. E., Jacobs, R., De Freitas, P. H., Lambrichts, I. & Boscolo, F. N. 2016. MicroCT assessment of bone microarchitecture in implant sites reconstructed with autogenous and xenogenous grafts: a pilot study. *Clin Oral Implants Res*.

De Sanctis, M., Vignoletti, F., Discepoli, N., Munoz, F. & Sanz, M. 2010. Immediate implants at fresh extraction sockets: an experimental study in the beagle dog comparing four different implant systems. Soft tissue findings. *J Clin Periodontol,* 37**,** 769-76.

De Sanctis, M., Vignoletti, F., Discepoli, N., Zucchelli, G. & Sanz, M. 2009. Immediate implants at fresh extraction sockets: bone healing in four different implant systems. *J Clin Periodontol,* 36**,** 705-11.

De Santis, E., Lang, N. P., Favero, G., Beolchini, M., Morelli, F. & Botticelli, D. 2015. Healing at mandibular block-grafted sites. An experimental study in dogs. *Clin Oral Implants Res,* 26**,** 516-22.

Donath, K. & Breuner, G. 1982. A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. *J Oral Pathol,* 11**,** 318-26.

Favero, G., Botticelli, D., Garcia, B., Mainetti, T. & Lang, N. P. 2013. Alveolar bony crest preservation at implants installed immediately after tooth extraction: an experimental study in the dog. *Clin Oral Implants Res,* 24**,** 7-12.

Feldkamp La, D. L., Krass Jw. 1984. Practical cone-beam algorithm. . *Journal of the Optical Society of America,* 1:612-619.

Ferrus, J., Cecchinato, D., Pjetursson, E. B., Lang, N. P., Sanz, M. & Lindhe, J. 2010. Factors influencing ridge alterations following immediate implant placement into extraction sockets. *Clin Oral Implants Res,* 21**,** 22-9.

Galindo-Moreno, P., Nilsson, P., King, P., Becktor, J., Speroni, S., Schramm, A. & Maiorana, C. 2012. Clinical and radiographic evaluation of early loaded narrow diameter implants - 1-year follow-up. *Clin Oral Implants Res,* 23**,** 609-16.

Gotfredsen, K. 2012. A 10-year prospective study of single tooth implants placed in the anterior maxilla. *Clin Implant Dent Relat Res,* 14**,** 80-7.

Ioannidis, A., Gallucci, G. O., Jung, R. E., Borzangy, S., Hammerle, C. H. & Benic, G. I. 2015. Titanium-zirconium narrow-diameter versus titanium regular-diameter implants for anterior and premolar single crowns: 3-year results of a randomized controlled clinical study. *J Clin Periodontol,* 42**,** 1060-70.

Khobragade, P., Jain, A., Setlur Nagesh, S. V., Andreana, S., Dziak, R., Sunkara, S. K., Sunkara, S., Bednarek, D. R., Rudin, S. & Ionita, C. N. 2015. Micro-Computed tomography (CT) based assessment of dental regenerative therapy in the canine mandible model. *Proc SPIE Int Soc Opt Eng,* 9417.

Laurell, L. & Lundgren, D. 2011. Marginal bone level changes at dental implants after 5 years in function: a meta-analysis. *Clin Implant Dent Relat Res,* 13**,** 19-28.

Mangano, C., Piattelli, A., Mangano, F., Rustichelli, F., Shibli, J. A., Iezzi, G. & Giuliani, A. 2013. Histological and synchrotron radiation-based computed microtomography study of 2 human-retrieved direct laser metal formed titanium implants. *Implant Dent,* 22**,** 175-81.

Merheb, J., Quirynen, M. & Teughels, W. 2014. Critical buccal bone dimensions along implants. *Periodontol 2000,* 66**,** 97-105.

Molina, A., Sanz-Sanchez, I., Martin, C., Blanco, J. & Sanz, M. 2016. The effect of one-time abutment placement on interproximal bone levels and peri-implant soft tissues: a prospective randomized clinical trial. *Clin Oral Implants Res*.

Neldam, C. A., Lauridsen, T., Rack, A., Lefolii, T. T., Jorgensen, N. R., Feidenhans'l, R. & Pinholt, E. M. 2015. Application of high resolution synchrotron micro-CT radiation in dental implant osseointegration. *J Craniomaxillofac Surg,* 43**,** 682-7.

Neldam, C. A. & Pinholt, E. M. 2014. Synchrotron muCT imaging of bone, titanium implants and bone substitutes - a systematic review of the literature. *J Craniomaxillofac Surg,* 42**,** 801-5.

Ostman, P. O., Hellman, M. & Sennerby, L. 2012. Ten years later. Results from a prospective single-centre clinical study on 121 oxidized (TiUnite) Branemark implants in 46 patients. *Clin Implant Dent Relat Res,* 14**,** 852-60.

Pessoa, R. S., Sousa, R. M., Pereira, L. M., Neves, F. D., Bezerra, F. J., Jaecques, S. V., Sloten, J. V., Quirynen, M., Teughels, W. & Spin-Neto, R. 2016. Bone Remodeling Around Implants with External Hexagon and Morse-Taper Connections: A Randomized, Controlled, Split-Mouth, Clinical Trial. *Clin Implant Dent Relat Res*.

Sanz, M., Cecchinato, D., Ferrus, J., Pjetursson, E. B., Lang, N. P. & Lindhe, J. 2010. A prospective, randomized-controlled clinical trial to evaluate bone preservation using implants with different geometry placed into extraction sockets in the maxilla. *Clin Oral Implants Res,* 21**,** 13-21.

Sanz Martin, I., Benic, G. I., Hammerle, C. H. & Thoma, D. S. 2016a. Prospective randomized controlled clinical study comparing two dental implant types: volumetric soft tissue changes at 1 year of loading. *Clin Oral Implants Res,* 27**,** 406-11.

Sanz-Martin, I., Sailer, I., Hammerle, C. H. & Thoma, D. S. 2016b. Soft tissue stability and volumetric changes after 5 years in pontic sites with or without soft tissue grafting: a retrospective cohort study. *Clin Oral Implants Res,* 27**,** 969-74.

Schneider, D., Grunder, U., Ender, A., Hammerle, C. H. & Jung, R. E. 2011. Volume gain and stability of peri-implant tissue following bone and soft tissue augmentation: 1-year results from a prospective cohort study. *Clin Oral Implants Res,* 22**,** 28-37.

Schwarz, F., Hegewald, A. & Becker, J. 2014. Impact of implant-abutment connection and positioning of the machined collar/microgap on crestal bone level changes: a systematic review. *Clin Oral Implants Res,* 25**,** 417-25.

Schwarz, F., Sahm, N. & Becker, J. 2012. Impact of the outcome of guided bone regeneration in dehiscence-type defects on the long-term stability of peri-implant health: clinical observations at 4 years. *Clin Oral Implants Res,* 23**,** 191-6.

Smeets, R., Stadlinger, B., Schwarz, F., Beck-Broichsitter, B., Jung, O., Precht, C., Kloss, F., Grobe, A., Heiland, M. & Ebker, T. 2016. Impact of Dental Implant Surface Modifications on Osseointegration. *Biomed Res Int,* 2016**,** 6285620.

Spray, J. R., Black, C. G., Morris, H. F. & Ochi, S. 2000. The influence of bone thickness on facial marginal bone response: stage 1 placement through stage 2 uncovering. *Ann Periodontol,* 5**,** 119-28.

Thoma, D. S., Jung, R. E., Schneider, D., Cochran, D. L., Ender, A., Jones, A. A., Gorlach, C., Uebersax, L., Graf-Hausner, U. & Hammerle, C. H. 2010. Soft tissue volume augmentation by the use of collagen-based matrices: a volumetric analysis. *J Clin Periodontol,* 37**,** 659-66.

Vignoletti, F. & Abrahamsson, I. 2012. Quality of reporting of experimental research in implant dentistry. Critical aspects in design, outcome assessment and model validation. *J Clin Periodontol,* 39 Suppl 12**,** 6-27.

Vignoletti, F., De Sanctis, M., Berglundh, T., Abrahamsson, I. & Sanz, M. 2009. Early healing of implants placed into fresh extraction sockets: an experimental study in the beagle dog. II: ridge alterations. *J Clin Periodontol,* 36**,** 688-97.

Wennerberg, A. & Albrektsson, T. 2010. On implant surfaces: a review of current knowledge and opinions. *Int J Oral Maxillofac Implants,* 25**,** 63-74.

Figure Legends

Figure 1a. Occlusal view after extraction of mesial roots of PM3 and PM4 and flap elevation at PM2 and M1.

Figure 1b. Implant installation at immediate and delayed sites.

Figure 1c. Test and control implants in post extraction sockets. Note the leg of the implant triangle faces the buccal plate.

Figure 2. Sections representing twelve weeks healing interval. Buccal sections appear on the right side of the image. (a) Delayed control implant in PM2 (b) delayed test implant in M1 (c) immediate test implant in PM3 (d) immediate control implant in PM4.

Figure 3. Three-dimensional image reconstruction of the Micro CT samples. 1a. Image reconstruction corresponds to an immediate control implant. 1b. Immediate test implant 1c. Delayed test implant 1d. Delayed control implant

Figure 4. Linear measurements performed to evaluate soft tissue changes. 4a. Image analysis in an immediate site. 4b. Image analysis at a delayed site. H0, Horizontal soft tissue changes at the level of the gingival margin or baseline alveolar crest; H2, 4 and 6, Horizontal soft tissue changes 2,4 and 6mm bellow H0.

Supplemental Figure 1. Histological sample depicting the vertical soft and hard tissue measurements together with the crestal width assessments.

Supplemental Figure 2. Volume of interest (VOI) selected. Cylindrical VOI is depicted in blue, buccal VOI is depicted in yellow and the buccal bone VOI appears in green.

Supplemental Figure 3. Sections representing four weeks healing interval. Buccal sections appear on the left side of the image. (a) delayed control implant in PM2 (b) delayed test implant in M1 (c) immediate test implant in PM3 (d) immediate control implant in PM4.

Supplemental Figure 4. STL image super-impositions. 3a. Surface scans of baseline (yellow) and follow up (green) models. 3b. Baseline and follow up models superimposed with the aid of a software program.

Table 1.

Header: Descriptive statistics of crestal width measurements stratified by implant type and site (Mean±SD).

Footer: *p<0.05, **p=0.06. CW: Crestal width at the level of the implant shoulder (CWO) and 1,2,3,4 and 5mm bellow the implant shoulder (CW1, CW2, CW3, CW4, CW5).

Table 2.

Header: Descriptive statistics of hard and soft tissue histometric measurements stratified by implant type, surgical approach and study timeline. (Mean±SD).

Footer: *p<0.05. I, implant shoulder; BC, most coronal aspect of bone crest; BIC, first bone to implant contact; buc, buccal; lin, lingual. PM: gingival margin; aJE most apical portion of the junctional epithelium.

Table 3.

Header: Descriptive statistics (Mean±SD) of the analysis performed in the cylindrical VOI, buccal VOI and STL image analysis stratified by implant site.

Footer: *p<0.05. BIC, bone to implant contact; BV/TV, Bone volume/tissue volume analysis. Vol bone, Volume of bone; Vol air, Volume of “air”; Vol imp, Volume of implant. H0, Horizontal soft tissue changes at the level gingival margin or baseline alveolar crest;. H2,4,6, Horizontal soft tissue changes 2,4 and 6mm bellow H0.

Suplemental Table 1.

Header: Descriptive statistics of soft and hard tissue values for implants grouped by study timeline and surgical approach. (Mean±SD).

Footer: *p<0.05. I, implant shoulder; BC, most coronal aspect of bone crest; BIC, first bone to implant contact; buc, buccal; lin: lingual. PM: gingival margin; aJE most apical portion of the junctional epithelium.

Suplemental Table 2.

Header: Descriptive statistics (Mean±SD) of the analysis performed in the cylindrical VOI stratified by study timeline.

Footer: *p<0.05. BIC, bone to implant contact; BV/TV, Bone volume/tissue volume analysis.

Suplemental Table 3.

Header: Descriptive statistics (Mean±SD) of the Buccal bone VOI stratified by implant site.

Footer: *p<0.05. Vol bone, Volume of bone; Vol air, Volume of “air”; Vol imp, Volume of implant.

Annex 1 - Supplementary methods

The local Regional Ethics Committee for Animal Research approved the study protocol (Code: ES280790000187). Eight animals were selected that fulfilled the inclusion criteria. Each animal provided four test and four control implant sites.

Anesthesia

Propofol (2mg/kg/i.v., Propovet, Abbott Laboratories, Kent, UK) was used to induction sedation and isofluorane gas at concentrations of 0.7–1.5% (Isoba-vet, Schering-Plough, Madrid, Spain) through an endotracheal tube was used as general anaesthetic. Local anaesthesia (Articaine 40mgr/ml, 0.01mgr/ml epinephrine. Inibsa Dental. Barcelona, Spain) was further infiltrated in the surgical areas for pain and bleeding control.

Post-surgical care

Animals were fed on a soft pellet diet and maintained in a 12:12 light/dark cycle and 21-22 Cº and daily monitored daily by an experienced veterinarian. A regular regimen of analgesic and antibiotic medication was used after each surgical intervention. Plaque control was provided with a solution of chlorhexidine 0.12% and CPC 0.05% (PerioAid Tratamiento, Laboratorios Dentaid. Barcelona, Spain) sprayed on all mandibular tooth sites 2 days per week. Further, once a week the surgical areas were brushed with a manual toothbrush soaked in chlorhexidine solution. Any sign of inflammation of peri-implant mucosa was documented during the postoperative healing.

Biopsy handling

The mandibles were freed from their attached tissues and cut into halves. Each hemi-mandible was placed into a sealable sample container with formalin 4% solution. These containers were immediately stored at 5ºC) until they were processed histologically.

Histological Processing

Specimens were prepared for ground sectioning, as described by Donath (Donath and Breuner, 1982) using a cutting–grinding unit (Exakts, Apparatebau, Norderstedt, Germany). In brief, specimens were dehydrated in a graded series of ethanol and embedded in methyl methacrylate. Blocks were cut in a bucco–lingual plane for each implant site and one central section was prepared and reduced to a final thickness of 20 mm by micro grinding and polishing (Exakts). Obtained sections were stained with the Levai Laczko method. One bucco-lingual section per implant was analysed using a Nikon Eclipse Ti microscope (Nikon, Heidelberg, Germany) equipped with image analysis software (Q-500MC; Nikon).

Image analysis

Impressions were taken using a one-step/two-viscosity technique with silicone impression materials (Express2 Putty Soft/Express2 Light Body, 3M Espe, St. Paul, MN, USA) and individualized acrylic impression trays. Dental stone casts were then fabricated (Elite Model, Zhermack. Rome, Italy) and evaluated for the presence of irregularities, porous areas, undefined gingival margins, broken cusps or undefined vestibulum. For matching the STL files, 3 clear and visible common reference points were selected in both the baseline and follow-up casts and the software automatically superimposed the models using a series of mathematical algorithms

(Sup Figs. 4a, 4b). In those sites where inproper fitting occured, manual adjustments were performed until the matching was deemed adequate.

Table 1. Descriptive statistics of crestal width measurements at T12 stratified by implant type and site. Mean±SD								
	DELAYED				IMMEDIATE			
	PM2(n= 4T,4C)		M1(n= 4T,4C)		PM3(n= 4T,4C)		PM4(n= 4T,4C)	
	TEST	CONTROL	TEST	CONTROL	TEST	CONTROL	TEST	CONTROL
CW0	0	0	0.44±0.99	0	0.22±0.49	0	0.47±0.94	0
CW1	0.31±0.41	0.12±0.15	1.49±0.86	1.67±0.47	0.37±0.51	0.30±0.24	1.49±0.41	0.96±0.34
CW2	0.93±0.81	0.79±0.62	1.99±0.27	1.56±0.43	0.83±0.33	0.41±0.29	1.22±0.46	0.86±0.38
CW3	1.30±0.62	1.35±0.63	1.92±0.46*	1.25±0.23	0.99±0.11*	0.47±0.46	0.84±0.48	0.65±0.38
CW4	1.32±0.68	1.56±0.67	1.48±0.61	0.81±0.49	1.24±0.10*	0.53±0.51	0.66±0.27	0.62±0.31
CW5	1.38±0.64	1.87±0.66	1.33±0.76	0.72±0.65	1.23±0.17*	0.60±0.58	0.43±0.24	0.67±0.32

Table 2. Descriptive statistics of soft and hard tissue histometric measurements stratified by implant type, surgical approach and study timeline. (Mean±SD)					
		DELAYED (n= 16T,16C)		IMMEDIATE (n= 16T,16C)	
		TEST	CONTROL	TEST	CONTROL
I-BC buc	T4	0.01±0.47	0.41±0.47	0.07±0.66	0.12±0.76
	T12	0.58±0.49	1.00±0.60	0.66±0.81	0.40±0.29
I-BIC buc	T4	0.67±0.47	0.83±0.49	1.55±1.21	1.70±0.80
	T12	1.24±0.72	1.08±0.88	1.54±0.89	1.18±0.47
BC-BIC buc	T4	0.66±0.3	0.42±0.3	1.48±1.09	1.57±0.83
	T12	0.65±0.71	0.07±0.56	0.87±1.01	0.78±0.47
I-BC lin	T4	-0.23±0.93	0.07±0.23	-0.14±0.84	0.09±0.8
	T12	0.43±0.61	0.32±0.56	0.48±0.58	0.62±0.56
I-BIC lin	T4	0.36±0.37	0.47±0.48	0.47±0.45	0.75±0.65
	T12	0.76±0.54	0.86±0.66	1.19±0.58	1.32±0.99
PM-aJE	T4	2.07±0.25	2.17±0.63	2.13±0.19	1.97±0.37
	T12	2.20±0.71	2.12±0.81	2.09±0.34	2.06±0.49
aJE-B	T4	2.15±1.36	1.9±0.79	2.97±1.01	2.96±1.08
	T12	2.22±1.07	2.26±1.04	2.66±0.73	2.23±0.58

Table 3. Descriptive statistics (Mean±SD) of the analysis performed in the cylindrical VOI, buccal VOI and STL image analysis stratified by implant site.

	DELAYED				IMMEDIATE			
	PM2 (n= 4T,4C)		M1 (n= 4T,4C)		PM3 (n= 4T,4C)		PM4 (n= 4T,4C)	
	TEST	**CONTROL**	**TEST**	**CONTROL**	**TEST**	**CONTROL**	**TEST**	**CONTROL**
Cylindrical VOI								
BIC	48.88±12.89	46.63±9.46	51.63±6.26	51.63±9.59	52.88±10.15	57.25±12.30	49.38±14.76	51.88±8.54
BV/TV	55.63±11.84	52.13±9.99	52.88±5.51	53.50±8.65	62.01±8.30	64.25±8.24	55.13±13.85	60.25±8.55
Buccal VOI								
Vol bone (mm3)	43.63±11.26	40.88±11.14	43.50±10.09	41.63±8.72	32.50±9.78	30.75±7.23	31±6.19	31±6.05
Vol air (mm3)	89.50±10.52	89.50±11.45	90.25±10.18	88.38±8.72	100.13±9.23	103.63±14.38	101.50±6.57	96±5.76
Vol imp (mm3)	18.25±2.25*	22±0.54	18.88±2.17*	22.50±1.20	19.88±0.84*	23.50±0.54	20.13±0.84*	23.25±1.28
%bone	27.38±6.78	26.88±7.20	28.50±6.74	27.38±5.98	21.25±6.41	20.88±7.36	20.38±4.07	20.50±3.59
%air	60±6.66	59±7.45	59.38±6.76	57.88±5.72	65.63±6.21	64.63±6.55	66.38±4.24	63.88±3.31
%imp	12.63±1.69*	14.50±0.54	12.38±1.51*	14.63±0.74	13±0.54*	14.63±1.41	13.13±0.64*	15.38±0.74
STL analysis								
H0	0.60±0.99	0.78±1.37	0.53±1.12	-2.11±2.01	-2.01±0.43	-1.65±0.97	-1.10±1.30	-1.94±1.15
H2	0.88±0.81	0.94±1.02	0.47±0.79	0.20±1.15	-0.63±0.51	-0.46±0.20	-0.44±0.40	-0.71±0.65
H4	0.43±0.31	0.46±1.36	0.43±0.56	0.43±1.00	-0.15±0.19	-0.26±0.19	-0.07±0.31	-0.23±0.36
H6	0.48±0.81	0.89±1.45	0.19±0.68	0.39±2.00	-0.33±0.21	0.00±0.62	-0.41±0.22	-0.19±0.34
Vertical	0.28±1.19	0.09±0.58	0.07±0.83	-0.56±0.36	-0.43±0.25	-0.27±0.27	-0.06±0.38	-0.32±0.48

Supl Table 1. Descriptive statistics of soft and hard tissue values for implants grouped by study timeline and surgical approach. (Mean±SD)

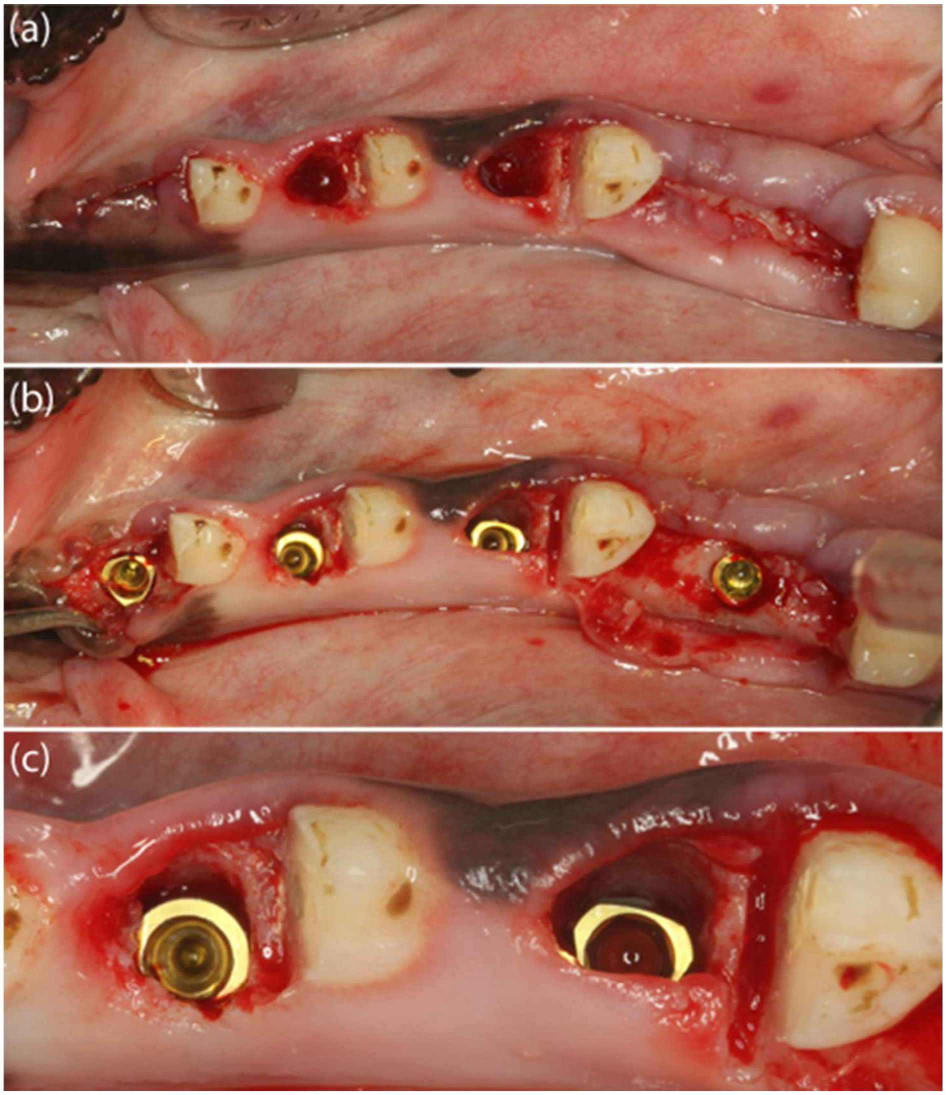
	T4		T12	
	DELAYED (n=8)	**IMMEDIATE (n=8)**	**DELAYED (n=8)**	**IMMEDIATE (n=8)**
I-BC buc	0.21±0.51	0.10±0.69	0.74±0.59	0.53±0.61
I-BIC buc	0.76±0.49	1.63±0.99*	1.11±0.81	1.36±0.72
BC-BIC buc	0.55±0.54	1.52±0.94*	0.37±0.72	0.83±0.77*
I-BC lin	-0.07±0.92	.0.26±0.80	0.28±0.51	0.55±0.56
I-BIC lin	0.44±0.42	0.61±0.56	0.73±0.52	1.25±0.79*
PM-aJE	2.13±0.48	2.05±0.30	2.15±0.74	2.07±0.41
aJE-B	2.02±1.06	2.96±1.01*	2.25±1.02	2.44±0.68

Supl Table 2. Descriptive statistics (Mean±SD) of the analysis performed in the cylindrical VOI stratified by study timeline.

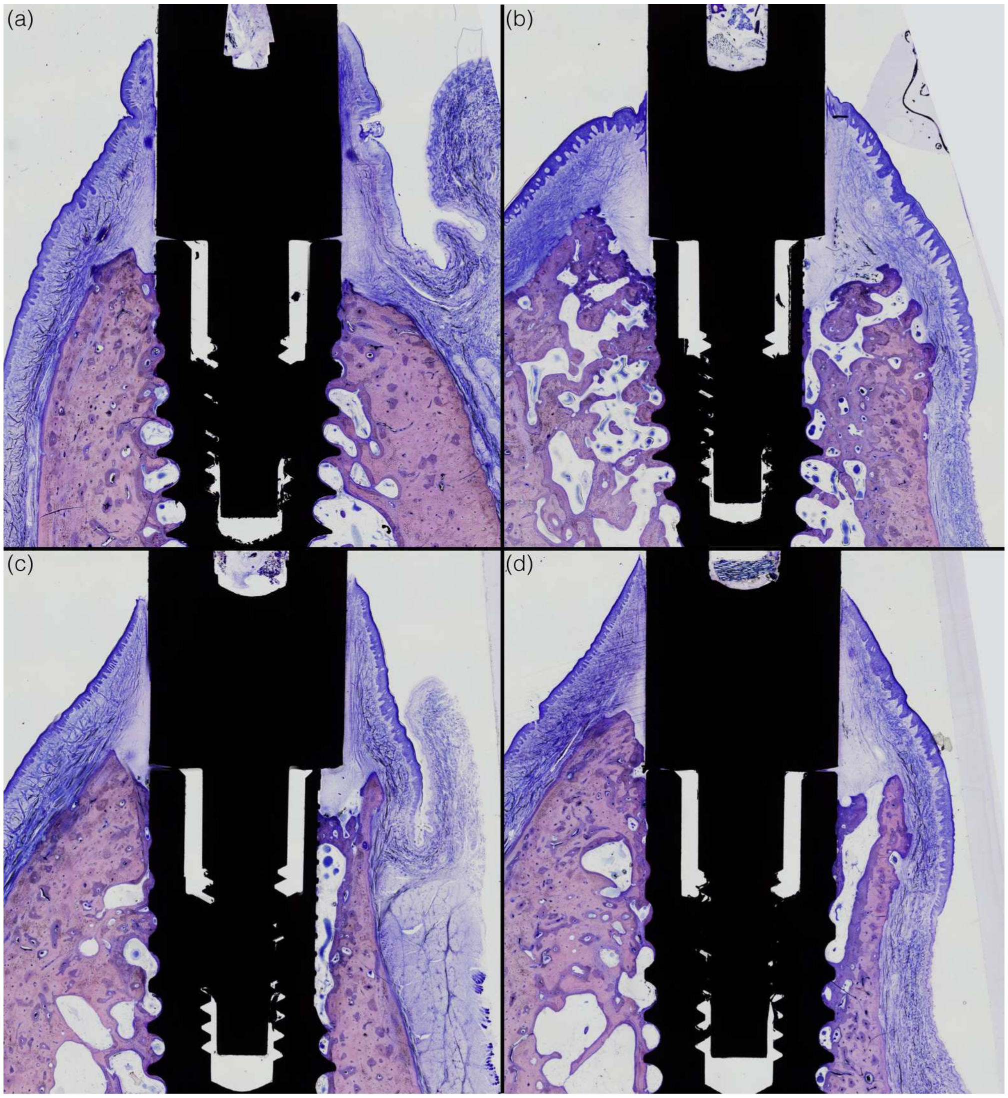
		DELAYED (n= 16T,16C)		IMMEDIATE (n= 16T,16C)	
		TEST	**CONTROL**	**TEST**	**CONTROL**
BIC	**T4**	44.00±7.75	49.18±11.56	48.13±14.10	51.13±9.92
	T12	56.50±7.76	49.13±7.88	54.13±10.41	58±10.74
BV/TV	**T4**	48.13±5.87	54.63±10.64	56.75±13.31	60.75±8.65
	T12	60.38±7.41*	51.00±7.43	60.38±10.16	63.75±8.38

Supl Table 3. Descriptive statistics (Mean±SD) of the buccal bone VOI stratified by implant site.

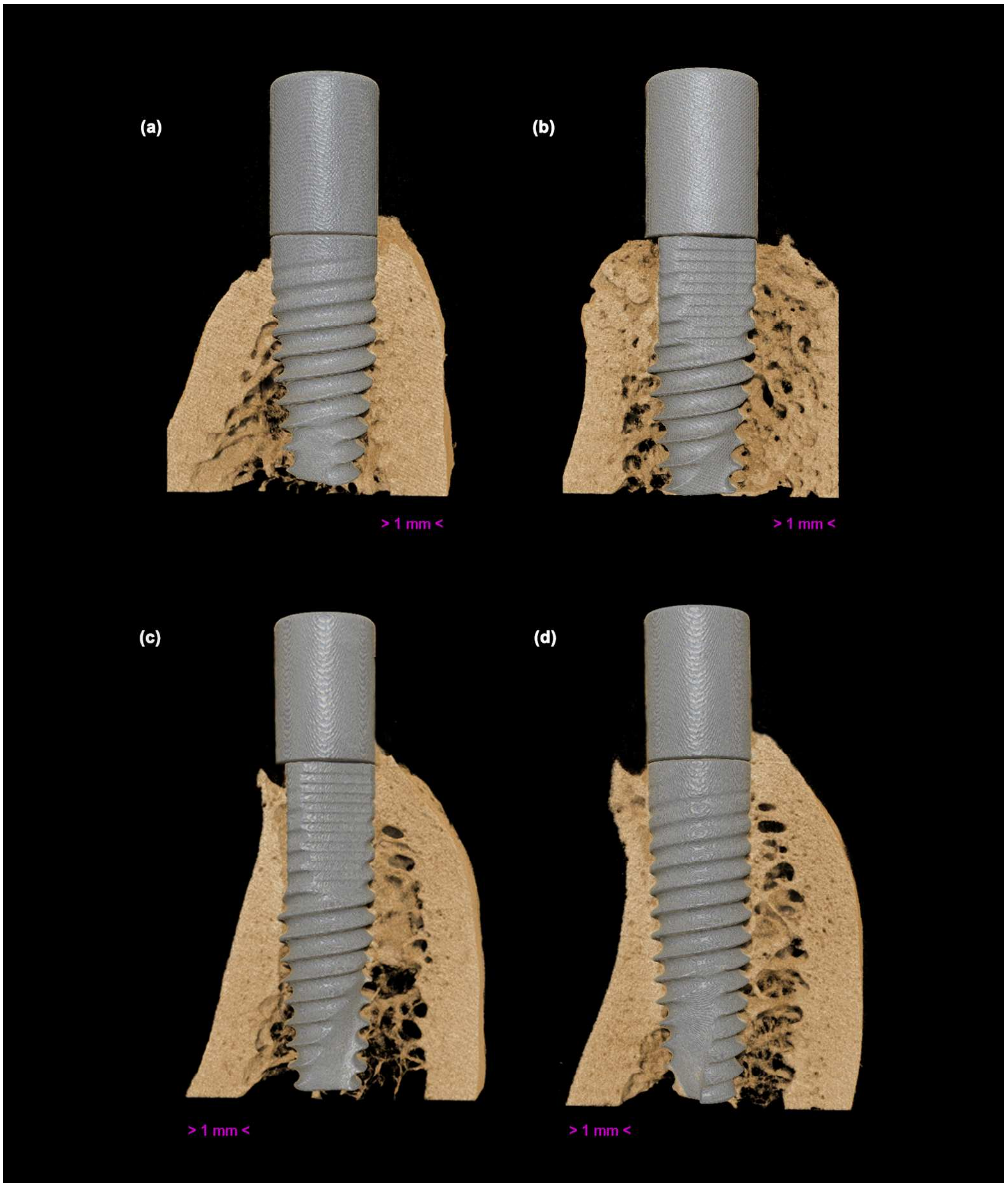
	DELAYED				IMMEDIATE			
	PM2 (n= 4T,4C)		**M1 (n= 4T,4C)**		**PM3 (n= 4T,4C)**		**PM4 (n= 4T,4C)**	
	TEST	**CONTROL**	**TEST**	**CONTROL**	**TEST**	**CONTROL**	**TEST**	**CONTROL**
Total Volume	75.75±12.27	78.63±13.39	83.50±13.78	86.88±12.18	64.38±9.95	65.50±8.53	70.09±12.15	70.88±9.41
Vol bone (mm3)	44.38±13.45	40.25±11.32	43.13±10.20	41.25±8.81	32.13±9.91	26.75±8.54	30.88±6.31	30.75±6.15
Vol air (mm3)	14.13±2.90	10.13±5.35	22.01±3.99	23.25±7.59	12.53±2.97	15.84±4.48	19.38±8.34	16.75±4.16
Vol imp (mm3)	18.25±2.25*	22.00±5.35	18.50±2.44*	22.38±1.30	19.71±4.27*	23.52±3.31	20.04±0.75*	23.13±1.24
%bone	56.02±7.05	50.75±7.72	50.88±4.54	47.25±6.36	48.75±8.11	45.75±5.72	43.88±5.38	43.00±4.69
%air	19.03±3.16	20.75±5.84	26.25±2.31	26.63±6.20	19.63±3.81	18.25±2.53	26.75±7.81	23.63±3.29
%imp	25.00±5.29	28.63±4.41	22.88±4.82	26.00±3.62	28.06±10.63	36.13±5.02	29.38±5.42	33.38±4.53

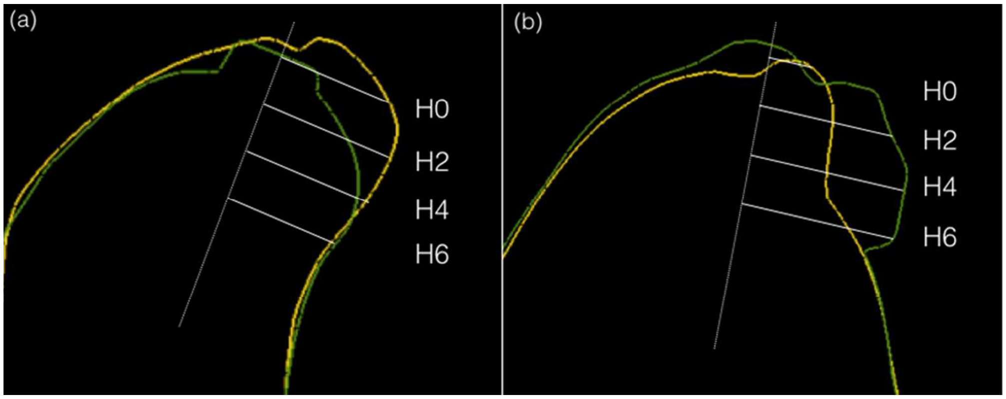


39x45mm (300 x 300 DPI)

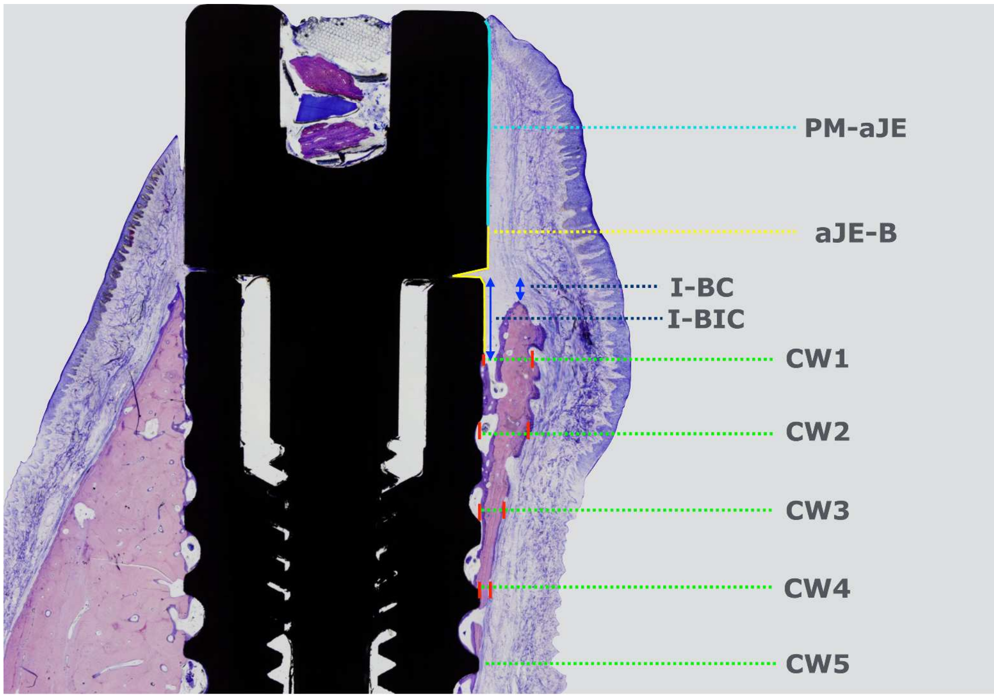


194x211mm (300 x 300 DPI)

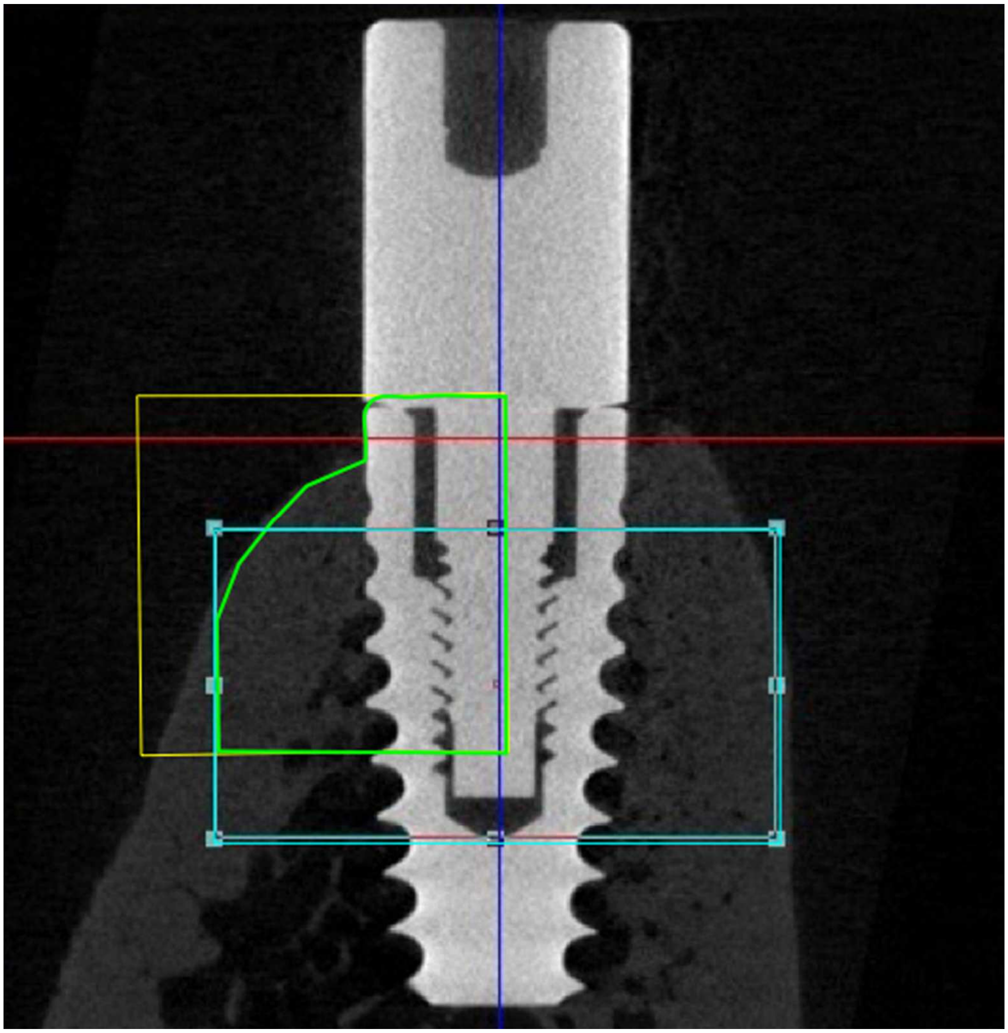

(a)
(b)
> 1 mm <
> 1 mm <
(c)
(d)
> 1 mm <
> 1 mm <



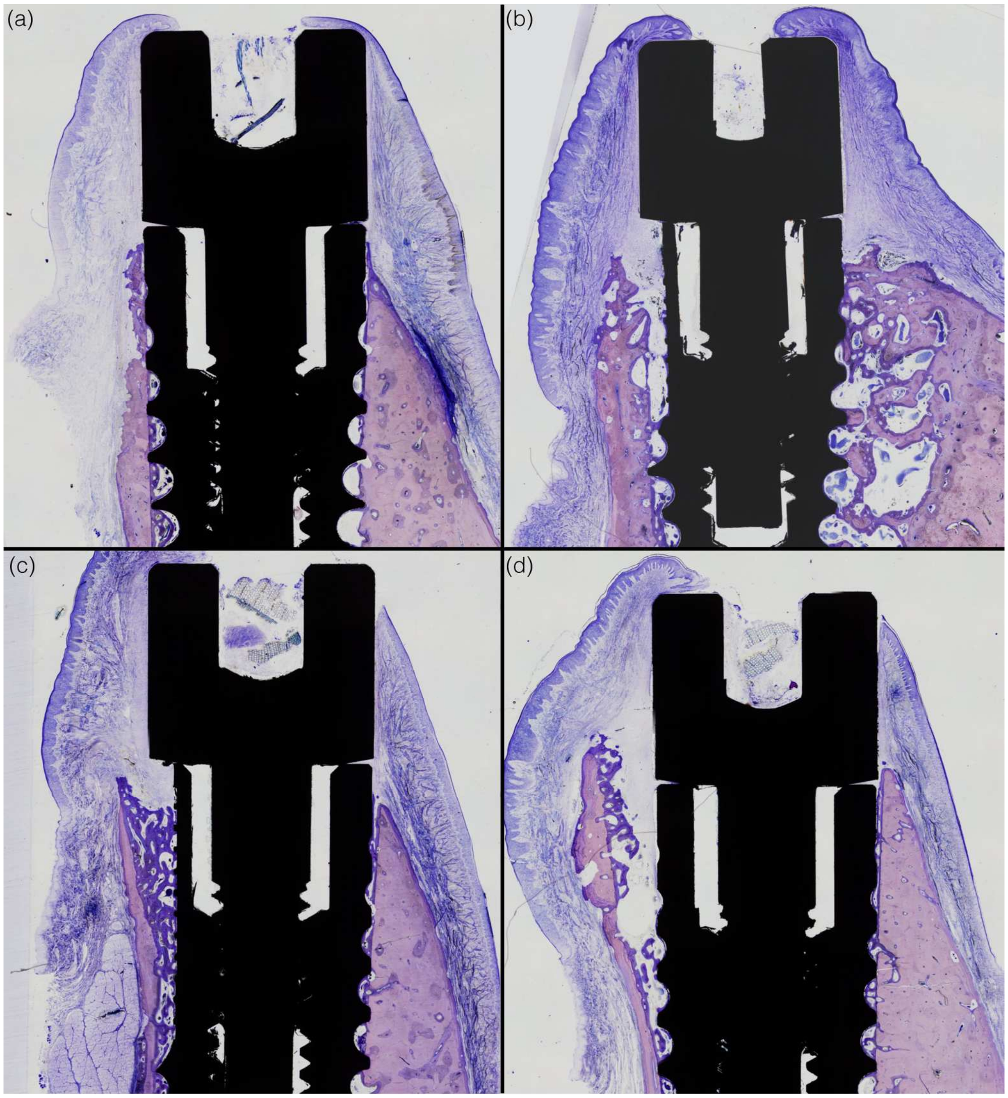
77x30mm (300 x 300 DPI)



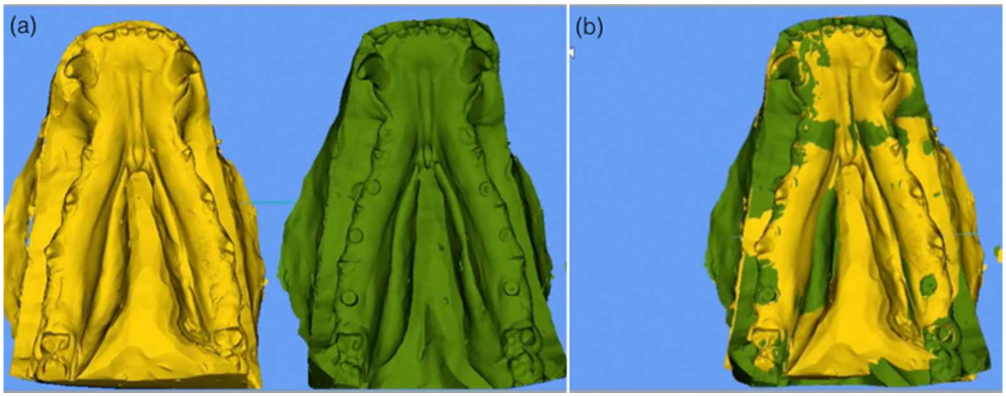
361x249mm (300 x 300 DPI)



76x78mm (300 x 300 DPI)



194x211mm (300 x 300 DPI)



68x26mm (300 x 300 DPI)

Artículo 2:

Hard and soft tissue volume analysis of immediate and delayed implants with different cervical design. A novel methodological approach using superimposed Micro-CT and STL images. Sanz-Martin I, Vignoletti F, Nuñez J, Permuy M, Muñoz F, Sanz M. Clinical Oral Implants Research. *Submitted for publication*.

Objetivo: Estudiar el volumen de tejido bucal en implantes con un diseño cervical o cilíndrico colocados en crestas cicatrizadas (DLI) o alveolos post extracción (IMI) mediante la superposición de imágenes de Micro CT y STL.

Material y métodos: Se colocaron implantes inmediatos y diferidos en 8 perros beagle. Las muestras se analizaron mediante Micro CT y se realizaron impresiones del tejido blando que se positivaron y fueron escaneadas para obtener archivos STL. Mediante un software de análisis se superpusieron las imágenes usando referencias comunes a ambas y se analizaron 3 volúmenes de interés; el volumen de tejido óseo en bucal del implante (B-BV), el volumen de tejido blando por debajo del hombro del implante (EC-STV) y el volumen de tejido blando por encima del hombro del implante (SC-STV). Además se obtuvieron mediciones lineales para calcular la altura del tejido blando por encima del hombro del implante (STH), la posición de la cresta ósea (I-BC) y el grosor del tejido blando a la altura del hombro del implante (MT-IS).

Resultados: No hubo diferencias significativas entre implantes T y C en cuanto al volumen de hueso vestibular aunque los implantes T obtuvieron mayor volumen de hueso bucal en 3 de las 4 localizaciones. El diseño del implante no afecto el volumen del tejido blando. El protocolo quirúrgico afecto el volumen de tejido blando y óseo. En los IMI la posición de la cresta ósea obtuvo una correlación positiva con B-BV, SC-STV, MT-IS y STH al contrario de lo ocurrido para los DLI.

Conclusiones: La superposición de imágenes de Micro CT y STL permitió analizar el volumen de tejido óseo y blando en las diferentes localizaciones. Los implantes con diseño triangular obtuvieron mayor volumen de hueso vestibular y un volumen de tejido blando similar. El enfoque quirúrgico influyó en el comportamiento del tejido blando.

CLINICAL ORAL IMPLANTS RESEARCH WILEY

Hard and soft tissue volume analysis of immediate and delayed implants with different cervical design. A novel methodological approach using superimposed Micro-CT and STL images.

Journal:	*Clinical Oral Implants Research*
Manuscript ID	Draft
Manuscript Type:	Novel Development
Date Submitted by the Author:	n/a
Complete List of Authors:	Sanz Martin, Ignacio; University Complutense of Madrid, Section of Periodontology, Faculty of Odontology Vignoletti, Fabio; Universida Complutense de Madrid, Periodontology Nunez, Javier Permuy-Mendaña, Maria; School of Veterinary. University of Santiago de Compostela, Veterinary clinical sciences MuÑoz, Fernando; School of Veterinary. University of Santiago de Compostela, Veterinary clinical sciences Sanz, Mariano; Universidad Complutense de Madrid, Periodontology
Keywords:	Animal Experiments, CT Imaging, Soft tissue-implant interactions, Wound healing, Bone implant interactions

Hard and soft tissue volume analysis of immediate and delayed implants with different cervical design. A novel methodological approach using superimposed Micro-CT and STL images.

Sanz-Martin I [1], Vignoletti F [1], Nuñez J [1], Permuy M[2], Muñoz F[2], Sanz M [1].

[1] Section of Periodontology, Faculty of Odontology, University Complutense of Madrid, Madrid, Spain.

[2] Faculty of Veterinary Lugo. University of Santiago de Compostela. Lugo, Spain

Key words: “dental implants”, “animal model”, “Immediate implant”, “soft tissue volume”, “MicroCT”, “dimensional alterations”, “volumetric analysis” “bone volume”.

Running title: Bone-soft tissue volume through MicroCT STL superimposition

Address for correspondence: Dr. Ignacio Sanz Martín

Facultad de Odontología

Universidad Complutense de Madrid

Plaza Ramón y Cajal

28040 Madrid

e-mail: ignaciosanzmartin@odon.ucm.es

Word Count Manuscript: 3338

Number of figures and tables: 5 figures, 3 tables

AIM

To study the hard and soft tissue volume after placing immediate (IMI) or delayed implants (DLI) with a modified triangular coronal macro-design (test) or a conventional cylindrical design.

MATERIAL AND METHODS:

Test and Control implants were inserted in healed ridges or in fresh extraction sockets of 8 beagle dogs. Tissue biopsies were processed for Micro-CT analysis and dental stone casts were optically scanned to obtain STL files revealing the soft tissue contours. Image analysis software was utilized to match common landmarks, which served as references. Three distinct volumes were calculated; Buccal bone volume (B-BV), soft tissue volume bellow the implant shoulder (EC-STV) and the soft tissue volume above the implant shoulder (SC-STV). Using linear measurements, the soft tissue height (STH), the mucosal thickness (MT-IS) and the distance from the implant shoulder to the Bone crest (I-BC) were assessed.

RESULTS:

There were no significant differences between test and control implants regarding the buccal bone volume, although test implants had greater B-BV in all locations except for PM2. The soft tissue volume was similar at test and control implants. The surgical approach (immediate or delayed) influenced the distribution of the total tissue volume. In the IMI, a low position of the bone crest was correlated with low values of B-BV, SC-STV, MT-IS and STH. This was not the case in DLI.

CONCLUSION:

The superimposition of DICOM-STL allowed the analysis of soft and hard tissue volumes. Triangular implants had higher bone volume and similar soft tissue volume. The surgical approach influenced tissue response.

INTRODUCTION

As treatments with dental implants have become more reliable and widespread with the general dental population the focus has shifted from osseointegration to the esthetic appearance of the implants restorations and the harmony with the adjacent tissues (Cairo et al., 2012). These aspects are heavily influenced by the widely studied physiologic changes that occur after tooth extraction, which often create soft and hard tissue deficiencies, hence influencing the aesthetic appearance of the prosthetic restorations and the peri-implant tissues.

To compensate or reduce these changes, different bone regenerative interventions have been proposed depending on the degree of crestal bone resorption (Sanz-Sanchez et al., 2015), as well as interventions aimed to increase the mucosal thickness by means of soft tissue grafts or soft tissue substitutes (Thoma et al., 2014). Recent research has pointed out that the dimensional changes occurring after dental extractions not only relate to changes in bone morphology but also to those occurring at the soft tissue level (Araujo et al., 2015). Chappuis and co-workers reported a 7.5-fold increase in soft tissue thickness after tooth extraction in patients with a thin biotype hypothesizing that a rapidly resorbing thin buccal plate favored soft tissue ingrowth and therefore increased soft tissue thickness (Chappuis et al., 2015). Moreover, experimental studies have shown an inverse correlation between peri-implant mucosal thickness and buccal bone thickness, indicating that vestibular bone deficiencies occurring at implant sites may be physiologically compensated by an increase in soft tissue thickness (Schwarz et al., 2016).

Interestingly, tissue thickness has also been shown to have an impact on the degree of bone resorption that occurs after abutment connection. In a prospective controlled clinical trial Puisys and Linkevicious concluded that; i) when mucosal tissues are of 2mm or less significantly more bone resorption might be expected and that ii) vertically thickened tissues seemed to behave similarly to natural thick soft tissue

(Puisys and Linkevicius, 2015). These results point out to the importance of having an adequate soft and hard tissue quantity around dental implants and, hence procedures aimed to provide enough hard and soft tissues have being routinely introduced in modern implant practices. Despite of this, the relative contribution of the soft and hard tissue to the total volume and their mutual interplay is poorly understood.

The introduction of soft tissue volumetric analysis using optical scanning and STL image superimposition has allowed the evaluation of changes in tissue contours (Benic et al., 2012). Using this methodology, we have gained knowledge on the impact of different treatment strategies aimed to augment tissue volume and further to assess its stability over time (Sanz Martin et al., 2016, Schneider et al., 2011). This technology, however, has certain limitations since it only provides information at the soft tissue level precluding the understanding of the soft and hard tissue interplay. In recent years, micro-level computed tomography (Micro-CT) has been extensively tested and proven as a valid method for the assessment of bone volumes (Bissinger et al., 2016, de Barros et al., 2016). Thus, we have hypothesized that by merging Micro-CT technology with STL image analysis we could obtain valid information on the relative interaction of the soft and hard tissues in the peri-implant tissue volume. It is, therefore, the purpose of this experimental in-vivo investigation to analyze the relative differences in soft and hard tissue volumes, when two types of implant cervical designs (cylindrical and triangular) were placed either immediately or in healed ridges.

MATERIAL AND METHODS

This pre-clinical in vivo investigation was designed as a prospective, randomized controlled study using eight adult beagle dogs with a weight ranging between 10 and 20 kg. The experimental study was carried out at the Experimental Surgical Centre of the Hospital ''Gomez-Ulla'' in Madrid, Spain, once the Regional Ethical Committee for Animal Research approved the study protocol (Code: ES280790000187). This investigation reports the results from a subset analysis of the specimens whose histological results are reported in a separate publication.

Implants and study design

Implant prototypes with cylindrical (control) and triangular (test) cervical design (MIS Implants Technologies Ltd., Bar-Lev Industrial Park, Israel) with a diameter of 3.5 mm and internal hexagonal connection were placed, either immediately after the extraction of the mesial root of third and fourth lower premolars (PM3 and PM4) with minimal flap elevation (IMI), or in the healed sites of the second premolars (PM2) and the mesial root of the first lower molar (M1) which had been previously extracted 8 weeks before (DLI) (Figures 1a-c). A random assignment by computer software (SPSS Version 20.0, IBM Corporation. New York, USA) allowed that both test and control implants were evenly distributed within IMI and DLI sites as well as in the different mandibular regions.

Twelve weeks after implant placement, animals were euthanized and tissue blocks containing the implants were obtained.

Each block was placed into a sealable sample container with formalin 4% solution at appropriate temperature (5ºC) for storage until processing. Then, samples were dehydrated in a graded series of ethanol solutions and embedded in a light-curing resin (Technovit 7200 VLC; Heraeus-Kulzer GMBH, Werheim, Germany).

Micro-CT analysis and DICOM image acquisition

The specimens were scanned using a high-resolution Micro-CT (Skyscan 1172, Bruker microCT NV, Kontich, Belgium). The X-ray source was set at 100Kv and 100μA with a voxel size of 12 μm and the use of an Aluminium/Copper filter (Al/Cu). The scanning was performed 360° around the specimens fixed on the object stage and images were obtained every 0.4°. Once scanned, the images were reconstructed with the NRecon® software (Bruker microCT NV, Kontich, Belgium) using the algorithm described by Feldkamp (Feldkamp LA, 1984). The obtained images were evaluated with the Data Viewer® software (Bruker microCT NV, Kontich, Belgium) and were rotated to ensure that the implant was perfectly in alignment.

STL image acquisition

Mandibular impressions were obtained at the time of sacrifice with individualized acrylic impression trays using the one-step/two-viscosity technique with silicone impression materials (Express2 Putty Soft/Express2 Light Body, 3M Espe, St. Paul, MN, USA). Dental stone casts were then obtained (Elite Model, Zhermack. Rome, Italy) resulting in 8 models, which once evaluated for the presence of irregularities, porosities, undefined gingival margins, broken cusps or undefined vestibulum were optically scanned with a desktop 3D scanner (Zfx Evolution Scanner, Zimmer Dental. Bolzano, Italy), thus generating STL files.

DICOM - STL image superimposition

DICOM and STL files were uploaded to an image analysis software with a specially developed purpose design plug-in (Swissmeda Software, Swissmeda AG, Zürich, Switzerland). The DICOM files obtained from the Micro CT containing the bone and implant information were compressed and uploaded into the software in order to be matched with the soft tissue information of the STL files. The implant abutment and the adjacent teeth were the common landmarks selected as references in both set of files to allow for an adequeate matching. If the abutment was submerged in the soft tissue and matching was not possible the implant was excluded from the analysis. Once these reference landmarks were selected, the software automatically superimposed the images using a series of mathematical algorithms (Figure. 2a). A further STL image depicting the implant was used with its specific cervical design and length and matched to the implant in the Micro CT image (Figure 2b). Care was taken that the triangle and internal hexagon fitted precisely in the cross-sectional views.

Volume computations

Once the matching was considered adequate, five curves outlining the buccal bony contours based on the information from the cross sectional images of the Micro CT were created. An area of interest was defined that extended 4mm apico-coronally bellow the implant shoulder. This area was drawn with the intent to cover the extent of the surface reduced in the triangular cervical design. Mesio-distally the volume analyzed extended the entire buccal width of the implant body (3.5mm). Volume computations resulted in values in mm^3.

Three distinct volumes of interest were defined; the volume enclosed between the implant suface and the outline of the buccal bone (Buccal bone volume/B-BV), the volume enclosed between the buccal soft tissue contour bellow the implant shoulder and the buccal bone (Epicrestal soft tissue volume/EC-STV) and the volume enclosed between the buccal soft tissue contour above the implant shoulder and the implant abutment (Supracretal soft tissue volume/SC-STV) (Figure 3). If there was bone coronal to the implant shoulder, the software calculated the volume of bone above the implant shoulder. This value was substracted from SC-STV and added to the B-BV.

Linear mesurements

A longitudinal slice that divided the implant mesio-distally into two equal parts was selected. A line coinciding with the axis of the implant was then drawn in the transversal images of the sections.

The following linear measurements were performed by a calibrated evaluator (Figure 3);

- I-BC: Vertical distalce from the implant shoulder to the most coronal extension of the bone crest.

- MT-IS: horizontal mucosal thickness at the implant shoulder.

- STH: vertical soft tissue heigth from the implant shoulder.

Statistical analysis

Descriptive statistics (means, standard deviations) of the continuous variables were analysed using a statistical software program (SPSS Version 20.0, IBM Corporation. New York, USA). The data was tested for normality by means of a Shapiro-Wilk test. A Generalized linear model test with Bonferroni correction was used to analyse differences for these continuous variables. To disclose associations between continuous variables, the Spearman correlation test was utilized. Statistical significance was set at the alpha level of 0.05.

RESULTS

During the experimental investigation the health status of the treated animals was considered as uneventful. There were no reported complications after the implant surgical procedures; all implant showed clinical evidence of integration at the time of sacrifice.

Of the initial 32 biopsies, 2 biopsies were excluded since matching was not possible due to the loss of the healing abutment, which prevented adequate references to perform the matching.

Volumetric assessment (primary outcome)

The image reconstructions of the volumes analyzed for the two implants (test and control) and the two sites (IMI and DLI) are shown in Figure 4. The results from the volumetric computations stratified by implant site are presented in Table 1.

There were no significant differences between test and control implants regarding bone volume, although test implants had greater B-BV in all locations except in PM2 were values showed similar volumes (15.77±7.29 mm^3 and 16.39±7.47 mm^3 for test and control respectively). In M1, these values were of 24.71±5.44mm^3 for the test implants and 21.89±4.28mm^3 for the control implants. In the immediate group, at the PM3 site the values were of 12.84±4.21 mm^3 in the triangular cervical design and 9.1±2.21 mm^3 for the cylindrical design. In the PM4 the volume of buccal bone was of 16.89±7.23 mm^3 for the test and 14.66±5.56 mm^3 for the control implants.

Delayed implants had greater buccal bone volume overall. Within each group, the DLI implants at the M1 sites had greater B-BV than in PM2, while IMI implants in PM4 had greater values than those in PM3 (Figure 5).

The total tissue volume under the implant shoulder (B-BV + EC-STV) was greater for delayed implants when compared to immediate implants, however these values were homogeneous between PM2 and M1 (DLI) and between PM3 and PM4 (IMI). Less voluminous ridges, such as those found in PM2 and PM3, tended to have greater volume of soft tissue bellow the implant shoulder (EC-STV) when compared to larger ridges such as those in M1 and PM4.

Above the implant shoulder, the supracrestal soft tissue volume did not seem to be influenced by the implant design. However, there were noticeable differences when comparing the implant sites. M1 sites had greater soft tissue volume when compared to PM2 sites. The values in the IMI were more similar although still favored the PM4 sites.

The distribution of the total tissue volume (TTV) shown in percentages can be found in Table 2. The percentage of the TTV occupied by the EC-STV remained rather stable in all implant sites representing about one third of the total volume. The percentage of B-BV had greater variability. Those sites that had initially lesser absolute values of B-BV (PM2 and PM3) had lower percentages (range of 19-26%) of the TTV occupied by bone. Sites that had more voluminous ridges (M1 and PM4) had higher percentages of the TTV occupied by bone, ranging between 30-36%. The greatest variability was observed in the percentage of SC-STV, where M1 and PM4 sites, despite of having higher absolute numbers, had a lower percentage of the TTV occupied by the soft tissue above the implant shoulder when compared to PM2 and PM3 sites.

Linear measurements

Descriptive statistics of the linear measurements stratified by implant site are depicted in Table 1. The implant cervical design did not seem to have an influence in the parameters analyzed. The I-BC ranged from 0-40-0.84mm in the delayed implants while in the immediate group this parameter ranged from 0.26-0.71mm. The soft tissue height above the implant shoulder was similar for all implant sites and cervical designs and ranged from 2.5-3.5mm. The horizontal mucosal thickness was also comparable with greater thickness at M1 sites and values with less that 0.5mm differences between test and control implants in all sites.

Correlations

The results of the correlation coefficients and p-values that relate volumetric and linear measurements can be found in Table 3. In the immediate implants, significant positive correlations were found between B-BV and SC-STV, B-BV and STH, B-BV and MT-IS. High I-BC values (low position of bone crest) were correlated with low values of B-BV, SC-STV, MT-IS and STH. In the delayed implants, significant positive correlations were observed between MT-IS and B-BV and SC-STV, while I-BC was positively correlated to EC-STV.

DISCUSSION

This experimental in-vivo study evaluated the differences in the buccal soft and hard tissue volumes when comparing two different implant designs at the cervical portion, which were placed either immediately in fresh extraction sockets or in healed sites. In most of the sites the buccal bone volume was higher for test implants in both surgical protocols. Implants with a triangular section in the cervical portion resulted in an increased distance to the socket buccal plate when placed in fresh extraction sockets and similarly, in an increased space to the buccal wall of the implant osteotomy when placed in healed ridges. This extra space, probably allowed for more bone ingrowth, what resulted in the greater bone volume attained in all sites except in PM2. In these sites, the thin buccal plate that resulted after the osteotomy preparation may have undergone extensive resorption and levelled off any possible differences between test and control implants.

This modified triangular implant cervical geometry, however, did not influence the soft tissue volume above or below the implant shoulder.

Differences in soft and hard tissue healing of immediate and delayed implants have recently received considerable attention, although mainly through the evaluation of two-dimensional changes by means of conventional ground histology (Passoni et al., 2016, Yi et al., 2016). The methodology employed in the present investigation using both STL and Micro-CT superimposed images allowed us to evaluate three-dimensional soft and hard tissue data. Micro-CT has recently been used to evaluate peri-implant bone volume changes (Becker et al., 2016), also the image processing technique used in the present investigation has been validated and extensively used and for the analysis of soft tissue volumetric changes around teeth and implants (Fickl et al., 2009, Schneider et al., 2011, Windisch et al., 2007). The superimposition of the obtained DICOM and STL files, has allowed us not only to quantify the soft tissue volume above and below the implant shoulder, but also to evaluate the effect of the implant design and the surgical protocol on the differential tissue volumes. While the modified implant cervical design had an impact on the hard tissue volume, the different surgical approaches (immediate vs. delayed) had a noticeable repercussion on both hard and soft tissues.

Moreover, the distinct characteristics of the four sites where the delayed and immediate implants were placed also resulted in differential outcomes. The M1 site, which corresponded to a wider crest at baseline, resulted in higher bone volumes when compared to PM2. Similarly, PM4 sites, with greater socket dimensions, resulted in higher bone volumes when compared to PM3 sites. Interestingly, in those sites with lesser buccal bone volumes, the soft tissue bellow the implant shoulder was higher. These findings are in agreement with a recently published investigation that evaluated three-dimensionally the fate of buccal soft and hard tissue eight weeks after extraction and before delayed implant placement (Chappuis et al., 2015). The authors observed a spontaneous soft tissue thickening in thin bone phenotypes 8 weeks after tooth extraction.

Similarly, delayed implants, in particular at M1 sites, resulted in greater soft tissue volume above the implant shoulder when compared to immediate implants. The impact of soft tissue volume above the implant shoulder has clear aesthetic implications as it influences the contour of the alveolar ridge and the colour of the peri-implant tissues (Jung et al., 2008, Bressan et al., 2011). Recently published clinical investigations have observed that delayed implants achieved superior aesthetic results when compared with immediate implants (Tonetti et al., 2017). The findings of the present study suggest that immediate implants may need additional soft tissue grafting to enhance the soft tissue volume on the transition zone from the implant shoulder to the gingival margin to compensate the dimensional changes that occur after tooth extraction. In fact, a recently published systematic review reported that the addition of connective tissue grafting at immediate implants was able to increase the facial gingival thickness and ridge dimensions although it concluded that more evidence was needed to asses its potential impact on implant aesthetics (Lee et al., 2016).

When looking at the correlations in the immediate implants, the position of the bone crest had a clear influence on the soft tissue volume above the implant shoulder, the soft tissue height and the mucosal thickness at the implant shoulder. These findings reinforce the need of clinical strategies aimed towards minimizing the hard tissue changes that occur after IMI through the use of scaffolding materials (Sanz et al., 2016). On the contrary, the position of the bone crest at delayed implants did not seem to influence the previously mentioned soft tissue parameters. These findings highlight the impact of the surgical approach on the interplay between soft and hard tissues. It can be hypothesized that displacement of the soft tissue towards the buccal aspect in the delayed implants may have masked the likely hard tissue changes. A recently published investigation that followed 22 patients over 18 months tested this particular hypothesis. All patients received delayed implants that showed a facial bone dehiscence <5mm at implant placement and were randomly allocated to the augmentation group or left for spontaneous healing. Small bony dehiscence defects allocated to spontaneous healing demonstrated high implant survival rates with healthy and stable soft tissues although these sites revealed greater vertical bone loss at the buccal aspect in the first 6 months after implant insertion (Jung et al., 2016).

It must be acknowledged that the present study had some limitations. The data presented was based on a low number of specimens from an experimental in-vivo study, what may be insufficient to draw conclusions that can be applied in clinical practice. Furthermore, due to the lack of baseline three dimensional hard tissue data, the analysis performed was an end-point assessment, which hindered the possibility of studying the dynamic changes of tissues over time. However, the methodology utilized combining the hard tissue information obtained from Micro CT with the optical scanning of the soft tissues around implants may open the door to the better understanding of the dynamics of tissue healing around dental implants and the reciprocal role that soft and hard tissue may have.

CONCLUSION

The results of the present investigation demonstrated that triangular implants resulted in non-significant higher buccal bone volumes and similar soft tissue volumes when compared to cylindrical implants. Marked differences were observed in the soft and hard tissue volume of delayed and immediate sites. The superimposition of MICRO-CT and optical surface scanning was effective in analyzing the volume of soft and hard tissue.

ACKNOWLEDGEMENTS

The authors highly appreciate the contribution and work of Stephan Schmälzle and Swissmeda on the image analysis software. The authors also acknowledge the help and support received from the staff at the Experimental Surgical Centre of the Hospital ‘‘Gomez-Ulla’’ and the work of Dr. Javier Sanz-Esporrín with the Generalized linear model. Also to Drs. Alejandro Coca, Juan Bollaín and Riccardo Di Raimondo for their contribution to the study.

CONFLICTS OF INTEREST AND FUNDING

This study was partially funded by a research grant from MIS implants technologies (Bar-Lev Industrial Park, Israel). Dr. Ignacio Sanz Martín and Dr. Mariano Sanz Alonso have received lecture fees from MIS Implants.

REFERENCES

Araujo, M. G., Da Silva, J. C., De Mendonca, A. F. & Lindhe, J. 2015. Ridge alterations following grafting of fresh extraction sockets in man. A randomized clinical trial. *Clinical Oral Implants Research,* 26**,** 407-12.

Becker, K., Klitzsch, I., Stauber, M. & Schwarz, F. 2016. Three-dimensional assessment of crestal bone levels at titanium implants with different abutment microstructures and insertion depths using micro-computed tomography. *Clinical Oral Implants Research*.

Benic, G. I., Wolleb, K., Sancho-Puchades, M. & Hammerle, C. H. 2012. Systematic review of parameters and methods for the professional assessment of aesthetics in dental implant research. *Journal of Clinical Periodontology,* 39 Suppl 12**,** 160-92.

Bissinger, O., Kirschke, J. S., Probst, F. A., Stauber, M., Wolff, K. D., Haller, B., Gotz, C., Plank, C. & Kolk, A. 2016. Micro-CT vs. Whole Body Multirow Detector CT for Analysing Bone Regeneration in an Animal Model. *PLoS One,* 11**,** e0166540.

Bressan, E., Paniz, G., Lops, D., Corazza, B., Romeo, E. & Favero, G. 2011. Influence of abutment material on the gingival color of implant-supported all-ceramic restorations: a prospective multicenter study. *Clinical Oral Implants Research,* 22**,** 631-7.

Cairo, F., Sanz, I., Matesanz, P., Nieri, M. & Pagliaro, U. 2012. Quality of reporting of randomized clinical trials in implant dentistry. A systematic review on critical aspects in design, outcome assessment and clinical relevance. *Journal of Clinical Periodontology,* 39 Suppl 12**,** 81-107.

Chappuis, V., Engel, O., Shahim, K., Reyes, M., Katsaros, C. & Buser, D. 2015. Soft Tissue Alterations in Esthetic Postextraction Sites: A 3-Dimensional Analysis. *Journal of Dental Research,* 94**,** 187S-93S.

De Barros, R. R., Novaes, A. B., Jr., De Carvalho, J. P. & De Almeida, A. L. 2016. The effect of a flapless alveolar ridge preservation procedure with or without a xenograft on buccal bone crest remodeling compared by histomorphometric and microcomputed tomographic analysis. *Clinical Oral Implants Research*.

Feldkamp La, D. L., Krass Jw. 1984. Practical cone-beam algorithm. . *Journal of the Optical Society of America,* 1:612-619.

Fickl, S., Schneider, D., Zuhr, O., Hinze, M., Ender, A., Jung, R. E. & Hurzeler, M. B. 2009. Dimensional changes of the ridge contour after socket preservation and buccal overbuilding: an animal study. *Journal of Clinical Periodontology,* 36**,** 442-8.

Jung, R. E., Herzog, M., Wolleb, K., Ramel, C. F., Thoma, D. S. & Hammerle, C. H. 2016. A randomized controlled clinical trial comparing small buccal dehiscence defects around dental implants treated with guided bone regeneration or left for spontaneous healing. *Clinical Oral Implants Research*.

Jung, R. E., Holderegger, C., Sailer, I., Khraisat, A., Suter, A. & Hammerle, C. H. 2008. The effect of all-ceramic and porcelain-fused-to-metal restorations on marginal peri-implant soft tissue color: a randomized controlled clinical trial. *International Journal of Periodontics Restorative Dentistry,* 28**,** 357-65.

Lee, C. T., Tao, C. Y. & Stoupel, J. 2016. The Effect of Subepithelial Connective Tissue Graft Placement on Esthetic Outcomes After Immediate Implant Placement: Systematic Review. *Journal of Periodontology,* 87**,** 156-67.

Passoni, B. B., Marques De Castro, D. S., De Araujo, M. A., De Araujo, C. D., Piatelli, A. & Benfatti, C. A. 2016. Influence of immediate/delayed implant placement and implant platform on the peri-implant bone formation. *Clinical Oral Implants Research,* 27**,** 1376-1383.

Puisys, A. & Linkevicius, T. 2015. The influence of mucosal tissue thickening on crestal bone stability around bone-level implants. A prospective controlled clinical trial. *Clinical Oral Implants Research,* 26**,** 123-9.

Sanz, M., Lindhe, J., Alcaraz, J., Sanz-Sanchez, I. & Cecchinato, D. 2016. The effect of placing a bone replacement graft in the gap at immediately placed implants: a randomized clinical trial. *Clinical Oral Implants Research*.

Sanz Martin, I., Benic, G. I., Hammerle, C. H. & Thoma, D. S. 2016. Prospective randomized controlled clinical study comparing two dental implant types: volumetric soft tissue changes at 1 year of loading. *Clinical Oral Implants Research,* 27**,** 406-11.

Sanz-Sanchez, I., Ortiz-Vigon, A., Sanz-Martin, I., Figuero, E. & Sanz, M. 2015. Effectiveness of Lateral Bone Augmentation on the Alveolar Crest Dimension: A Systematic Review and Meta-analysis. *Journal of Dental Research,* 94**,** 128S-42S.

Schneider, D., Grunder, U., Ender, A., Hammerle, C. H. & Jung, R. E. 2011. Volume gain and stability of peri-implant tissue following bone and soft tissue augmentation: 1-year results from a prospective cohort study. *Clinical Oral Implants Research,* 22**,** 28-37.

Schwarz, F., Sager, M., Golubovic, V., Iglhaut, G. & Becker, K. 2016. Horizontal mucosal thickness at implant sites as it correlates with the integrity and thickness of the buccal bone plate. *Clinical Oral Implants Research,* 27**,** 1305-1309.

Thoma, D. S., Buranawat, B., Hammerle, C. H., Held, U. & Jung, R. E. 2014. Efficacy of soft tissue augmentation around dental implants and in partially edentulous areas: a systematic review. *Journal of Clinical Periodontology,* 41 Suppl 15**,** S77-91.

Tonetti, M. S., Cortellini, P., Graziani, F., Cairo, F., Lang, N. P., Abundo, R., Conforti, G. P., Marquardt, S., Rasperini, G., Silvestri, M., Wallkamm, B. & Wetzel, A. 2017. Immediate versus delayed implant placement after anterior single tooth extraction: the timing randomized controlled clinical trial. *Journal of Clinical Periodontology,* 44**,** 215-224.

Windisch, S. I., Jung, R. E., Sailer, I., Studer, S. P., Ender, A. & Hammerle, C. H. 2007. A new optical method to evaluate three-dimensional volume changes of alveolar contours: a methodological in vitro study. *Clinical Oral Implants Research,* 18**,** 545-51.

Yi, H. Y., Park, Y. S., Pippenger, B. E., Lee, B., Miron, R. J. & Dard, M. 2016. Dimensional Changes Following Immediate and Delayed Implant Placement: A Histomorphometric Study in the Canine. *International Journal of Oral Maxillofacial Implants*.

LEGENDS

Figures

-Figure 1

a) Occlusal image showing the crestal incisions at PM2 and M1 delayed sites, and the extraction of the mesial root of PM3 and PM4.

b) Test and control implants were installed in delayed and immediate sites. Test implants in this specimen were randomized to PM2 and PM4 while control implants were at PM3 and M1

c) Healing abutments were secured and flaps sutured with resorbable sutures.

-Figure 2

a) Superimposition of Micro CT which provides information on the hard tissue structures to the optical scan of the dental casts revealing the soft tissue anatomy.

b) Cross sectional images of the Micro CT. In color blue can be appreciated the outline of the soft tissues and the matching with the implant abutment. Matching of the implant STL can also be appreciated in blue.

-Figure 3. Linear and Volumetric measurements. The buccal bone volume can be appreciated in dark blue, while the epicrestal volume of soft tissue can be appreciated in magenta and the supracrestal volume of soft tissue in light blue. The outline of the soft tissues can be appreciated in orange. STH, Soft tissue height; MT-IS, mucosal thickness at the implant shoulder; I-BC, distance from the implant shoulder to the most coronal part of the bone crest.

Figure 4. Three-dimensional reconstruction of the volumetric computations. The buccal bone volume can be appreciated in dark blue, while the epicrestal volume of soft tissue can be appreciated in magenta and the supracrestal volume of soft tissue in light blue.

4a. Image reconstruction corresponds to an immediate test implant.

4b. Immediate control implant

4c. Delayed control implant

4d. Delayed test implant

-Figure 4: Histogram representing the volumes analyzed in the 4 different implant sites. The buccal bone volume can be appreciated in dark blue, while the epicrestal volume of soft tissue can be appreciated in magenta and the supracrestal volume of soft tissue in light blue.

a) Histogram of volumes at control implants.

b) Histogram of volumes at test implants.

Tables

-Table 1

Header: Descriptive statistics (Mean±SD) of volumetric measurements in mm^3 and linear measurements in mm stratified by implant site.

Footer: Buccal Bone Vol, buccal bone volume; SC Soft tissue Vol, Supracrestal volume of soft tissue; EC Soft tissue Vol, Epicrestal volume of soft tissue; I-BC, distance from the implant shoulder to the most coronal aspect of bone crest; STH, soft tissue height; MT-IS, mucosal thickness at the implant shoulder.

Table 2

Header: Distribution of total tissue volume and tissue volume bellow the implant shoulder shown in percentages.

Footer: Buccal Bone Vol, buccal bone volume; SC Soft tissue Vol, Supracrestal volume of soft tissue; EC Soft tissue Vol, Epicrestal volume of soft tissue.

Table 3

Header: Correlation between volumetric and linear measurements in delayed (blue cells) and immediate (red cells) implants.

Footer: Buccal Bone Vol, buccal bone volume; SC Soft tissue Vol, Supracrestal volume of soft tissue; EC Soft tissue Vol, Epicrestal volume of soft tissue; I-BC, distance from the implant shoulder to the most coronal aspect of bone crest; STH, soft tissue height; MT-IS, mucosal thickness at the implant shoulder.

Table 1. Descriptive statistics (Mean±SD) of volumetric measurements in mm^3 and linear measurements in mm stratified by implant site.

	DELAYED				IMMEDIATE			
	PM2		M1		PM3		PM4	
	TEST (n=3)	CONTROL (n=4)	TEST (n=3)	CONTROL (n=4)	TEST (n=4)	CONTROL (n=4)	TEST (n=4)	CONTROL (n=4)
Volumetric measurements								
Buccal Bone Vol	15.77±7.29	16.39±7.47	24.71±5.44	21.89±4.28	12.84±4.21	9.1±2.21	16.89±7.23	14.66±5.56
SC Soft tissue Vol	20.47±1.03	16.69±8.00	22.73±7.96	26.75±4.98	15.58±6.37	17.06±5.74	18.91±8.96	17.51±5.69
EC Soft tissue Vol	24.41±9.05	26.74±4.2	20.21±3.64	20.22±8.52	20.14±2.49	22.74±6.92	18.06±5.96	15.11±4.13
Linear measurements								
I-BC	0.73±0.81	0.84±0.34	0.50±0.10	0.40±0.48	0.71±0.85	0.53±0.37	0.26±0.30	0.32±0.15
STH	2.47±0.29	3.03±0.68	3.42±1.02	2.82±0.2	2.58±0.71	2.56±0.33	2.81±0.24	2.79±0.41
MT-IS	2.51±0.21	2.09±1.70	2.90±0.16	2.93±0.36	1.80±0.46	2.16±0.43	2.59±0.55	1.94±0.43
***p<0.05**								
Buccal Bone Vol, buccal bone volume; SC Soft tissue Vol, Supracrestal volume of soft tissue; EC Soft tissue Vol, Epicrestal volume of soft tissue								
I, implant shoulder; BC,most coronal aspect of bone crest; STH, soft tissue height; MT-IS, mucosal thickness at the implant shoulder.								

Table 2. Distribution of total tissue volume and tissue volume bellow the implant shoulder shown in percentages.

	DELAYED				IMMEDIATE			
	PM2		M1		PM3		PM4	
	TEST (n=3)	CONTROL(n=4)	TEST(n=3)	CONTROL(n=4)	TEST(n=4)	CONTROL(n=4)	TEST(n=4)	CONTROL(n=4)
Distribution of the total tissue volume								
Buccal Bone Vol	24.61	26.32	36.78	32.23	26.24	19.18	32.1	30.66
SC Soft tissue Vol	39.23	46.97	29.91	28.94	42.75	46.36	33.92	32.36
EC Soft tissue Vol	36.14	26.69	33.3	38.81	30.99	34.44	33.97	36.97
Distribution of the tissue volume bellow the implant shoulder.								
Buccal Bone Vol	38.77	35.63	54.86	53.37	38.44	29.73	48.11	48.36
EC Soft tissue Vol	61.22	64.36	45.13	46.62	61.55	70.26	51.88	51.63
***p<0.05**								
Bone Vol Buccal, buccal bone volume; SC Soft tissue Vol, Supracrestal volume of soft tissue; EC Soft tissue Vol, Epicrestal volume of soft tissue								
I, implant shoulder; BC,most coronal aspect of bone crest; STH, soft tissue height; MT-IS, mucosal thickness at the implant shoulder.								

Table 3. Correlation between volumetric and linear measurements in delayed (blue cells) and immediate (red cells) implants.

		Buccal Bone Vol	SC Soft tissue Vol	EC Soft tissue Vol	I-BC	STH	MT-IS
Buccal Bone Vol	Pearson Corr.	1	.318	-.348	-.304	.029	.592*
	p value		.268	.222	.290	.922	.026
SC Soft tissue Vol	Pearson Corr.	.601*	1	-.295	-.328	.499	.651*
	p value	.014		.306	.252	.069	.012
EC Soft tissue Vol	Pearson Corr.	-.200	.360	1	.658*	-.036	-.114
	p value	.457	.171		.011	.902	.698
I-BC	Pearson Corr.	-.581*	-.546*	.227	1	-.044	-.180
	p value	.018	.029	.397		.883	.539
STH	Pearson Corr.	.549*	.511*	-.008	-.746**	1	.112
	p value	.028	.043	.977	.001		.703
MT-IS	Pearson Corr.	.637**	.772**	.175	-.636**	.562*	1
	p value	.008	.000	.518	.008	.023	

Immediate = red cells. Delayed = blue cells. *. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

Bucca Bone Vol, buccal bone volume; SC Soft tissue Vol, Supracrestal volume of soft tissue; EC Soft tissue Vol, Epicrestal volume of soft tissue

I, implant shoulder; BC,most coronal aspect of bone crest; STH, soft tissue height; MT-IS, mucosal thickness at the implant shoulder.

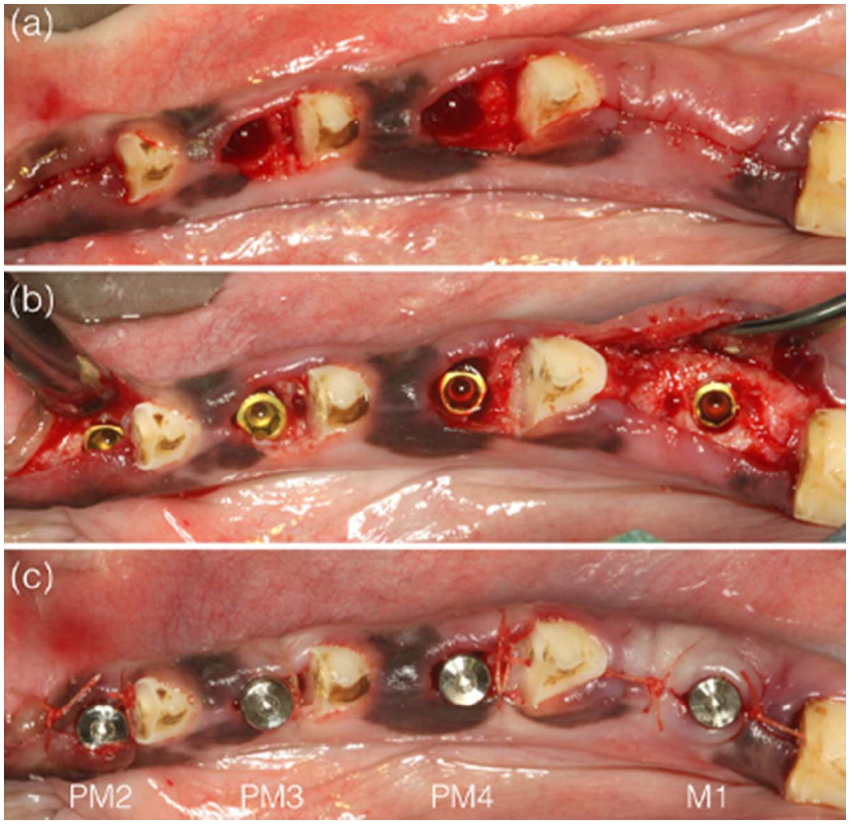


Figure 1

35x34mm (300 x 300 DPI)

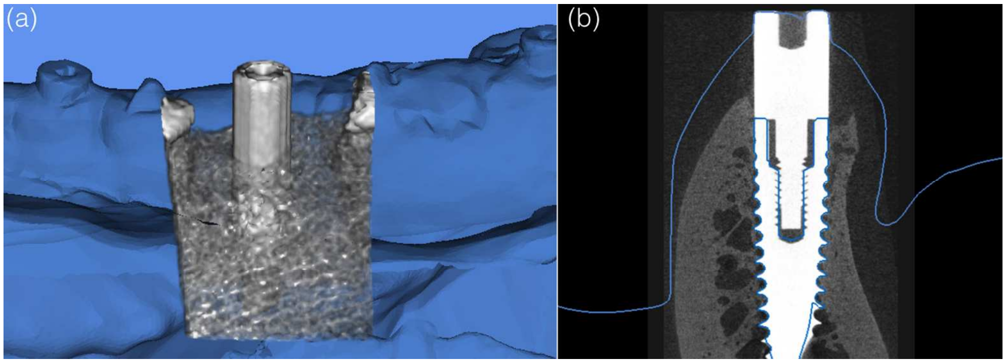


Figure 2

267x94mm (300 x 300 DPI)

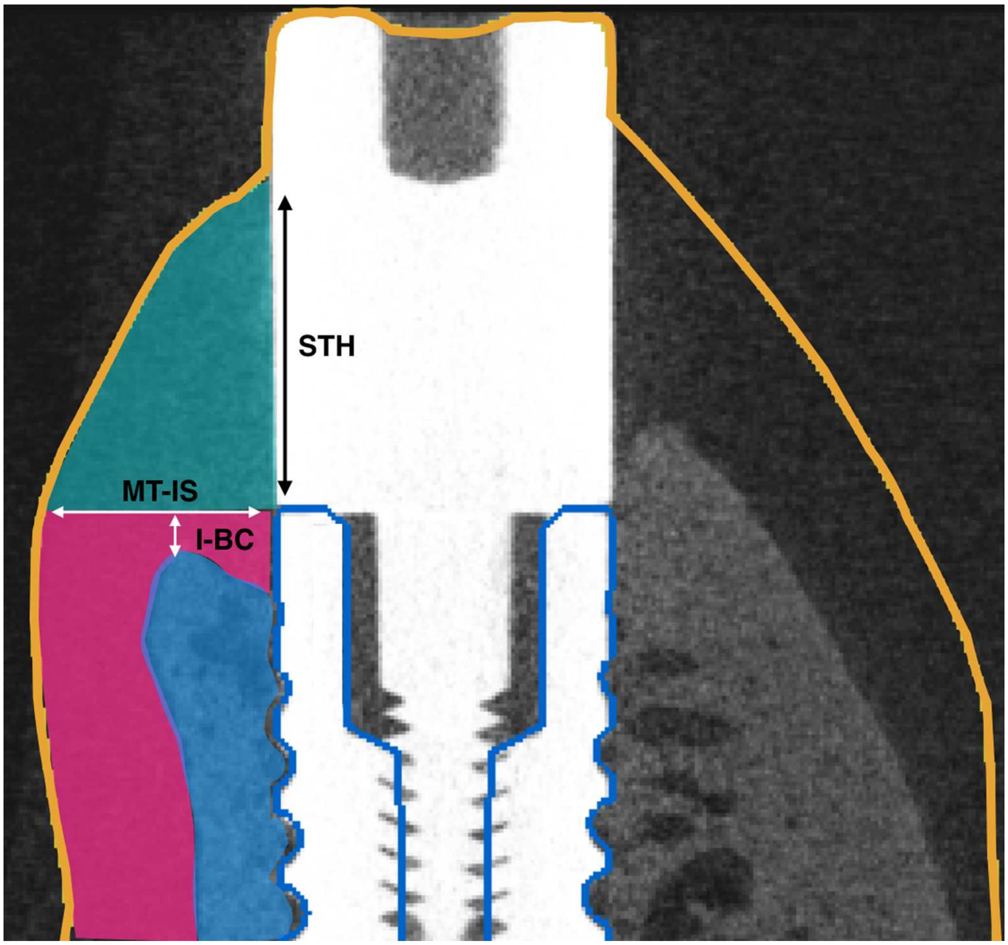


Figure 3

160x150mm (300 x 300 DPI)

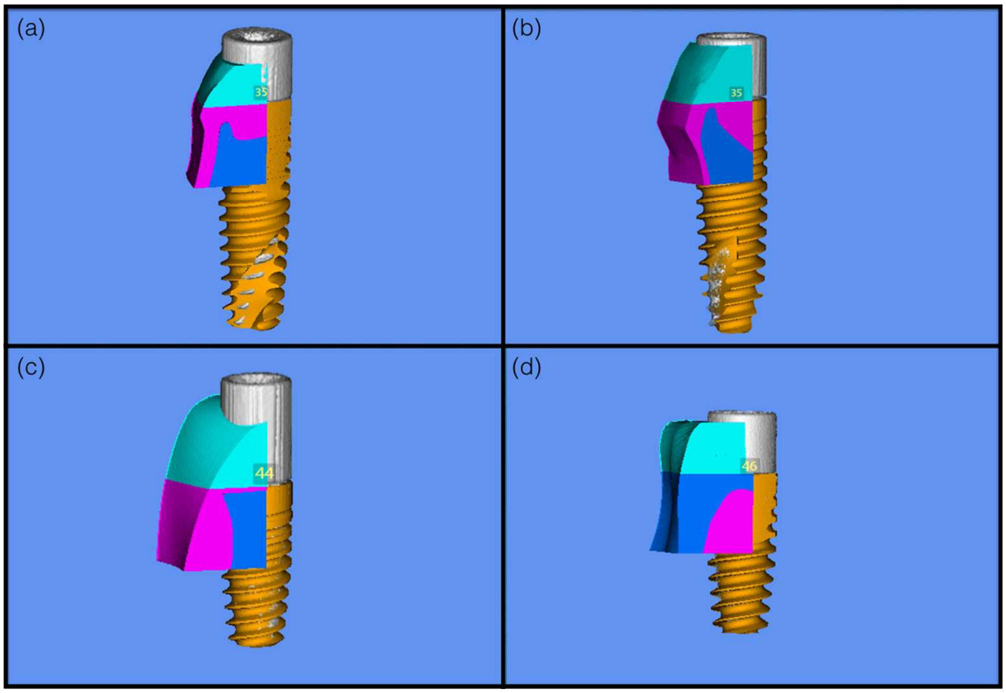


Figure 4

222x152mm (300 x 300 DPI)

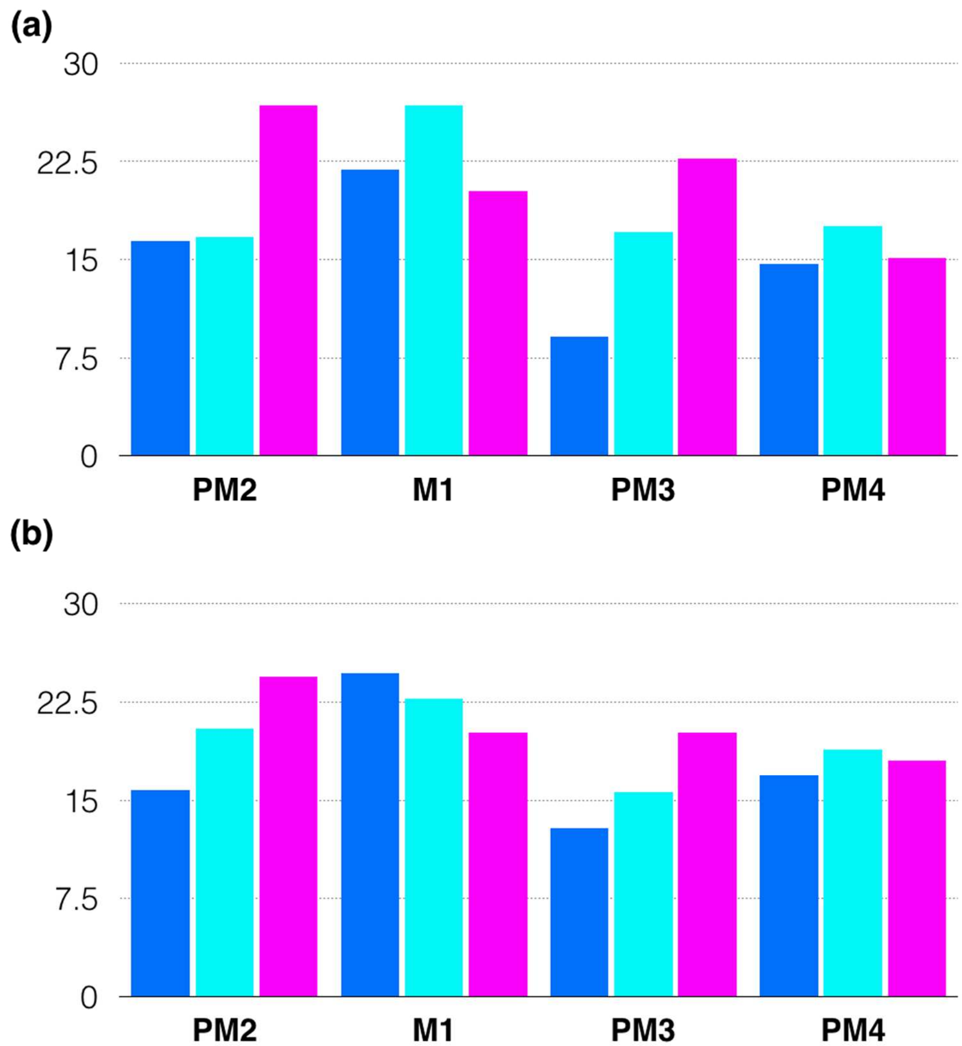


Figure 5

141x154mm (300 x 300 DPI)

Artículo 3:

Contour changes after guided bone regeneration of large non-contained mandibular buccal bone defects using deproteinized bovine bone mineral and a porcine-derived collagen membrane. An experimental in vivo investigation. Sanz-Martin I, Ferrantino L, Vignoletti F, Nuñez J, Baldini N, Duvina M, Sanz M. *Clinical Oral Investigations. Submitted for publication*.

Objetivo: Analizar el contorno del tejido blando después de realizar tres procedimientos regenerativos diferentes en defectos óseos crónicos.

Material y métodos: Se crearon defectos en la mandibular de 9 perros beagle. Tres meses después se realizaron procedimientos regenerativos que fueron aleatorizados a los siguientes grupos i) uso de xenoinjerto óseo (BRG) ii) membrana reabsorbible de colágeno (MBG) y iii) combinación de los dos anteriores (CBG). Se tomaron impresiones de silicona antes de la extracción (T1), antes del procedimiento de aumento (T2) y tres meses después del procedimiento regenerativo (T3).

Resultados: Después de la extracción ocurrieron cambios significativos en el reborde alveolar. Después de la regeneración en el defecto mesial (D1) en T3, la ganancia media horizontal en MBG fue de 0.47±0.34mm, 0.79±0.67mm en BRG y 0.87±0.69mm para CBG. En el defecto medio (D2), la ganancia media para MBG fue de 0.11±0.31; 1.01±0.91 para BRG y 0.98±0.49 para CBG. La ganancia media en el defecto más distal (D3) fue de 0.24±0.72 para MBG, 1.04±0.92 para BRG y 0.86±0.56 para CBG. Las diferencias fueron significativas para la comparación de MBG-BRG y MBG-CBG mientras que BRG-CBG obtuvieron valores similares.

Conclusiones: BRG y CBG tuvieron una eficacia similar y superior a MBG en el aumento horizontal del contorno de los tejidos. El contorno después de la regeneración rara vez alcanzó los valores previos a la extracción.

Clinical Oral Investigations

Contour changes after guided bone regeneration of large non-contained mandibular buccal bone defects using deproteinized bovine bone mineral and a porcine-derived collagen membrane. An experimental in vivo investigation.

--Manuscript Draft--

Manuscript Number:	CLOI-D-17-00172
Full Title:	Contour changes after guided bone regeneration of large non-contained mandibular buccal bone defects using deproteinized bovine bone mineral and a porcine-derived collagen membrane. An experimental in vivo investigation.
Article Type:	Original Article
Corresponding Author:	Ignacio Sanz Martín Universidad Complutense de Madrid Facultad de Odontologia SPAIN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Universidad Complutense de Madrid Facultad de Odontologia
Corresponding Author's Secondary Institution:	
First Author:	Ignacio Sanz Martín
First Author Secondary Information:	
Order of Authors:	Ignacio Sanz Martín
	Luca Ferrantino
	Fabio Vignoletti
	Javier Nuñez
	Nicola Baldini
	Marco Duvina
	Mariano Sanz
Order of Authors Secondary Information:	
Funding Information:	Geistlich Pharma (13560220) — Prof Mariano Sanz
Abstract:	OBJECTIVES: To evaluate soft tissue contour changes after three different regenerative therapies in chronic ridge defects. MATERIAL AND METHODS: Buccal chronic bone defects were created in the mandible of nine beagle dogs. Augmentation procedures were performed 3 months later using a bone replacement graft (BRG), an resorbable collagen membrane (MBG) or a combination of both procedures (CBG). Silicone impressions were taken before tooth extraction (T1), before the augmentation procedure (T2) and three months after the regenerative surgeries (T3). Casts were optically scanned and STL files were superimposed to analyse the horizontal changes in ridge contours. RESULTS: After defect creation the majority of the horizontal changes occurred 4 and 6mm below the gingival margin. In the mesial defect (D1) at T3, the mean horizontal gain in MBG amounted to 0.47±0.34mm, 0.79±0.67mm in the BRG and 0.87±0.69mm for the CBG. In the middle defect (D2), the mean changes for the MBG the values were 0.11±0.31; 1.01±0.91 for the BRG and 0.98±0.49 for the CBG. The mean changes in the distal defect (D3) amounted to 0.24±0.72 for the MBG, 1.04±0.92 for the BRG and 0.86±0.56 for the CBG. The differences reached significance in all defects for the comparison MBG-BRG and MBG-CBG, while similar parameters were observed for the comparison BRG-CBG. CONCLUSION: BRG and CBG were equally effective and superior to MBG in increasing the horizontal tissue contours. The augmentation seldom reached the

Contour changes after guided bone regeneration of large non-contained mandibular buccal bone defects using deproteinized bovine bone mineral and a porcine-derived collagen membrane. An experimental in vivo investigation.

Sanz-Martin I [1], Ferrantino L [2], Vignoletti F [1], Nuñez J [1],Baldini N[3], Duvina M[4], Sanz M [1].

[1] Section of Periodontology, Faculty of Odontology, University Complutense of Madrid, Madrid, Spain.

[2] Università di Milano, Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, Milan, Italy.

[3] Department of Periodontics and Fixed Prosthodontics, University of Siena, Italy

[4] Oral Surgery Department. University of Florence, Italy

Key words: Bovine bone mineral, collagen membrane, bone regeneration, experimental study, wound healing.

Running title: Contour changes after guided bone regeneration.

Address for correspondence: Dr. Ignacio Sanz Martín

Facultad de Odontología

Universidad Complutense de Madrid

Plaza Ramón y Cajal

28040 Madrid

e-mail: ignaciosanzmartin@odon.ucm.es

Word Count Manuscript: 4010. Number of figures and tables: 4 figures, 3 tables.

ABSTRACT

OBJECTIVES: To evaluate soft tissue contour changes after three different regenerative therapies in chronic ridge defects.

MATERIAL AND METHODS: Buccal chronic bone defects were created in the mandible of nine beagle dogs. Augmentation procedures were performed 3 months later using a bone replacement graft (BRG), an resorbable collagen membrane (MBG) or a combination of both procedures (CBG). Silicone impressions were taken before tooth extraction (T1), before the augmentation procedure (T2) and three months after the regenerative surgeries (T3). Casts were optically scanned and STL files were superimposed to analyse the horizontal changes in ridge contours.

RESULTS: After defect creation the majority of the horizontal changes occurred 4 and 6mm below the gingival margin. In the mesial defect (D1) at T3, the mean horizontal gain in MBG amounted to 0.47±0.34mm, 0.79±0.67mm in the BRG and 0.87±0.69mm for the CBG. In the middle defect (D2), the mean changes for the MBG the values were 0.11±0.31; 1.01±0.91 for the BRG and 0.98±0.49 for the CBG. The mean changes in the distal defect (D3) amounted to 0.24±0.72 for the MBG, 1.04±0.92 for the BRG and 0.86±0.56 for the CBG. The differences reached significance in all defects for the comparison MBG-BRG and MBG-CBG, while similar parameters were observed for the comparison BRG-CBG.

CONCLUSION: BRG and CBG were equally effective and superior to MBG in increasing the horizontal tissue contours. The augmentation seldom reached the values before extraction.

CLINICAL RELEVANCE: Scaffolding materials are needed for contour augmentation when using resorbable membranes.

INTRODUCTION

In humans, the loss of horizontal ridge contour as a consequence of tooth loss may account for more than 50% of the ridge width [1, 2], and the resulting lack of adequate crestal bone availability may significantly affect the successful implant placement in an ideal, prosthetically driven position [3]. Bone augmentation procedures are, therefore, aimed to compensate these changes and to reconstruct deficient alveolar ridges to permit the accurate placement of dental implants. Different regenerative interventions, such as the use of autogenous bone grafts, distraction osteogenesis, ¨split¨ ridge osteotomy and guided bone regeneration (GBR) have shown efficacy in augmenting the alveolar ridge [4].

GBR with barrier membranes is based on the biological principle of compartmentalized healing by preventing the ingrowth of cells from the overlying mucosa into the membrane-protected space and allowing the colonization of competent osteogenic cells [5, 6]. It is currently the standard of care for horizontal bone augmentation, due to its predictability and minimal invasiveness [7]. Barrier membranes of different designs and compositions have been tested in preclinical and clinical models to provide evidence that GBR predictably results in bone regeneration when applied over critical size osseous defects [8, 9]. Residual crests, however, usually result in non-contained bone defects where the use of barrier membranes, mainly those being resorbable, will collapse into the defect and will reduce the space available for the colonization of osteogenic cells [10]. In addition, the blood clot tends to shrink during healing, what amplifies this effect [11].

Current GBR approaches, therefore, combine the use of barrier membranes with bone grafts and bone substitutes, which serve as scaffolds to fill the defect volume and to stabilize the blood clot, thus preventing its tendency to shrinkage. Moreover, the current understanding of bone biology and the biological behavior of modern biomaterials have resulted in less invasive surgical approaches and the attainment of better clinical results [12, 13]. A recent systematic review from our research group has reported that the combination of a xenogeneic bone replacement graft with a resorbable native collagen membrane is the GBR procedure most widely used and the one that achieves more consistent results [14]

In spite of this body of evidence on the efficacy of GBR combining bone replacement grafts and bio-absorbable membranes, their respective wound healing patterns and their specific tissue response when used either alone or in combination are still partially known [15]. This existing knowledge has been mainly derived from pre-clinical studies using histological outcomes to evaluate the healing patterns [16]. These studies have demonstrated the ability of these biomaterials of guiding new bone formation (osteoconduction) and being gradually replaced by new bone, although there is high variability in the degree of biomaterial bioabsorption and its replacement by new bone formation (Sanz and Vignoletti, 2015). Histological methods, however, are unable to assess the reconstruction of the whole alveolar process since they can only focus on selected sections of varying thicknesses depending on the method of histologic processing.

The use of digital image analysis has been recently introduced in implant dentistry to study changes in contours and tissue volume. This has been particularly useful in the assessment of the tissue changes after bone augmentation procedures, either simultaneous or staged with implant placement [17, 18]. The outcome of implant supported reconstructions is not assessed anymore solely on the basis of implant survival but on how peri-implant tissues are in harmony with its adjacent structures [19].

It is therefore the aim of this pre-clinical in-vivo investigation to evaluate the changes in tissue contour occurring after a GBR procedure combining a xenogeneic bone replacement graft and a natural collagen resorbable membrane, for the lateral augmentation of critical size defects.

MATERIAL AND METHODS

This pre-clinical *in vivo* investigation was designed following the modified ARRIVE guidelines for pre-clinical research [20] with a randomised block, examiner-blind experimental study evaluating 4 stages of healing in two groups of dogs.

Sample and facilities

The experimental phase of the study was carried out at the “Veterinary Teaching Hospital” in the University of Santiago in Lugo, Spain after the study protocol being approved by the Ethical Committee of the Rof Codina Foundation (Lugo, Spain) (Ref AE-LU-001/12/INVMED (02)/Outros/04). Nine female beagle dogs, between 1.5 and 2 years old, with a weight ranging between 10 and 20 kilograms, were used. This investigation was conducted according to Spanish and European Union regulations (European Communities Council Directive 86/609/EEC) on experimental in vivo experimentation. All animals were fed on a soft pellet diet and maintained in individual kennels in a 12:12 light/dark cycle and 22-21 Cº as well as daily monitored during the entire course of the experiment by an experienced veterinarian.

Surgical Procedure

After animal sedation with propofol (2mg/kg/i.v., Propovet, Abbott Laboratories, Kent, UK), general anaesthesia was maintained under mechanical induced respiration of 2.5-4% of isoflurane (Isoba-vet, Schering-Plough, Madrid, Spain). The animals were pre-medicated with acepromazine (0.05 mg/kg/i.m., Calmo Meosan, Pfeizer, Madrid, Spain) and morphine (0.3 mg/kg/i.m., Morfina Braun 2%, B. Braun Medical, Barcelona, Spain) was administered as analgesic medication. Lidocaine 2% with epinephrine 1:100000 (2% Xylocaine Dental, Dentsply, York, PA, USA) were infiltrated locally to reduce bleeding during surgery.

Defect preparation and augmentation procedures

The experimental model used in this study is outlined in Figure 1. On both sides of the mandible, buccal and lingual mucoperiosteal flaps were raised. The second, third and fourth lower premolars (P) and the first molar (M) were hemisected by means of a Lindemann bur. The mesial root of M1, the mesial root of P4, the distal root of P3 and booth roots of P2 where extracted. A pulpotomy was made with a sterile bur and a pulp cap with calcium hydroxide was applied (Dycal, Dentsply, York, PA, USA) and a glass-ionomer filling (Ketac. 3M ESPE. Berkshire, UK) in each of the residual roots. In the three edentulous regions of each side of the mandible buccal bone defects were created with diamond burs under copious sterile saline irrigation (Figure 1a). The defect sizes were about 10 mm in height (apico-coronally), 10 mm in width (mesio-distally) and 5 mm in depth (buco-lingually). Flaps were then repositioned and sutured (Vicryl" 5.0, Johnson & Johnson, St-Stevens-Woluwe, Belgium). A period of three months was given for healing of the surgically created defects. Then the augmentation procedures were carried out with the elevation of full thickness flaps from 1M1 to 1P1 fully exposing the bone defects (Figure 1b).

Each defect was randomly allocated to one of three augmentation procedures using a computer-generated list. In the bone replacement group (BRG) the defect was filled with a bone replacement graft composed of 90% of deproteinized bovine bone mineral with 10% collagen (DBBM-C) (Geistlich Bio-Oss® Collagen; Geistlich Pharma AG, 6110 Wolhusen, Switzerland). This bone replacement graft was hydrated with saline and well adapted to fill the residual crest defect by means of resorbable sutures (Vicryl" 4.0, Johnson & Johnson, St-Stevens-Woluwe, Belgium). In the membrane alone group (MBG) the defect was covered with an absorbable Native Bilayer Collagen Membrane (NBCM) (Geistlich Bio-Gide®; Geistlich Pharma AG, Wolhusen, Switzerland). The membrane was trimmed and adapted over the ridge to completely cover the defect and extended beyond the defect margins by 2–3mm. The NBCM was secured by attaching four titanium pins (Frios® membrane tacks, Dentsply, York, PA, USA) in the buccal and lingual bone. In the combination group (CBG), both interventions were combined and the defect was filled with the DBBM-C and the NBCM was adapted to cover the defect and extended beyond the defect margins by 2–3mm. The membrane was secured as previously described (Figure 1c).

Releasing incisions were made in the periosteum at the base of the buccal and lingual flaps and the augmented defects were carefully covered by tension-free flaps and secured by horizontal internal mattress sutures alternated with interrupted 4/0 e-PTFE sutures (Goretex Suture, W. L. Gore & Associates Inc. Newark, DE, USA). For postoperative pain control, morphine (0.3 mg/kg/i.m.) was administered for the first 24 hours and meloxicam (0.1 mg/kg/s.i.d./p.o., Metacam, Boehringer Ingelheim España, Barcelona, Spain) for 3 following days. Amoxicillin (22mg/kg/s.i.d./s.c., Amoxoil retard, Syva, León, Spain) was used as post-operative antibiotic therapy for 7 days. During two weeks postoperatively, the animals were fed with water-softened food and surgical wounds were cleaned three times a week using gauzes impregnated with a chlorhexidine solution (0.12%). The sutures were removed after 14 days.

Soft tissue contour changes

Impressions of the lower jaws were obtained before the extractions (T1), prior to the augmentation surgery (T2) (i.e. three months after the extractions) and 3 months after the augmentation procedure (T3). For this purpose, a one-step/two-viscosity technique with silicone impression materials (Express2 Putty Soft/Express2 Light Body, 3M Espe, St. Paul, MN, USA) and individualized acrylic impression trays were used. Dental stone casts were fabricated (Elite Model, Zhermack. Rome, Italy), resulting in a total of 27 casts, 9 casts for each of the three different timelines (T1, T2, T3). Models were evaluated for the presence of irregularities such as porous areas, undefined gingival margins, broken cusps or undefined vestibulum.

The cast models were optically scanned with a desktop 3D scanner (Zfx Evolution Scanner, Zimmer Dental. Bolzano, Italy) resulting in individual STL files for each time period (Fig. 2), which were uploaded to an image analysis software (Swissmeda Software, Swissmeda AG, Zürich, Switzerland) (Fig. 3a). To match the STL files, three clear and visible common reference points were selected in both the baseline and follow-up casts. After the selection of these references, the software automatically superimposed the three models using a series of mathematical algorithms. In those sites where inproper fitting occured, manual adjustments were performed until the matching was deemed adequate (Fig. 3b).

Once the matching was deemed adequate a longitudinal slice perpendicular to the ridge that divided defect into two equal parts was selected. A line coinciding with the axis of tooth at baseline was then drawn in the transversal images of the sections. A screenshot was then exported to an image processing software to perform the horizonal measurements (ImageJ, National Institutes of Health. Maryland, USA).

Since the three defects had distinct anatomical characteristics, data were analyzed separetely. Defect D1 corresponded to the most mesial defect and was created after the extraction of P2 and had mesialy to it P1 and distally to it the mesial root of P3. Defect D2 was created after the extraction of the distal root of P3 and the mesial root of P4 and had mesially to it the mesial root of P3 and distally to it the distal root of P4. Defect D3 was the most distal defect, was created after the extraction of the mesial root of M1 and had mesially to it the distal root of P4 and distal to it the distal root of M1.

The following linear measurements were performed by a blinded calibrated examiner (JA), independent from the investigator undertaking the analysis (Fig. 4):

i) The *horizontal soft tissue changes* were assessed 2, 4 and 6mm below the gingival margin (GM) by measuring the distance between the line coinciding with the axis of the tooth at baseline and the buccal soft tissue outline at the three diferent timelines (T1, T2 and T3)[21, 22]. Once the horizontal measurements (HM) were calcualted at the three different heights (2,4, and 6mm) at T1, the horizontal changes (HC) were calculated by substracting the HM at T1 from the HM at T2 to obtain the HC from T1-T2 which gave information on the degree of ridge collapse. To assess the changes in HM after the augmentation procedure the HM at T3 were substracted from those at T2 to obtain the HC from T2-T3. HM at T1 were substracted from the HM at T3 to obtain the HC from T1-T3 which assessed the differences between the regenerated and the baseline tissue contours.

ii) The *vertical soft tissue changes* were assessed by measuring the distance between two lines perpendicular to the axis of the tooth. The first line was coinciding with the buccal gingival margin of the tooth at T1 and the other lines were coinciding with the edentulous crest at T2 and T3 (VC T1-T2, VC T1-T3).

iii) Mean linear changes between T2 and T3 provided information on the mean distance between the two surfaces in a selected area of interest. For this purpose, an area that encompassed the center of each defect was selected by means of a dedicated software (Swissmeda Software, Swissmeda AG, Zürich, Switzerland). The area extended 10mm mesio distally and had an apico-coronal height of 5mm. The software then calculated, by mean of a series of mathematical algorythms, the mean distance between T2 and T3 surfaces in each defect.

Statistical analysis

Descriptive statistics (means, standard deviations) of continuous variables were computed for each site separately using a statistical software program (SPSS Version 18.0, IBM corporation. New York, USA). The data was tested for normality by means of a Shapiro-Wilk test and found to be non-normally distributed. The Kruskall-Wallis test was used to determine differences at baseline and to analyze if the regenerative treatment had an impact in the continuous variables. Post hoc analysis was further performed with the Kruskall-Wallis test to check for pairwise comparisons between the three regenerative approaches. Statistical significance was set at the alpha level of 0.05.

RESULTS

All animals healed uneventfully after both surgical interventions without occurrence of infections or evident membrane dehiscences after the regenerative procedures.

Changes in horizontal and vertical measurements after defect creation (T1-T2)

Table 1 depicts the baseline (T1) horizontal widths (BW) at the level of the GM and 2,4 and 6 below the GM, the horizontal and vertical changes from T1 and T2 at 4 and

6 mm from the GM and the percentage of loss that occurred from baseline values. At baseline, there were no significant differences in horizontal measurements between the three treatment groups in each of the three defects at the level of the GM and 2,4 and 6 mm below. In the most distal defect (D3) the baseline horizontal measurements at the level of the GM and 2mm below presented marked differences between the three treatment groups.

After the extraction and defect creation major changes occurred in the alveolar ridge. All groups had a mean loss in height that ranged from 2.04mm to 3.22mm from the GM with no significant differences between the treatment groups. The majority of the horizontal changes occurred at 4 and 6mm below the gingival margin with greater changes occuring in the most posterior sites (D2 and D3) than in the anterior defects (D1). The HC at 4 and 6mm below the GM were similar for the three groups, ranging in losses between 30 to 60%. Six mm below the GM the percentage bone loss was more homogeneous among the defects (D1:20%, D2:26 to 42% and D3:32-34%).

Changes in horizontal measurements from defect healing to 3 months after the regenerative intervention (T2-T3)

Since vertical gains did not occur in any of the three augmentation procedures, these values were not reported. In fact, in all groups there was a mean loss in height ≥ 2mm, which obliged us to report the horizontal changes at the levels 4 and 6mm below the GM. In the three defects (D1, D2 and D3) the amount of tissue augmentation was greater in the BRG and CBG compared to the MBG groups, at both 4 and 6mm levels, although these differences were not statistically significant.

The mean horizontal contour changes in mm (HC T2-T3) and mean percentages of regenerated contours are reported in Table 2 for the three defect sites. At the 4 mm level in the intermediate defect (D2) they were 0.57±0.19 mm (14%) in the MBG; 1.26±0.89 mm (27%) in the BRG and 1.18±0.51 mm (35%) in the CBG groups. At the 6 mm level these changes were respectively for the MBG, BRG and CBG groups 0.40±1.19 mm (14%), 1.14±1.67 mm (55%) and 1.05±0.07 mm (48%).

The mean linear changes between T2 and T3 are also reported in Table 2 for the three defect sites. These values provide more comprehensive information regarding the changes that occured between T2 and T3 as they report on the mean horizontal changes that occur in the selected area of interest as opossed to the HC which report on values from a single sagittal slide. In D2, the mean changes for the MB, BRG and CBG groups were respectively: 0.11±0.31; 1.01±0.91and 0.98±0.49 mm. The pairwise analysis showed statistically significant differences between MBG-BRG and MBG-CBG in all three defect sites, while no differences where observed for the comparison BRG-CBG.

Comparisons between baseline and post regenerative horizontal and vertical measurements (T1-T3)

The mean horizontal contour changes in mm (HC T1-T3) are reported in Table 3 for the three defect sites. Positive values indicate that the values in T1 were greater than in T3 while negative values indicate the opposite. In D1, since the initial loss was less pronounced than in D2 and D3, the HC at T3 was closer to baseline values. At the 4mm level, the HM did not reach the baseline values in any of the three augmentation modalities although in the BRG the differences between T1 and T3 were closer to the baseline values when compared to the MBG and CBG.

At the 4 mm level in the intermediate defect (D2) HC values were 3.40±0.63 mm in the MBG; 3.59±2.27 mm in the BRG and 2.17±0.66 mm in the CBG groups. At the 6 mm level these changes were respectively for the MBG, BRG and CBG groups 2.94±0.94 mm, 0.57±1.14 mm and 2.71±0.05 mm.

The VC from T1-T3 were very similar from the values obtained from T1-T2 proving minimal vertical gains after the regenerative procedures without significant differences between the three interventions.

DISCUSSION

The present experimental *in vivo* investigation measured the soft tissue contour changes occurring after tooth extraction and defect creation and then assessed the efficacy of three augmentation procedures to reconstruct these soft tissue contours. Extraction and defect creation caused a marked reduction in ridge height and width. Horizontal loss ranged from 30-60% at 4mm below the GM and from 20-40% at 6mm. The horizontal changes after the augmentation surgeries favoured the bone replacement graft and the combination of bone replacement graft and membrane. The BRG recovered 42% of the loss that occurred after defect creation at 4mm below the GM and 69% at 6mm. The CBG recovered 37% and 63% at the 4 and 6mm levels respectively. In the MBG theses values were of 13% and 22%.

The analysis of the mean changes in tissue contours in all defects (D1, D2 and D3 combined) before and after the augmentation surgeries showed significantly higher gains for the BRG and CBG when compared to the MBG (0.94mm, 0.90mm and 0.27mm respectively). The lesser gains observed in the MBG can be explained by the lack of bone replacement graft which prevented space maintenance and appropriate clot stabilization. This effect was also due to the native collagen membrane utilized, which has scarce memory and tends to collapse over the surrounding tissues. On the other hand, this barrier membrane easily adapts to the contours provided by the bone replacement grafts when used as scaffolds [23, 24]. In similar defects in experimental studies, the use of scaffolds giving support to collagen barriers, prevented the ridge collapse when compared with sham operated areas [25].

The lack of differences between the BRG and CBG groups could be explained by the proven slow bioabsorbability of DBBM [26, 27]. In this study the bone replacement graft was able to prevent soft tissue collapse, irrespective of whether it was covered by a membrane or not. The likely biological effect of the membrane on the new bone formation can only be assessed histologically and hence, in this study assessing the ridge contours, whether the biomaterial is in full contact with new bone or with connective tissue did not affect this outcome.

These results, however, are in contrast with recent preclinical data showing that the addition of a collagen barrier fixed with titanium pins was able to better withstand the compression applied to the biomaterial at wound closure [28]. These discrepancies could be explained by the different methods used to assess the outcome, since in this study the effect was studied on non-living pig jaws with cone-bean tomography (CBCT) before and immediately after wound closure.

Clinical studies have shown that the combination of resorbable membranes and particulate bone grafts was able to predictably reconstruct the alveolar ridge in single tooth defects and achieve esthetically pleasing tissue contours that remained stable at the 6-year follow-up [29]. The present investigation dealt with large non-contained defects with wide mesio-distal edentulous spaces, which probably compared better to defects after extraction of multiple teeth, rather than single tooth anterior defects. Although the objective of the regenerative interventions was not to achieve vertical growth but to attempt horizontal augmentation solely, complete horizontal reconstruction at 4 and 6mm below the GM was rarely achieved. This partial reconstruction of the alveolar ridge was probably due to the use of non-space maintaining membranes. Space containing non-resorbable membranes have demonstrated in clinical and experimental studies their ability to attain both vertical and horizontal regeneration [30-32], although their use may lead to more post-operative and soft tissue complications, mainly if the membranes become exposed during healing [33].

In terms of methodology, the modified healed defect model used in this pre-clinical study is a validated experimental mode to test alveolar ridge regenerative interventions [34, 35]. Similarly, the methodology used to assess the changes in tissue contours is a well-established method to investigate the impact of different regenerative therapies in mucosal contours [36-38]. In fact, soft tissue assessment by means of optical scanning of dental impressions in combination with image analysis software is the method of choice for the three dimensional assessment of the soft tissue changes after implant placement and augmentation procedures [22, 39]. This analysis however, focuses on the soft tissue changes which does not allow to draw inferences on the interplay with the hard tissues changes which may impact tissue thickness [40]

The high variability observed in the horizontal measurements is in line with other preclinical investigations utilizing similar methodology [41]. Similarly, human clinical studies utilizing comparable image analysis technology, have also reported high variability when evaluating the changes that occur in ridge contours after ridge augmentation procedures with autologous block grafts in the maxillary anterior region [17, 42]. In this study, the changes in tissue contours were evaluated before grafting, before implant placement, before abutment connection, at crown placement and 1 and 5 years later. The authors observed a marked increase in ridge width after the augmentation procedure and after crown insertion, which clearly indicated that the implant supported restorations, had a significant influence on the final horizontal ridge contours. In this investigation only the changes before and after different bone augmentation procedures were assessed without evaluating the impact of dental implants or restorations, what makes the comparisons with the previously mentioned studies impossible. Moreover, in light of the inherent limitations with the present animal model resulting in defects of different sizes from mesial to distal and on marked changes after the healing period, the obtained results should not be fully extrapolated to clinical situations. Our randomized experimental design, however, assured that all treatment strategies were equally distributed in the different sites. Furthermore, the data extracted from the image analysis was stratified according to the different defects locations to allow for site-specific comparisons. The methodology used for image analysis has shown a high reproducibility and excellent accuracy for measuring contour changes in a methodological study [43]. This method has been widely used in a variety of clinical and experimental investigations proving to be a non-invasive and reliable technique to assess changes in soft tissue after reconstructive therapy [44-46].

CONCLUSION

The gains in horizontal tissue contours achieved by bone replacement grafts or by the combination of bone replacement grafts with resorbable collagen membranes was superior to the membrane group alone although none of the treatment strategies was able to completely restore the ridge width to baseline values before tooth extraction. Additional therapy may be required if the goal is to completely reestablish the alveolar tissue contours.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to Dr. Nicola Discepoli, Dr. Fernando Luengo who participated in the experimental surgeries and the staff from the veterinary hospital of Rof Codina in Lugo in particular Prof. Fernando Muñoz. A high appreciation is also expressed to Prof. Massimo De Sanctis and Prof. Raul Caffesse for their contribution to the study.

COMPLIANCE WITH ETHICAL STANDARS

CONFLICTS OF INTEREST

Dr. Sanz-Martín, Dr. Ferrantino, Dr. Vignolettu, Dr. Nuñez, Dr. Baldini and Dr. Duvina report no conflict of interest. Dr. Sanz reports to have received research grants through the University Complutense of Madrid and lecture fees from Geistlich Pharma.

FUNDING

This investigation was partially supported with a research contract between Geistlich Pharma AG and the Universities of Goteborg, Complutense of Madrid and University of Siena.

ETHICAL APPROVAL

The study protocol was approved by the Ethical Committee of the Rof Codina Foundation (Lugo, Spain) (Ref AE-LU-001/12/INVMED (02)/Outros/04)

INFORMED CONSENT

No informed consent was obtained since the present was an animal study.

REFERENCES

1. Schropp, L., A. Wenzel, L. Kostopoulos, and T. Karring, *Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study*. Int J Periodontics Restorative Dent, 2003. 23(4): p. 313-23.
2. Tan, W.L., T.L. Wong, M.C. Wong, and N.P. Lang, *A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans*. Clin Oral Implants Res, 2012. 23 Suppl 5: p. 1-21.
3. Araujo, M.G., C.O. Silva, M. Misawa, and F. Sukekava, *Alveolar socket healing: what can we learn?* Periodontol 2000, 2015. 68(1): p. 122-34.
4. Chiapasco, M., M. Zaniboni, and M. Boisco, *Augmentation procedures for the rehabilitation of deficient edentulous ridges with oral implants*. Clin Oral Implants Res, 2006. 17 Suppl 2: p. 136-59.
5. Melcher, A.H., *Role of the periosteum in repair of wounds of the parietal bone of the rat*. Arch Oral Biol, 1969. 14(9): p. 1101-9.
6. Nyman, S., J. Gottlow, J. Lindhe, T. Karring, and J. Wennstrom, *New attachment formation by guided tissue regeneration*. J Periodontal Res, 1987. 22(3): p. 252-4.
7. Donos, N., N. Mardas, and V. Chadha, *Clinical outcomes of implants following lateral bone augmentation: systematic assessment of available options (barrier membranes, bone grafts, split osteotomy)*. J Clin Periodontol, 2008. 35(8 Suppl): p. 173-202.
8. Dahlin, C., L. Sennerby, U. Lekholm, A. Linde, and S. Nyman, *Generation of new bone around titanium implants using a membrane technique: an experimental study in rabbits*. Int J Oral Maxillofac Implants, 1989. 4(1): p. 19-25.
9. Schmid, J., C.H. Hammerle, L. Fluckiger, J.R. Winkler, A.J. Olah, S. Gogolewski, and N.P. Lang, *Blood-filled spaces with and without filler materials in guided bone regeneration. A comparative experimental study in the rabbit using bioresorbable membranes*. Clin Oral Implants Res, 1997. 8(2): p. 75-81.
10. Schenk, R.K., D. Buser, W.R. Hardwick, and C. Dahlin, *Healing pattern of bone regeneration in membrane-protected defects: a histologic study in the canine mandible*. Int J Oral Maxillofac Implants, 1994. 9(1): p. 13-29.
11. Hammerle, C.H., J. Schmid, A.J. Olah, and N.P. Lang, *A novel model system for the study of experimental guided bone formation in humans*. Clin Oral Implants Res, 1996. 7(1): p. 38-47.
12. Gruber, R., B. Stadlinger, and H. Terheyden, *Cell-to-cell communication in guided bone regeneration: molecular and cellular mechanisms*. Clin Oral Implants Res, 2016.
13. Sanz, M. and F. Vignoletti, *Key aspects on the use of bone substitutes for bone regeneration of edentulous ridges*. Dent Mater, 2015. 31(6): p. 640-7.

14. Sanz-Sanchez, I., A. Ortiz-Vigon, I. Sanz-Martin, E. Figuero, and M. Sanz, *Effectiveness of Lateral Bone Augmentation on the Alveolar Crest Dimension: A Systematic Review and Meta-analysis*. J Dent Res, 2015. 94(9 Suppl): p. 128S-42S.
15. Benic, G.I. and C.H. Hammerle, *Horizontal bone augmentation by means of guided bone regeneration*. Periodontol 2000, 2014. 66(1): p. 13-40.
16. Thoma, D.S., I.S. Martin, S. Muhlemann, and R.E. Jung, *Systematic review of pre-clinical models assessing implant integration in locally compromised sites and/or systemically compromised animals*. J Clin Periodontol, 2012. 39 Suppl 12: p. 37-62.
17. Jemt, T. and U. Lekholm, *Measurements of buccal tissue volumes at single-implant restorations after local bone grafting in maxillas: a 3-year clinical prospective study case series*. Clin Implant Dent Relat Res, 2003. 5(2): p. 63-70.
18. Schneider, D., U. Grunder, A. Ender, C.H. Hammerle, and R.E. Jung, *Volume gain and stability of peri-implant tissue following bone and soft tissue augmentation: 1-year results from a prospective cohort study*. Clin Oral Implants Res, 2011. 22(1): p. 28-37.
19. Benic, G.I., M. Elmasry, and C.H. Hammerle, *Novel digital imaging techniques to assess the outcome in oral rehabilitation with dental implants: a narrative review*. Clin Oral Implants Res, 2015. 26 Suppl 11: p. 86-96.
20. Vignoletti, F. and I. Abrahamsson, *Quality of reporting of experimental research in implant dentistry*. *Critical aspects in design, outcome assessment and model validation*. J Clin Periodontol, 2012. 39 Suppl 12: p. 6-27.
21. Sanz Martin, I., G.I. Benic, C.H. Hammerle, and D.S. Thoma, *Prospective randomized controlled clinical study comparing two dental implant types: volumetric soft tissue changes at 1 year of loading*. Clin Oral Implants Res, 2016. 27(4): p. 406-11.
22. Sanz-Martin, I., I. Sailer, C.H. Hammerle, and D.S. Thoma, *Soft tissue stability and volumetric changes after 5 years in pontic sites with or without soft tissue grafting: a retrospective cohort study*. Clin Oral Implants Res, 2016. 27(8): p. 969-74.
23. Meloni, S.M., S.A. Jovanovic, I. Urban, L. Canullo, M. Pisano, and M. Tallarico, *Horizontal Ridge Augmentation using GBR with a Native Collagen Membrane and 1:1 Ratio of Particulated Xenograft and Autologous Bone: A 1-Year Prospective Clinical Study*. Clin Implant Dent Relat Res, 2016.
24. Schwarz, F., A. Schmucker, and J. Becker, *Long-term outcomes of simultaneous guided bone regeneration using native and cross-linked collagen membranes after 8 years*. Clin Oral Implants Res, 2016.
25. Benic, G.I., D.S. Thoma, F. Munoz, I. Sanz Martin, R.E. Jung, and C.H. Hammerle, *Guided bone regeneration of peri-implant defects with particulated and block xenogenic bone substitutes*. Clin Oral Implants Res, 2016. 27(5): p. 567-76.
26. Araujo, M., E. Linder, and J. Lindhe, *Effect of a xenograft on early bone formation in extraction sockets: an experimental study in dog*. Clin Oral Implants Res, 2009. 20(1): p. 1-6.

27. Schmitt, C.M., H. Doering, T. Schmidt, R. Lutz, F.W. Neukam, and K.A. Schlegel, *Histological results after maxillary sinus augmentation with Straumann(R) BoneCeramic, Bio-Oss(R), Puros(R), and autologous bone. A randomized controlled clinical trial*. Clin Oral Implants Res, 2013. 24(5): p. 576-85.
28. Mir-Mari, J., H. Wui, R.E. Jung, C.H. Hammerle, and G.I. Benic, *Influence of blinded wound closure on the volume stability of different GBR materials: an in vitro cone-beam computed tomographic examination*. Clin Oral Implants Res, 2016. 27(2): p. 258-65.
29. Buser, D., V. Chappuis, M.M. Bornstein, J.G. Wittneben, M. Frei, and U.C. Belser, *Long-term stability of contour augmentation with early implant placement following single tooth extraction in the esthetic zone: a prospective, cross-sectional study in 41 patients with a 5- to 9-year follow-up*. J Periodontol, 2013. 84(11): p. 1517-27.
30. Naenni, N., D. Schneider, R.E. Jung, J. Husler, C.H. Hammerle, and D.S. Thoma, *Randomized clinical study assessing two membranes for guided bone regeneration of peri-implant bone defects: clinical and histological outcomes at 6 months*. Clin Oral Implants Res, 2016.
31. Schneider, D., F.E. Weber, U. Grunder, C. Andreoni, R. Burkhardt, and R.E. Jung, *A randomized controlled clinical multicenter trial comparing the clinical and histological performance of a new, modified polylactide-co-glycolide acid membrane to an expanded polytetrafluorethylene membrane in guided bone regeneration procedures*. Clin Oral Implants Res, 2014. 25(2): p. 150-8.
32. Urban, I.A., S.A. Jovanovic, and J.L. Lozada, *Vertical ridge augmentation using guided bone regeneration (GBR) in three clinical scenarios prior to implant placement: a retrospective study of 35 patients 12 to 72 months after loading*. Int J Oral Maxillofac Implants, 2009. 24(3): p. 502-10.
33. Simion, M., M. Baldoni, P. Rossi, and D. Zaffe, *A comparative study of the effectiveness of e-PTFE membranes with and without early exposure during the healing period*. Int J Periodontics Restorative Dent, 1994. 14(2): p. 166-80.
34. Araujo, M.G., M. Sonohara, R. Hayacibara, G. Cardaropoli, and J. Lindhe, *Lateral ridge augmentation by the use of grafts comprised of autologous bone or a biomaterial. An experiment in the dog*. J Clin Periodontol, 2002. 29(12): p. 1122-31.
35. von Arx, T., D.L. Cochran, J.S. Hermann, R.K. Schenk, and D. Buser, *Lateral ridge augmentation using different bone fillers and barrier membrane application. A histologic and histomorphometric pilot study in the canine mandible*. Clin Oral Implants Res, 2001. 12(3): p. 260-9.
36. Capelli, M., T. Testori, F. Galli, F. Zuffetti, A. Motroni, R. Weinstein, and M. Del Fabbro, *Implant-buccal plate distance as diagnostic parameter: a prospective cohort study on implant placement in fresh extraction sockets*. J Periodontol, 2013. 84(12): p. 1768-74.
37. Friberg, B. and T. Jemt, *Soft tissue augmentation in connection to dental implant treatment using a synthetic, porous material--a case series with a 6-month follow-up*. Clin Implant Dent Relat Res, 2012. 14(6): p. 872-81.

38. Schneider, D., P.R. Schmidlin, A. Philipp, B.M. Annen, V. Ronay, C.H. Hammerle, T. Attin, and R.E. Jung, *Labial soft tissue volume evaluation of different techniques for ridge preservation after tooth extraction: a randomized controlled clinical trial*. J Clin Periodontol, 2014. 41(6): p. 612-7.
39. Akcali, A., D. Schneider, F. Unlu, N. Bicakci, T. Kose, and C.H. Hammerle, *Soft tissue augmentation of ridge defects in the maxillary anterior area using two different methods: a randomized controlled clinical trial*. Clin Oral Implants Res, 2015. 26(6): p. 688-95.
40. Chappuis, V., O. Engel, K. Shahim, M. Reyes, C. Katsaros, and D. Buser, *Soft Tissue Alterations in Esthetic Postextraction Sites: A 3-Dimensional Analysis*. J Dent Res, 2015. 94(9 Suppl): p. 187S-93S.
41. Thoma, D.S., R.E. Jung, D. Schneider, D.L. Cochran, A. Ender, A.A. Jones, C. Gorlach, L. Uebersax, U. Graf-Hausner, and C.H. Hammerle, *Soft tissue volume augmentation by the use of collagen-based matrices: a volumetric analysis*. J Clin Periodontol, 2010. 37(7): p. 659-66.
42. Jemt, T. and U. Lekholm, *Single implants and buccal bone grafts in the anterior maxilla: measurements of buccal crestal contours in a 6-year prospective clinical study*. Clin Implant Dent Relat Res, 2005. 7(3): p. 127-35.
43. Windisch, S.I., R.E. Jung, I. Sailer, S.P. Studer, A. Ender, and C.H. Hammerle, *A new optical method to evaluate three-dimensional volume changes of alveolar contours: a methodological in vitro study*. Clin Oral Implants Res, 2007. 18(5): p. 545-51.
44. Fickl, S., D. Schneider, O. Zuhr, M. Hinze, A. Ender, R.E. Jung, and M.B. Hurzeler, *Dimensional changes of the ridge contour after socket preservation and buccal overbuilding: an animal study*. J Clin Periodontol, 2009. 36(5): p. 442-8.
45. Rebele, S.F., O. Zuhr, D. Schneider, R.E. Jung, and M.B. Hurzeler, *Tunnel technique with connective tissue graft versus coronally advanced flap with enamel matrix derivative for root coverage: a RCT using 3D digital measuring methods*. *Part II*. *Volumetric studies on healing dynamics and gingival dimensions*. J Clin Periodontol, 2014. 41(6): p. 593-603.
46. Thalmair, T., S. Fickl, D. Schneider, M. Hinze, and H. Wachtel, *Dimensional alterations of extraction sites after different alveolar ridge preservation techniques - a volumetric study*. J Clin Periodontol, 2013. 40(7): p. 721-7.

FIGURE LEGENDS

-Figure 1: 1a. Facial view of box shaped defects after the extraction of the mesial root of M1, the mesial root of P4, the distal root of P3 and booth roots of P2. 1b. Occlusal view 3 months after defect creation. 1c. Augmentation procedures after randomization mesial defect (D1) received the collagen native membrane alone.

Central defect (D2) received the combination therapy while the distal defect (D3) received the bone replacement graft.

-Figure 2: Three-dimensional reconstructions of STL files after optical scanning of models before tooth extraction (T1/yellow), before augmentation procedure (T2/green) and 3 months after the augmentation therapy (T3/grey).

-Figure 3: 3a. Outline of models before tooth extraction (T1/yellow), before augmentation procedure (T2/green) and 3 months after the augmentation therapy (T3/grey). 3b. STL image superimposition with the aid of image analysis software. Notice the different in ridge with from T1 to T2 and T3.

-Figure 4: Linear measurements performed to evaluate soft tissue changes. The dotted line represents the axis of the tooth, the most coronal line perpendicular to it link the facial and lingual gingival margin. Horizontal linear measurements are taken at the level of the gingival margin, 2,4 and 6mm below it.

-Table 1

Header: Vertical and Horizontal measurements at baseline (T1) and changes between T1-T2.

Footer: MBG, membrane group; BRG, bone replacement graft group; CBG, combination group; BW GM T1, baseline width at gingival margin in T1; BW 2,4,6mm T1, baseline width 2,4,6mm below gingival margin at T1; HC 4-6 T1-T2, Horizontal changes 4 and 6mm below the gingival margin from T1 to T2; VC T1-T2, vertical changes from T1 to T2; % Loss 4, 6mm, percentage of loss from the baseline horizontal values.

-Table 2:

Header: Vertical and Horizontal changes from T2 to T3.

Footer: MBG, membrane group; BRG, bone replacement graft group; CBG, combination group; HC 4-6 T2-T3, Horizontal changes 4 and 6mm below the gingival margin from T2 to T3; % Gain 4, 6mm, percentage of gain from the initial loss.

-Table 3:

Header: Vertical and Horizontal changes from T1 to T3.

Footer: MBG, membrane group; BRG, bone replacement graft group; CBG, combination group; HC 4-6 T1-T3, Horizontal changes 4 and 6mm below the gingival margin from T1 to T3; VC T1-T3, vertical changes from T1 to T3.

Table 1. Vertical and Horizontal measurements at baseline (T1) and changes between T1-T2 (n=9)									
DEFECT 1 (D1)	**BW GM T1**	**BW 2mm T1**	**BW 4mm T1**	**BW 6mm T1**	**HC 4mm**	**HC 6mm**	**VC**	**% Loss4mm**	**% Loss6mm**
MBG	4.34±0.24	6.10±0.35	7.07±0.41	8.56±0.65	3.07±0.25	1.73±0.42	3.22±0.51	43.42	20.21
BRG	5.02±0.50	6.52±0.89	7.27±0.24	8.15±0.45	2.18±0.43	1.70±0.27	2.98±0.38	29.98	20.85
CBG	5.30±0.88	6.72±0.43	7.47±0.14	7.54±1.32	4.32±0.70	1.53±0.40	2.57±0.52	57.83	20.29
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.729/0.245/0.300	0.353/0.246/0.758	0.426/0.146/0.384	0.657/0.288/0.459	0.425/0.487/0.130	0.988/0.909/0.922	0.519/0.555/0.948		
DEFECT 2 (D2)	**BW GM T1**	**BW 2mm T1**	**BW 4mm T1**	**BW 6mm T1**	**HC 4mmT1-T2**	**HC 6mm**	**VC**	**% Loss 4mm**	**% Loss 6mm**
MBG	5.05±1.7	7.36±0.56	8.12±0.46	8.91±1.19	4.03±0.43	3.76±1.22	2.57±0.35	49.63	42.19
BRG	5.05±1.6	6.83±1.09	7.61±0.57	7.87±0.39	4.52±0.78	2.04±0.6	2.66±1.76	59.39	25.92
CBG	5.6±0.16	8.20±0.94	9.40±1.16	10.61±0.62	3.35±0.15	3.75±0.2	2.08±0.21	35.63	35.34
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.989/0.688/0.696	0.494/0.345/0.151	0.416/0.103/0.101	0.191/0.080/0.065	0.400/0.828/0.579	0.234/0.994/0.236	0.778/0.439/0.319		
DEFECT 3 (D3)	**BW GM T1**	**BW 2mm T1**	**BW 4mm T1**	**BW 6mm T1**	**HC 4mmT1-T2**	**HC 6mm**	**VC**	**% Loss 4mm**	**% Loss 6mm**
MBG	8.80±1.7	9.91±1.3	10.07±0.72	10.26±1.07	6.5±2.25	3.34±0.92	2.86±1.84	64.54	32.55
BRG	5.15±2.1	8.25±0.85	9.07±0.30	9.69±0.77	5.43±3.47	3.24±2.02	1.99±2.34	59.86	33.43
CBG	6.09±1.27	8.27±0.93	9.56±0.14	10.09±0.87	5.04±2.88	3.49±0.77	2.41±0.82	52.71	34.58
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.086/0.066/0.494	0.081/0.101/0.975	0.073/0.257/0.312	0.535/0.831/0.662	0.746/0.625/0.907	0.933/0.833/0.830	0.576/0.740/0.788		

Table 2. Vertical and Horizontal changes from T2 to T3 (n=9)					
DEFECT 1 (D1)	**HC 4mm**	**HC 6mm**	**Mean changes**	**% Gain 4mm**	**% Gain 6mm**
MBG	0.59±0.69	0.75±0.08	0.47±0.34	19.21	43.35
BRG	1.76±1.18	2.25±1.37	0.79±0.67	80.73	132.35
CBG	1.89±1.60	1.75±0.69	0.87±0.69	43.75	114.37
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.586/0.323/0.590	0.152/0.229/0.736	0.049*/0.025*/0.546		
DEFECT 2 (D2)	**HC 4mm**	**HC 6mm**	**Mean changes**	**% Gain 4mm**	**% Gain 6mm**
MBG	0.57±0.19	0.54±0.58	0.11±0.31	14.14	14.36
BRG	1.26±0.89	1.14±1.67	1.01±0.91	27.87	55.88
CBG	1.18±0.51	1.05±0.07	0.98±0.49	35.22	28
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.166/0.246/0.896	0.397/0.492/0.934	0.001*/0.002*/0.833		
DEFECT 3 (D3)	**HC 4mm**	**HC 6mm**	**Mean changes**	**% Gain 4mm**	**% Gain 6mm**
MBG	0.41±1.18	0.40±1.19	0.24±0.72	6.3	11.97
BRG	1.18±0.17	0.58±1.29	1.04±0.92	21.73	17.9
CBG	1.79±0.55	1.68±0.41	0.86±0.56	35.51	48.13
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.358/0.098/0.458	0.848/0.172/0.276	0.026*/0.044*/0.507		

Table 3. Vertical and Horizontal changes from T1 to T3 (n=9)			
DEFECT 1 (D1)	**HC 4mm**	**HC 6mm**	**VC**
MBG	2.48±0.43	0.98±1.50	2.97±0.15
BRG	0.42±0.74	-0.52±1.1	2.15±1.03
CBG	2.43±1.20	-0.22±1.74	2.51±1.03
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.045*/0.955/0.031	0.219/0.397/0.614	0.169/0.500/0.370
DEFECT 2 (D2)	**HC 4mm**	**HC 6mm**	**VC**
MBG	3.4±0.63	3.22±1.81	2.91±0.66
BRG	3.59±2.27	0.57±1.14	2.32±1.90
CBG	2.17±0.66	2.71±0.05	2.16±1.61
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.491/0.861/0.435	0.246/0.746/0.189	0.934/0.693/0.640
DEFECT 3 (D3)	**HC 4mm**	**HC 6mm**	**VC**
MBG	6.11±2.23	2.94±0.94	2.94±1.56
BRG	1.72±1.66	2.66±0.73	2.48±2.46
CBG	3.08±2.3	1.81±0.75	1.95±0.36
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.074/0.147/0.520	0.730/0.157/0.312	0.753/0.456/0.713

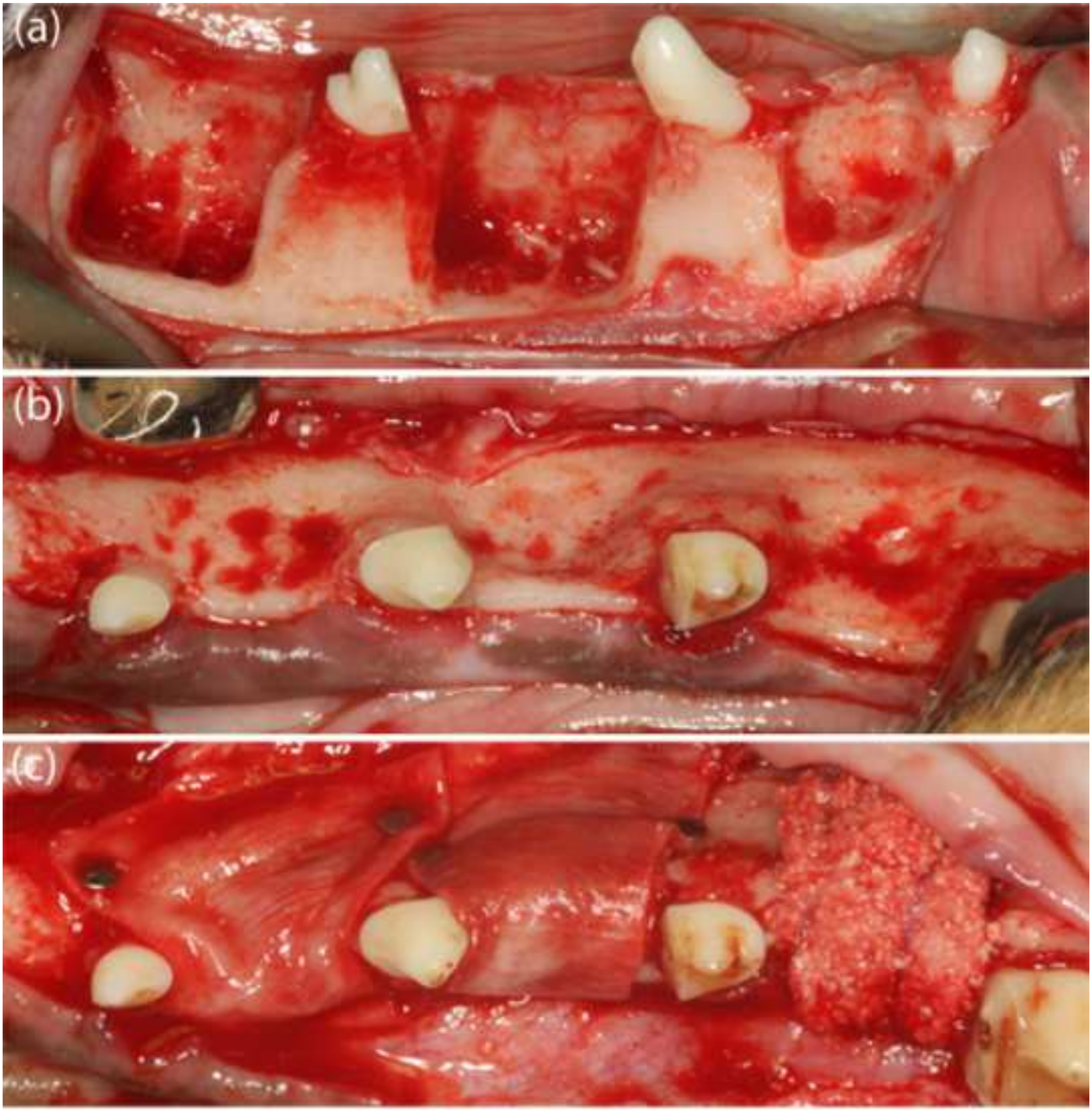

(a)
(b)
(c)

Figure 2

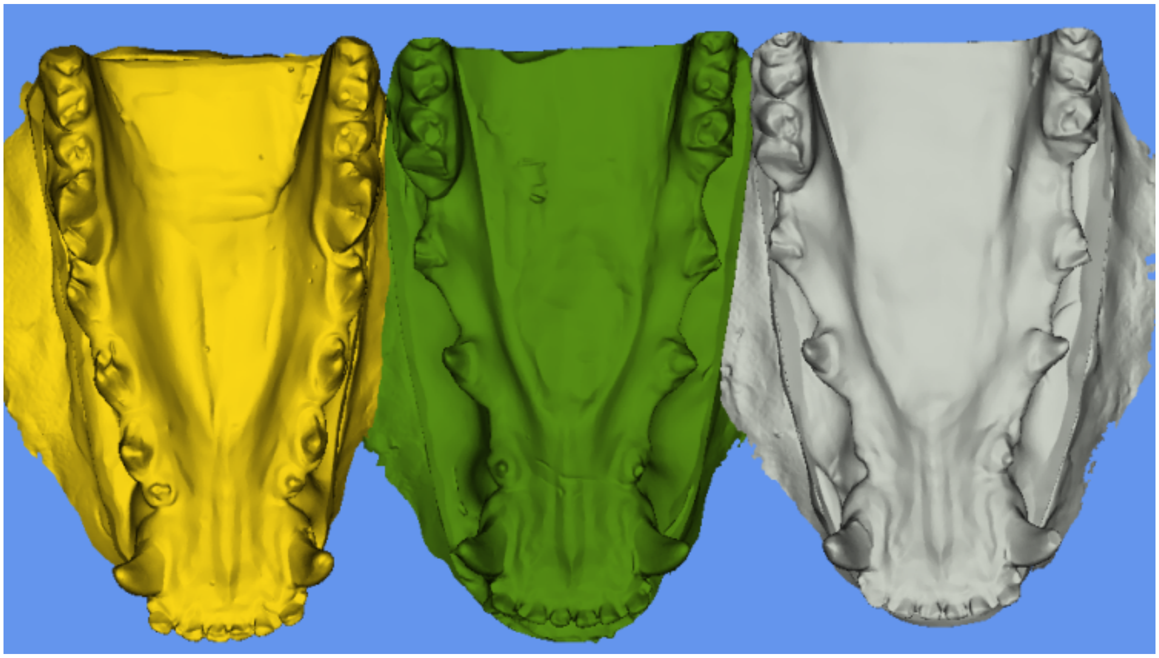


Figure 3

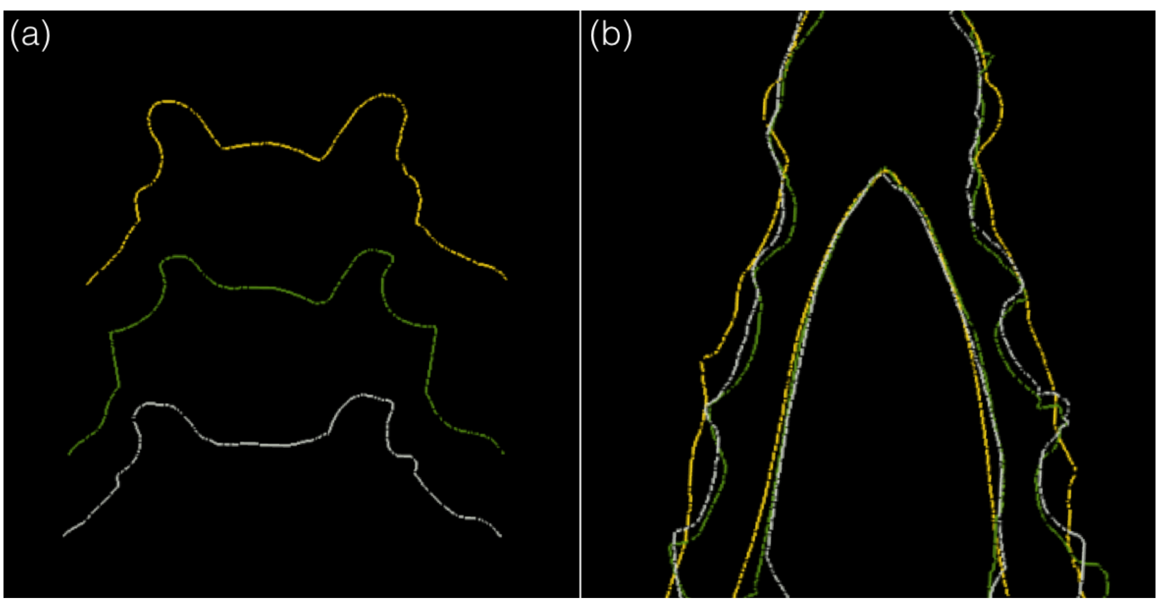
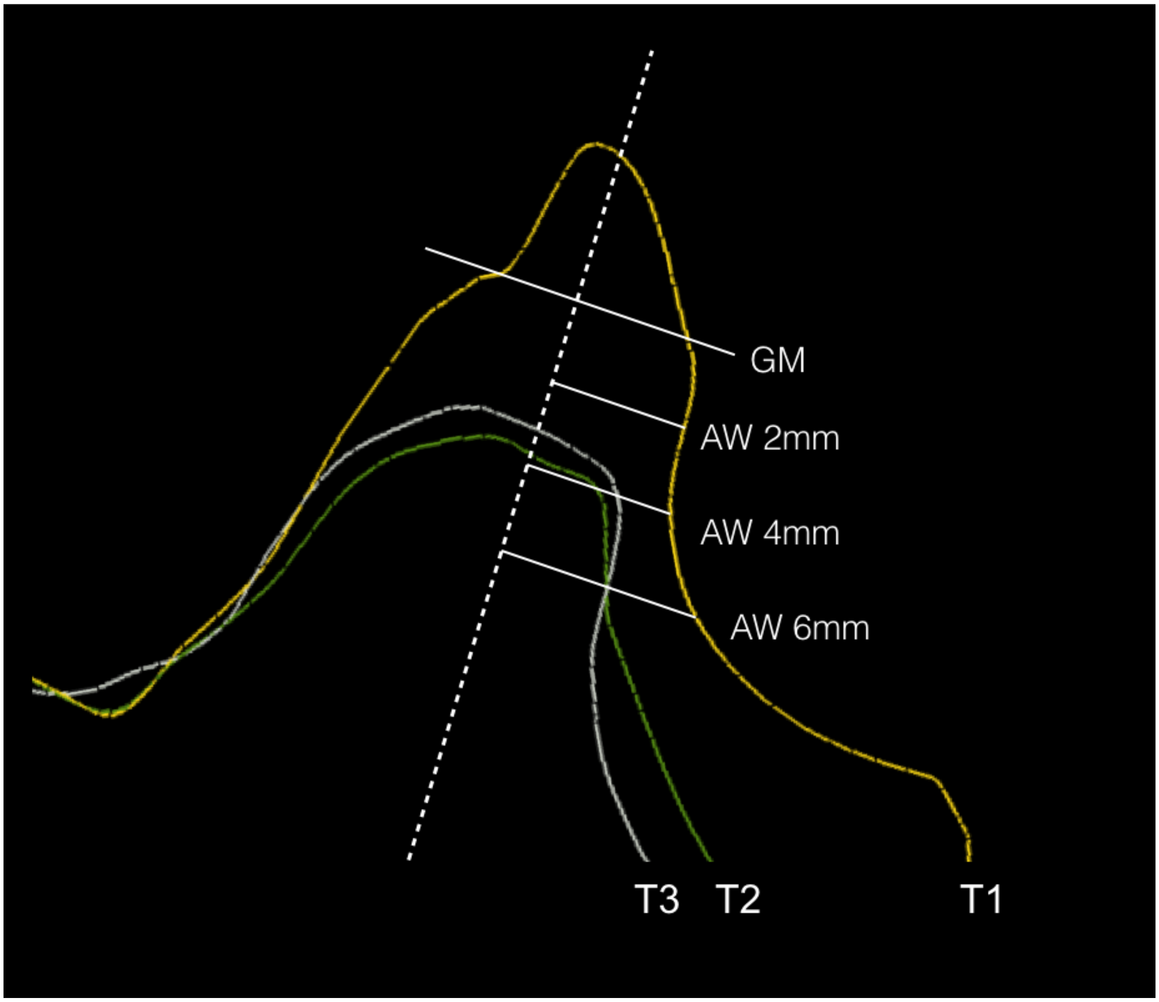


Figure 4



VII. DISCUSIÓN

Los resultados del estudio comparativo entre implantes cilíndricos y triangulares demostraron que los valores histomorfométricos verticales referentes a las dimensiones de la anchura biológica y a la distancia de hombro del implante al primer contacto hueso-implante así como la distancia de hombro del implante al aspecto coronal del la cresta ósea fueron similares para ambos grupos. El componente horizontal de la cresta ósea obtuvo valores similares en implantes T y C a la altura del hombro del implante y 1mm por debajo de él. En los aspectos más apicales (2, 3, 4 y 5mm) bajo el hombro del implante los implantes Test obtuvieron mejores resultados y de manera más acentuada en los implantes inmediatos.

El porcentaje de contacto hueso-implante (BIC), los contornos del tejido blando bucal así como el volumen de componentes de la muestra fueron similares en cuanto al porcentaje de hueso con un menor componente de Titanio en los implante Test.

La superposición de las imágenes de Micro CT y las imágenes del escaneado óptico de los modelos demostraron que la metodología utilizada fue capaz de aportar información en cuanto al volumen de tejido blando y óseo. El volumen de hueso vestibular fue mayor en los implantes triangulares aunque el diseño del implante no afectó al volumen de tejido blando. El modo de cicatrización (inmediato o diferido) demostró afectar el comportamiento e interacción entre volumen de tejido blando y óseo.

Análisis histológico

En cuanto a los valores de anchura de cresta, los resultados obtenidos tanto en implantes inmediatos como diferidos concuerdan con aquellos obtenidos en otras publicaciones con protocolos experimentales similares (De Santis, et al. 2015, Favero, et al. 2013). El hecho de que los implantes Test consiguiesen una mayor anchura de cresta en los aspectos más apicales indica que el espacio provisto en los implantes Test entre la cara vestibular del implante y el tejido óseo se rellenó de hueso y favoreció la anchura de hueso vestibular. Estos hallazgos se han replicado en la literatura en humanos con diferentes diseños de implantes (Ferrus, et al. 2010). En un estudio clínico controlado, se colocaron implantes en alveolos post extracción de premolares, caninos e incisivos. Al estudiar el grosor de la tabla vestibular se concluyó que esta era mayor en aquellos implantes colocados en zonas de caninos e incisivos donde la distancia entre el hombro del implante y la pared vestibular fue mayor.

Independientemente del diseño del implante, la colocación de los implantes no consiguió prevenir los cambios dimensionales en la cresta, lo que coincide con datos publicados en estudios experimentales con perros beagle y estudios clínicos en humanos (Botticelli, et al. 2004, Sanz, et al. 2010).

Los resultados en cuanto a la posición vertical de la cresta ósea, en los que no se encontraron diferencias entre Test y Control difieren que los publicados por Caneva y colaboradores (Caneva, et al. 2012). En un modelo experimental en perro beagle se colocaron implantes inmediatos de dos diámetros diferentes (3.3 y 5mm). Los autores reportaron menor pérdida ósea, y por lo tanto una posición vertical de la cresta más coronal, en los implantes con diámetro estrecho concluyendo que cuanto más cerca estaba la superficie del implante de la tabla vestibular mayor era la pérdida ósea. Las diferencias con los resultados de la presente investigación se pueden explicar por los diseños de implantes utilizados. Mientras que Caneva y cols. utilizaron implantes con un mismo diseño cervical y diferentes diámetros, el presente estudio utilizó implantes con un mismo diámetro y un diseño cervical modificado que daba un aspecto triangular a la porción coronal al reducir en los 4mm coronales 0.4mm en cada una de las caras del triángulo.

De igual manera, otro factor importante a tener en cuenta al valorar los resultados de este estudio son los pilares utilizados que pueden haber limitado el beneficio otorgado por el diseño cervical. Al haber utilizado el mismo diseño de pilar para implantes Test y Control, los pilares de cicatrización en los implantes Test sobresalían en las caras planas del triángulo actuando como un cambio de plataforma inverso. Estas limitaciones han sido solventadas en la versión comercial de estos implantes que portan pilares de cicatrización con un perfil cóncavo o diseño de tulipa.

Es importante destacar que los implantes utilizados fueron hechos específicamente para este estudio pre-clínico. El uso de prototipos de implantes es necesario para testar sus hipotéticas ventajas y realizar cambios si se detectan limitaciones en el diseño de los mismos.

En el presente estudio la reducción en cada una de las caras del triángulo fue de 0.4mm para implantes de un diámetro de 3.5mm, lo que contrasta con los 0,2mm para los implantes de 3.9 mm en la versión comercial.

Este aumento en la reducción vestibular de los implantes puede haber comprometido su resistencia a la fractura al haber dejado una porción de titanio demasiado delgada en los tres lados del triángulo. Esto puede explicar las fracturas que se produjeron durante la inserción de los mismos en huesos corticales que ocasionaban un elevado torque de inserción.

Es relevante señalar, de igual manera, que la conexión del prototipo de implante utilizado difiere con la versión comercial. Mientras que los implantes utilizados en el estudio experimental tenían un hexágono interno, la versión comercial del mismo presenta una conexión de cono Morse. La conexión del componente protético con el implante ha demostrado tener un marcado efecto sobre la preservación del hueso crestal en estudio clínicos en humanos, existiendo evidencia que indica que diseños cónicos en conexiones internas son capaces de limitar la pérdida inicial que ocurre después de la conexión (Pessoa, et al. 2016).

En cuanto a las diferencias entre implantes inmediatos y diferidos, existe limitada evidencia en la literatura científica que permita comparaciones histológicas. En un artículo recientemente publicado encontró una pérdida de hueso crestal y patrones de remodelado similares (Mainetti, et al. 2016). Mientras que en los implantes inmediatos la distancia del hombro del implante al primer contacto hueso implante disminuyó de 2.6mm a 1.2mm a los 3 meses, en los implantes diferidos los valores de la posición de la cresta aumentaron desde la colocación aunque su valor a los 3 meses fue similar al encontrado en implantes inmediatos.

Los hallazgos de la presente investigación demostraron que los implantes inmediatos presentaban mayores valores de I-BIC en los estadios iniciales de cicatrización que se redujeron en la evaluación a las 12 semanas. Investigaciones experimentales que han valorado la secuencia de cicatrización de implantes inmediatos han coincidido al encontrar cambios similares a los cambios observados de las 4 a las 12 semanas en los valores de I-BC e I-BIC (Vignoletti, et al. 2009)

Por otra parte, se ha de tener en cuenta que el gap creado tras la colocación de los implantes inmediatos puede necesitar un tiempo prolongado para rellenarse completamente por tejido óseo y los tiempos de cicatrización utilizados pueden ser insuficientes para valorar su relleno (Vignoletti & Sanz 2014).

De hecho, en ambos diseños de implantes, el espacio entre el hombro del implante y el primer contacto hueso implante se hallaba relleno de un tejido conectivo denso. La posición del hombro del implante en los alveolos post extracción, situado al nivel de la cortical vestibular puede haber influido en estos resultados. Estudios preclínicos con modelos similares al utilizado han concluido que la posición corono-apical del hombro del implante puede influir en el grado de remodelado que ocurre (Caneva, et al. 2010), concluyendo que una posición más apical del hombro del implante en implantes inmediatos puede compensar la pérdida inicial que ocurre y limitar de esta manera una sobre exposición de la superficie rugosa del implante.

Por otra parte, los valores hallados en esta investigación en cuanto a la posición de la cresta ósea son similares a los reportados en investigaciones experimentales (Araujo, et al. 2005).

Análisis mediante Micro CT

En lo que se refiere al análisis mediante Micro CT, el porcentaje de contacto hueso implante no fue diferente al comparar los implantes T y C. Esto era de esperar ya que se utilizó la misma superficie y el único componente que difería era el diseño cervical de los implantes. Aquellos factores que pueden influir en el % de BIC, como el grado de rugosidad, no variaron en los implantes utilizados (Smeets, et al. 2016, Wennerberg & Albrektsson 2010). Los valores de BIC reportados en este estudio, que tuvieron un rango de 48-57%, coinciden con los publicados en la literatura científica para superficies similares a la utilizada (Choi, et al. 2015, Mangano, et al. 2013).

El uso de imágenes de Micro CT para analizar el % de BIC ha sido recientemente validado como un método fiable para asesorar la interacción hueso-implante (Neldam & Pinholt 2014). Estudios comparativos han demostrado que el análisis de la integración a través de Micro CT posee un grado de variabilidad menor, al observar la totalidad de la muestra en 3 dimensiones, en comparación con técnicas histológicas convencionales que analizan cortes únicos (Bernhardt, et al. 2012).

Al analizar la fracción de volumen de tejido óseo sobre el volumen de tejido total de la muestra se observó que estos valores eran superiores para los implantes Test en los implantes diferidos (60.38±7.41 y 51.00±7.43) aunque no en los inmediatos. Los implantes Test mostraron en todas las localizaciones un menor componente de Titanio en la parte vestibular de la muestra.

Los valores superiores de BV/TV encontrados en los implantes test a los 12 meses, comparados con los controles cilíndricos, contrastan con el análisis del aspecto vestibular de la muestra, que se extendía 5mm mesio-ditalmente, y que no halló diferencias entre Test y Control.

Estas diferencias se pueden explicar por la metodología empleada para el análisis de estos componentes. Mientras que la fracción BV/TV se calculaba incluyendo un anillo circunferencial de la muestra del implante, en las mediciones bucales sólo se incluía la parte vestibular de la misma extendiéndose hacia mesial y distal 0,75mm respectivamente. Es probable por lo tanto que en los implantes diferidos se halla producido un relleno más consistente de los espacios creados en cada una de las caras del triángulo lo que le ha otorgado valores mayores de BV/TV. Esta ventaja no se ha replicado en el análisis de los volúmenes vestibulares de hueso, es posible que el haber extendido la región de análisis hacia mesial y distal las posibles diferencias entre implantes T y C se hayan disipado.

Las diferencias halladas entre implantes inmediatos y diferidos en estas variables responde a los diferentes patrones de cicatrización anteriormente explicados al valorar los resultados histológicos. Por lo tanto, dada la variabilidad existente y los factores relacionados con el relleno del GAP se explica que el relleno de los espacios creados en los implantes Test haya sido menor en los implantes inmediatos al compararlos con los diferidos.

El análisis de los componentes de la fracción bucal evaluó el porcentaje de hueso, titanio y "espacio vacío" y se realizó en dos volúmenes de interés; el volumen bucal y el volumen de hueso bucal. El "espacio vacío" es la manera del software de identificar estructuras como tejido conectivo, matriz osteoide o aire. Ya que el análisis de los diferentes componentes del tejido incluía en bucal un importante componente de espacio vacío que no correspondía con el tejido óseo se delineó el límite del contorno bucal del tejido óseo para crear un nuevo volumen de análisis denominado "volumen de hueso bucal". El análisis del mismo reveló valores similares en los diferentes componentes lo que permitió concluir que no existían diferencias en la estructura ósea de los implantes test y control.

La ausencia de diferencias entre T y C en el componente de hueso contrasta con las diferencias encontradas en el análisis histológico que halló un mayor grosor de cresta en los implante Test. De nuevo, la inclusión de un volumen mesial y distal al implante puede hacer incluido zonas donde el diseño del implante no ejercía una influencia detectable lo que puede explicar que las medidas lineales no se correlacionen con la volumétricas.

Sin embargo, el porcentaje de componente de Titanio en la parte bucal fue obviamente menor para los implantes Test en todas las localizaciones lo que valida, en parte, el objetivo del implante de proveer mayor espacio al tejido óseo.

El análisis del volumen óseo se ha usado en investigaciones recientes como alternativa a las mediciones lineales ya que permite evaluar un volumen de interés aportando información tridimensional que resulta clave para valorar las características del tejido óseo o el resultado de procedimientos regenerativos (Beck-Broichsitter, et al. 2015, de Barros, et al. 2016, Khobragade, et al. 2015).

Una investigación recientemente publicada ha validado esta tecnología para analizar tridimensionalmente los cambios óseos a nivel del hombro del implante (Becker, et al. 2016), concluyendo que la tecnología aplicada permitió valorar los cambios en el hueso crestal 360º alrededor del hombro del implante. Lamentablemente, los volúmenes de interés y la metodología difiere con el presente estudio, lo que impide establecer comparaciones.

El análisis de volúmenes de hueso a través de cortes bidimensionales obtenidos mediante tomografía computerizada ha demostrado ser una metodología fiable para estudiar los cambios en este tejido siempre que las imágenes tengan una resolución y un tamaño de voxel adecuado (Osechinskiy & Kruggel 2011, Sarve, et al. 2011). Las superposición de las imágenes 2D se realiza a través de programas informáticos que utilizan algoritmos matemáticos para crear reconstrucciones tridimensionales que se utilizan para los cálculos de volumen (Varnavas, et al. 2013, Varnavas, et al. 2015). Para asegurar, por lo tanto, la veracidad de estos análisis es fundamental utilizar imágenes de gran resolución y un elevado número de cortes, siendo el Micro CT la metodología de imagen mejor contrastada para este propósito.

Análisis del contorno del tejido blando

En cuanto al análisis del contorno de los tejidos blandos mediante la superposición de archivos STL provenientes del escaneado óptico de los modelos de escayola antes y después de la colocación de implantes, el diseño del implante no influyó en los cambios observados. Estos resultados parecen concluir que el diseño cervical del implante no tuvo un impacto sobre el contorno del tejido blando.

En los implantes inmediatos, al comparar el contorno del tejido antes y después de la extracción se apreció una reducción en altura y anchura evidentes. Estos hallazgos concuerdan con los publicados por Caneva y cols. (Caneva, et al. 2012) utilizando una metodología similar.

Al contrario de lo observado en los implante inmediatos, en los diferidos se observó una ganancia consistente en el contorno del tejido blando. Este hallazgo se puede explicar por el desplazamiento hacia vestibular del tejido que se realizó en estos implantes tras la incisión crestal.

El impacto del protocolo quirúrgico en el contorno del tejido blando observado mediante la superposición de las imágenes STL sugirió un análisis más profundo de estas observaciones.

Superposición de Micro CT y STL

Por lo explicado anteriormente, se desarrolló una metodología capaz de solventar las limitaciones del anterior análisis incluyendo información sobre el tejido óseo con el objetivo de analizar la posible reciprocidad entre ambos componentes y la interacción que ocurre entre ellos.

Para ello, las imágenes obtenidas con el análisis de Micro CT de las muestras se superpusieron a las imágenes del escaneado óptico (STL) utilizando referencias comunes a través de un software ya contrastado en mediciones volumétricas (Fickl, et al. 2009, Schneider, et al. 2011, Windisch, et al. 2007).

Esta metodología permitió estudiar el volumen vestibular de hueso, así como el volumen de tejido blando por encima y debajo del hombro del implante. De igual manera, se pudo analizar la interpelación entre los diferentes volúmenes y el impacto del protocolo quirúrgico y diseño cervical del implante.

Los resultados del análisis de volumen vestibular encontraron que los implantes Test obtuvieron un mayor volumen de hueso en todas las localizaciones menos en PM2. Estos hallazgos contrastan con los resultados volumétricos del análisis de volúmenes óseos mediante Micro CT. La razón de estas diferencias yace en las regiones analizadas; mientras el análisis de volumen óseo mediante Micro CT estudió un volumen de interés que se extendía 5mm mesio-distalmente, este último análisis que superponía Micro CT y STL se centro únicamente en los 3.5mm que corresponden a la anchura mesio-distal del cuerpo del implante siendo por lo tanto más probable que en esta zona el diseño cervical del implante tuviese un mayor efecto.

Los implantes Test colocados en PM2 no obtuvieron diferencias al compararlos con los controles. Esto se puede deber al grosor de la cortical vestibular en esta localización tras la ostectomía, ya que la cresta tenía una anchura buco-lingual inicial no superior a 5mm. El trauma quirúrgico puede haber ocasionado una pérdida de altura en corticales finas que haya igualado los volúmenes de hueso vestibular y por lo tanto impedido encontrar diferencias entre Test y Control.

El diseño cervical del implante no tuvo un impacto en el volumen de tejido blando sin embargo, el protocolo quirúrgico utilizado influyó en el comportamiento de los tejidos blandos y la interacción con el tejido óseo.

Como se ha explicado anteriormente, las diferencias en la cicatrización de implantes inmediatos y diferidos han recibido considerable atención aunque la evidencia existente hasta la fecha ha evaluado su comportamiento únicamente mediante mediciones histológicas que limitan el análisis del tejido a una o dos secciones por implante (Passoni, et al. 2016, Yi, et al. 2016).

En el presente estudio las cuatro localizaciones utilizadas obtuvieron resultados diferentes dada su heterogeneidad anatómica. En M1, que representaba la cresta de mayor anchura en implantes diferidos al comienzo del estudio, el volumen de hueso fueron superior al hallado en PM2. De igual manera, los implantes inmediatos colocados en los alveolos de PM4, que presentaba mayores dimensiones que PM3, obtuvieron mayor volumen de hueso vestibular.

Fue interesante observar como en aquellas zonas donde el volumen de hueso vestibular era menor, el volumen de tejido blando debajo del hombro del implante era mayor. Estos resultados concuerdan con los reportados en una publicación reciente que evaluó tridimensionalmente el comportamiento de los tejidos blandos y duros después de la extracción y antes de la colocación de implantes diferidos (Chappuis, et al. 2015). Los autores reportaron un engrosamiento espontáneo del tejido de manera espontánea que multiplicaba por siete sus dimensiones tras la extracción dentaria demostrando que la reabsorción de la cortical vestibular era ocupada por tejido blando produciendo un engrosamiento del mismo.

De manera similar, los implantes diferidos y en particular aquellos colocados en M1 obtuvieron un mayor volumen de tejido blando por encima del hombro del implante. El impacto del volumen de tejido en esta localización es de una gran importancia ya que tiene un influencia contrastada sobre el contorno del proceso alveolar y el color de la mucosa peri-implataria (Bressan, et al. 2011, Jung, et al. 2008). Esto puede justificar los resultados de investigaciones recientemente publicadas que han concluido que los implantes diferidos presentan mejores resultados estéticos al compararlos con implantes inmediatos (Tonetti, et al. 2017). Los resultados de este estudio, por lo tanto, parecen indicar que los implantes inmediatos necesitan un mayor volumen de tejido vestibular en la zona de transición del hombro del implante al margen gingival para poder compensar los cambios dimensionales que ocurren tras la extracción dentaria. De hecho, una revisión sistemática recientemente publicada ha concluido que los injertos de tejido conectivo autólogo fueron capaces de engrosar el tejido peri-implantario y mejorar el contorno del proceso alveolar aunque su impacto en la estética precisa de más estudios que lo evalúen de manera objetiva (Lee, et al. 2016).

Al valorar el resultado de las correlaciones entre el volumen de tejido óseo y el volumen de tejido blando en los implante inmediatos, la posición de la cresta ósea tuvo un impacto evidente en el volumen de tejido óseo por encima del hombro del implante, la altura de tejido y el grosor del tejido a la altura del hombro del implante. Estos resultados hacen énfasis en la necesidad de estrategias clínicas que minimicen los cambios dimensionales que ocurren en la tabla vestibular tras la extracción dentaria y colocación de implantes inmediatos (Sanz, et al. 2016).

Por el contrario la posición de la cresta ósea en implantes diferidos no influyó en los parámetros anteriormente mencionados para los implantes inmediatos lo que demuestra el impacto del protocolo quirúrgico en la interacción entre tejido blando y tejido duro. Se podría hipotetizar que el desplazamiento hacia vestibular del tejido blando tras la incisión crestal enmascaró los posibles cambios óseos a nivel del hombro del implante en los implantes diferidos.

Un estudio aleatorizado ha testado recientemente esta hipótesis en 22 pacientes seguidos durante un periodo de 18 meses. Todos los pacientes recibieron implantes diferidos en los que se encontraron dehiscencias óseas vestibulares menores de

5mm que se aleatorizaron al grupo de aumento mediante técnicas regenerativas convencionales o al grupo de cicatrización espontánea (Test). Todos los implantes demostraron condiciones estables y salud peri-implantaria en las citas de seguimiento aunque los implantes en el grupo Test presentaron una mayor pérdida ósea vertical vestibular (Jung, et al. 2016).

Es evidente que se necesita más evidencia para comprender la interacción entre el tejido óseo y el tejido blando y su impacto en la estética y el mantenimiento de los niveles óseos peri-implantarios.

Análisis del contorno del tejido blando tras regeneración ósea lateral

En cuanto al estudio comparativo de técnicas regenerativas, los resultados demostraron que la extracción y creación de defectos produjo un colapso horizontal y vertical significativo en el reborde alveolar. La anatomía del defecto tuvo un impacto claro sobre el aumento conseguido. Además, el aumento del contorno del reborde fue superior para los grupos BRG y CBG, sin diferencias entre ambos. Sin embargo, la anchura del proceso alveolar después de la terapia regenerativa no alcanzó los valores previos a la extracción dentaria.

En cuanto la porcentaje de contorno horizontal recuperado después de la creación de los defectos, el BRG recuperó el 42% de la anchura perdida a 4mm bajo el margen gingival y 69% a 6mm bajo el margen gingival. El CBG recuperó 37% y 63% a 4 y 6mm bajo el margen gingival respectivamente. En el MBG esos valores fueron de 13% y 22%.

La ganancia media de los tres defectos regenerados (D1, D2 y D3) después de los procedimientos regenerativos favoreció al BRG y CBG al compararlo con el MBG (0.94mm, 0.90mm y 0.27mm).

La menor ganancia observada en el MBG se puede explicar por la falta de un sustituto óseo que estabilizase el coágulo y limitase el colapso de la membrana sobre el defecto. Las propiedades de la membrana de colágeno nativo utilizado favorecen la adaptación sobre los márgenes de la herida, lo que la otorga un sencillo manejo. Como contrapartida, esta membrana carece de estabilidad dimensional o capacidad de mantenimiento del espacio por lo que necesita de sustitutos óseos que actúen como matriz y permitan estabilizar el coágulo (Meloni, et al. 2016, Schwarz, et al. 2016). Es estudios experimentales con diseños similares, el uso de sustitutos óseos impidió el colapso de la membrana sobre el defecto maximizando la cantidad de tejido regenerando (Benic, et al. 2016).

La ausencia de diferencias entre BRG y CBG puede haber ocurrido como consecuencia de la lenta tasa de reabsorción del xenoinjerto utilizado (Araujo, et al. 2009, Schmitt, et al. 2013). En este estudio, el uso de un xenoinjerto fue capaz de limitar el colapso del tejido blando sobre el defecto independientemente del uso de una membrana o no. El efecto de la membrana reabsorbible sobre los tejidos sólo se puede asesorar de manera histológica, en este análisis el hecho de que el biomaterial haya sido realmente sustituido por nuevo hueso o esté embebido en el tejido blando no puede afectar el resultado del análisis realizado.

Los resultados derivados del análisis del presente estudio contrastan con los publicados recientemente referentes a el beneficio de estabilizar las membranas de colágeno con pines de titanio limitando de esta manera la compresión del biomaterial durante el cierre de la herida y la consecuente reducción de volumen (Mir-Mari, et al. 2016). Estas discrepancias se pueden explicar por las diferencias en los métodos analíticos utilizados, ya que el estudio anteriormente mencionado utilizó un modelo in-vitro de mandíbulas de cerdo y análisis mediante CBCT antes e inmediatamente después de la técnica regenerativa.

Los resultados clínicos del uso de membranas reabsorbibles combinadas con el uso de sustitutos óseos ha demostrado ser capaz de reconstruir el proceso alveolar de manera predecible y conseguir resultados estéticos estables a largo plazo (Buser, et al. 2013).

Esta investigación evaluó defectos horizontales que presentaban amplias distancias mesio-distales, lo que les hacía más similares a los defectos creados después de la extracción de varias piezas dentales contiguas en los que existe un pequeño componente vertical además del colapso horizontal.

El objetivo de las técnicas de aumento no fue conseguir ningún tipo de crecimiento vertical si no recuperar los contornos horizontales de la cresta, a pesar de ello no se consiguió la reconstrucción completa del proceso alveolar 4 y 6mm bajo el margen gingival.

Esta reconstrucción parcial del proceso alveolar puede haber resultado por el tipo de membranas utilizadas. Las membranas no reabsorbibles, con capacidad de mantener el espacio, han demostrado buenos resultados en la reconstrucción de defectos verticales (Naenni, et al. 2016, Schneider, et al. 2014, Urban, et al. 2009), aunque su uso puede derivar en complicaciones originadas por la exposición de la membrana durante el periodo de cicatrización (Simion, et al. 1994).

En cuanto a la metodología aplicada, el modelo experimental utilizado ha sido contratado en numerosas investigaciones para testar diferentes técnicas regenerativas y biomateriales (Araujo, et al. 2002, von Arx, et al. 2001).

La variabilidad encontrada en los resultados presentados coincide con estudios que han valorado procedimientos de aumento de cresta con modelos y herramientas experimentales similares (Thoma, et al. 2010).

Estudios en humanos también han reportado alta variabilidad en la medición de los cambios del contorno del reborde alveolar tras el aumento lateral de cresta mediante bloques de hueso autólogo en el sector anterior del maxilar (Jemt & Lekholm 2003, Jemt & Lekholm 2005). En esta serie de estudios los cambios en el contorno de la cresta se analizaron antes y después del procedimiento de aumento, antes de la colocación de implantes, antes de la cirugía de conexión del pilar, en el momento de colocación de la corona y 1 y 5 años después. Los autores observaron un marcado aumento en el grosor de la cresta después de la cirugía de aumento y después de la cirugía de conexión por el desplazamiento bucal del tejido blando y la colocación del pilar. En la presente investigación sólo se estudiaron los cambios después del procedimiento regenerativo por lo que no se pueden establecer comparaciones, aunque se ha de tener en cuenta que la cirugía de conexión podría haber aumentado el grosor de la cresta y quizás haberlo aproximado más a los valores antes de la extracción 4 y 6mm bajo el margen gingival.

Relevancia clínica

El uso de implantes con modificaciones del diseño cervical puede ayudar a mejorar las dimensiones de la tabla vestibular en implantes inmediatos y diferidos. Los cambios en la geometría del implante no mostraron un beneficio sobre los tejidos blandos lo que parece indicar que las técnicas de manejo de tejido blando tienen una importancia destacada para restaurar los contornos del reborde alveolar en casos donde la apariencia de la restauración sea relevante. Estos aumentos se pueden realizar mediante el uso de injertos de tejido blando en implantes inmediatos o mediante el desplazamiento del tejido crestal hacia vestibular en los implantes diferidos.

El volumen de tejido blando y duro debajo del hombro del implante parecen estar interrelacionados, ya que los implantes con volumen limitado de tejido óseo vestibular demostraron tener mayor volumen de tejido blando y viceversa. El volumen de tejido blando en la zona de transición del hombro del implante al margen gingival demostró tener mayor variabilidad y ser más susceptible a los protocolos quirúrgicas de colocación de implantes.

En cuanto a las técnicas de aumento del reborde alveolar, la utilización de membranas y biomateriales obtuvo mejores resultados en cuanto a la ganancia media aunque sólo fue capaz de reconstruir aproximadamente 2/3 del volumen perdido. En casos en los que se desee recuperar completamente el contorno del reborde alveolar se deben usar, así mismo, técnicas mucogingivales capaces de aumentar el volumen del tejido blando.

Limitaciones

Los estudios presentados como parte de esta trabajo de tesis tienen limitaciones inherentes a la aplicabilidad de los resultados derivados de estudios preclínicos en animales (Vignoletti & Abrahamsson 2012).

Es importante señalar que a pesar de las evidentes ventajas del modelo animal que permitían analizar en una misma hemimandíbula implantes inmediatos y diferidos, las situaciones clínicas eran diferentes. Los implantes diferidos colocados en M1 presentaban una cresta de amplias dimensiones y abundante tejido queratinizado, a diferencia de lo ocurrido en PM2. Estas situaciones clínicas eran diferentes de lo que se hubiese encontrado si se hubiesen extraído las raíces mesiales en PM3 y PM4 que representaba las zonas donde se colocaban los implantes inmediatos.

Así mismo, se ha de tener en cuenta que los resultados histológicos y del análisis de Micro CT derivan de muestras estudiadas después del sacrificio de los animales lo que impide realizar un análisis dinámico de los cambios en los tejidos.

Imágenes tridimensionales del tejido óseo y blando anteriores a la extracción hubiesen permitido calcular el porcentaje de tejido perdido después de las extracciones y estudiar de manera más informativa los cambios en el tejido blando y óseo.

Por otra parte los implantes estudiados no llevaron ningún tipo de restauración provisional o definitiva que hubiese ayudado a analizar un escenario más próximo a las situaciones clínicas reales. En la parte de procedimientos regenerativos hubiese sido útil analizar el porcentaje del contorno que se recupera tras la colocación de implantes y restauraciones.

Implicaciones para futuras investigaciones

La presente investigación ha supuesto un paso adelante en la comprensión de la interacción entre tejido blando y óseo en implantes, el impacto de la geometría y protocolos quirúrgicos. Sin embargo, las limitaciones de los estudios animales obligan a contrastar esos hallazgos en estudios clínicos en humanos que permitan analizar de manera dinámica los cambios que ocurren después de la colocación de implantes.

Esto puede aportar información en cuanto a la importancia de las técnicas de aumento de tejido blando así como las técnicas regenerativas óseas en la consecución de resultados harmónicos con los tejidos adyacentes. De igual manera, aportar información sobre la importancia de esta técnicas en el mantenimiento de la salud peri-implantaria.

Sería pertinente también conocer cuál es la relación entre tejidos blandos gruesos y un hueso subyacente fino además de si un tejido blando grueso puede compensar la falta de hueso en el aspecto bucal de los implantes

Desde el punto de vista científico, sería relevante poder aplicar la tecnología desarrollada para superponer imágenes de Micro CT y STL a archivos DICOM convencionales obtenidos tras realizar escáneres de pacientes. Para ello es necesario testar la reproducibilidad de esta técnica ya que el Micro CT es capaz de aportar un alto número de cortes lo que otorga una elevada definición a las reconstrucciones tridimensionales y fiabilidad a los cálculos de volumen. Las imágenes DICOM obtenidas de escáneres tomográficos convencionales no suelen tener este grado de definición y además se acompañan frecuentemente de artefactos que impiden realizar mediciones de volumen fiables.

VIII. CONCLUSIONES

Las conclusiones de la serie de trabajos realizados son las siguientes:

1. El uso de implantes de diseño cervical triangular fue efectivo a la hora de conseguir un mayor grosor de cresta. No existieron diferencias significativas en cuanto a las medidas histomorfométricas verticales de tejido blando y óseo.

2. Los implantes Test presentaron un menor componente de Titanio en el análisis del volumen de la muestra mediante Micro CT con porcentajes similares de BIC entre Test y Control.

3. El diseño del implante no afecto al contorno del tejido blando aunque si lo hizo el protocolo quirúrgico observando en los implantes inmediatos un colapso horizontal y vertical del tejido blando mientras que en los diferidos se produjo una ganancia horizontal tras desplazar el tejido crestal hacia vestibular.

4. La metodología desarrollada para la superposición de imágenes de Micro CT y STL fue capaz de medir los volúmenes de tejido óseo y blando por encima y debajo del hombro del implante.

5. Los implantes Test presentaron mayor volumen óseo vestibular que los controles en tres de las cuatro localizaciones estudiadas.

6. El protocolo quirúrgico tuvo un influencia directa en la interacción entre el volumen de tejido blando por encima y debajo del hombro del implante con el volumen de tejido óseo.

7. Las técnicas regenerativas que utilizaron sustitutos óseos o sustitutos combinados con membranas obtuvieron los mejores resultados aunque sólo fueron capaces de reconstruir el 60% de total del contorno del reborde antes de la extracción.

IX. REFERENCIAS

Abrahamsson, I., Berglundh, T., Wennstrom, J. & Lindhe, J. (1996) The peri-implant hard and soft tissues at different implant systems. A comparative study in the dog. *Clin Oral Implants Res* **7**: 212-219.

Abrahamsson, I., Zitzmann, N. U., Berglundh, T., Wennerberg, A. & Lindhe, J. (2001) Bone and soft tissue integration to titanium implants with different surface topography: An experimental study in the dog. *Int J Oral Maxillofac Implants* **16**: 323-332.

Annen, B. M., Ramel, C. F., Hammerle, C. H. & Jung, R. E. (2011) Use of a new cross-linked collagen membrane for the treatment of peri-implant dehiscence defects: A randomised controlled double-blinded clinical trial. *Eur J Oral Implantol* **4**: 87-100.

Araujo, M., Linder, E. & Lindhe, J. (2009) Effect of a xenograft on early bone formation in extraction sockets: An experimental study in dog. *Clin Oral Implants Res* **20**: 1-6.

Araujo, M. G. & Lindhe, J. (2005) Dimensional ridge alterations following tooth extraction. An experimental study in the dog. *J Clin Periodontol* **32**: 212-218.

Araujo, M. G., Sonohara, M., Hayacibara, R., Cardaropoli, G. & Lindhe, J. (2002) Lateral ridge augmentation by the use of grafts comprised of autologous bone or a biomaterial. An experiment in the dog. *J Clin Periodontol* **29**: 1122-1131.

Araujo, M. G., Sukekava, F., Wennstrom, J. L. & Lindhe, J. (2005) Ridge alterations following implant placement in fresh extraction sockets: An experimental study in the dog. *J Clin Periodontol* **32**: 645-652.

Beck-Broichsitter, B. E., Garling, A., Koehne, T., Barvencik, F., Smeets, R., Mehl, C., Jeschke, A., Wiltfang, J. & Becker, S. T. (2015) 3d-tracking the regenerative potential of the mandible with micro-cts. *Oral Maxillofac Surg* **19**: 29-35.

Becker, K., Klitzsch, I., Stauber, M. & Schwarz, F. (2016) Three-dimensional assessment of crestal bone levels at titanium implants with different abutment microstructures and insertion depths using micro-computed tomography. *Clin Oral Implants Res*.

Becker, K., Stauber, M., Schwarz, F. & Beissbarth, T. (2015) Automated 3d-2d registration of x-ray microcomputed tomography with histological sections for dental implants in bone using chamfer matching and simulated annealing. *Comput Med Imaging Graph* **44**: 62-68.

Belser, U. C., Grutter, L., Vailati, F., Bornstein, M. M., Weber, H. P. & Buser, D. (2009) Outcome evaluation of early placed maxillary anterior single-tooth implants using objective esthetic criteria: A cross-sectional, retrospective study in 45 patients with a 2- to 4-year follow-up using pink and white esthetic scores. *J Periodontol* **80**: 140-151.

Benic, G. I., Bernasconi, M., Jung, R. E. & Hammerle, C. H. (2017) Clinical and radiographic intra-subject comparison of implants placed with or without guided bone regeneration: 15-year results. *J Clin Periodontol* **44**: 315-325.

Benic, G. I., Elmasry, M. & Hammerle, C. H. (2015) Novel digital imaging techniques to assess the outcome in oral rehabilitation with dental implants: A narrative review. *Clin Oral Implants Res* **26 Suppl 11**: 86-96.

Benic, G. I. & Hammerle, C. H. (2014) Horizontal bone augmentation by means of guided bone regeneration. *Periodontol 2000* **66**: 13-40.

Benic, G. I., Jung, R. E., Siegenthaler, D. W. & Hammerle, C. H. (2009) Clinical and radiographic comparison of implants in regenerated or native bone: 5-year results. *Clin Oral Implants Res* **20**: 507-513.

Benic, G. I., Thoma, D. S., Munoz, F., Sanz Martin, I., Jung, R. E. & Hammerle, C. H. (2016) Guided bone regeneration of peri-implant defects with particulated and block xenogenic bone substitutes. *Clin Oral Implants Res* **27**: 567-576.

Benic, G. I., Wolleb, K., Sancho-Puchades, M. & Hammerle, C. H. (2012) Systematic review of parameters and methods for the professional assessment of aesthetics in dental implant research. *J Clin Periodontol* **39 Suppl 12**: 160-192.

Berglundh, T. & Lindhe, J. (1996) Dimension of the periimplant mucosa. Biological width revisited. *J Clin Periodontol* **23**: 971-973.

Bernhardt, R., Kuhlisch, E., Schulz, M. C., Eckelt, U. & Stadlinger, B. (2012) Comparison of bone-implant contact and bone-implant volume between 2d-histological sections and 3d-srmicroct slices. *Eur Cell Mater* **23**: 237-247; discussion 247-238.

Bernhardt, R., Scharnweber, D., Muller, B., Thurner, P., Schliephake, H., Wyss, P., Beckmann, F., Goebbels, J. & Worch, H. (2004) Comparison of microfocus- and synchrotron x-ray tomography for the analysis of osteointegration around ti6al4v implants. *Eur Cell Mater* **7**: 42-51; discussion 51.

Botticelli, D., Berglundh, T. & Lindhe, J. (2004) Hard-tissue alterations following immediate implant placement in extraction sites. *J Clin Periodontol* **31**: 820-828.

Braut, V., Bornstein, M. M., Belser, U. & Buser, D. (2011) Thickness of the anterior maxillary facial bone wall-a retrospective radiographic study using cone beam computed tomography. *Int J Periodontics Restorative Dent* **31**: 125-131.

Bressan, E., Paniz, G., Lops, D., Corazza, B., Romeo, E. & Favero, G. (2011) Influence of abutment material on the gingival color of implant-supported all-ceramic restorations: A prospective multicenter study. *Clin Oral Implants Res* **22**: 631-637.

Buser, D., Chappuis, V., Bornstein, M. M., Wittneben, J. G., Frei, M. & Belser, U. C. (2013) Long-term stability of contour augmentation with early implant placement following single tooth extraction in the esthetic zone: A prospective, cross-sectional study in 41 patients with a 5- to 9-year follow-up. *J Periodontol* **84**: 1517-1527.

Buser, D., Janner, S. F., Wittneben, J. G., Bragger, U., Ramseier, C. A. & Salvi, G. E. (2012) 10-year survival and success rates of 511 titanium implants with a sandblasted and acid-etched surface: A retrospective study in 303 partially edentulous patients. *Clin Implant Dent Relat Res* **14**: 839-851.

Buser, D., Martin, W. & Belser, U. C. (2004) Optimizing esthetics for implant restorations in the anterior maxilla: Anatomic and surgical considerations. *Int J Oral Maxillofac Implants* **19 Suppl**: 43-61.

Caneva, M., Botticelli, D., Morelli, F., Cesaretti, G., Beolchini, M. & Lang, N. P. (2012) Alveolar process preservation at implants installed immediately into extraction sockets using deproteinized bovine bone mineral - an experimental study in dogs. *Clin Oral Implants Res* **23**: 789-796.

Caneva, M., Botticelli, D., Rossi, F., Cardoso, L. C., Pantani, F. & Lang, N. P. (2012) Influence of implants with different sizes and configurations installed immediately into extraction sockets on peri-implant hard and soft tissues: An experimental study in dogs. *Clin Oral Implants Res* **23**: 396-401.

Caneva, M., Salata, L. A., de Souza, S. S., Baffone, G., Lang, N. P. & Botticelli, D. (2010) Influence of implant positioning in extraction sockets on osseointegration: Histomorphometric analyses in dogs. *Clin Oral Implants Res* **21**: 43-49.

Cardaropoli, G., Araujo, M., Hayacibara, R., Sukekava, F. & Lindhe, J. (2005) Healing of extraction sockets and surgically produced - augmented and non-augmented - defects in the alveolar ridge. An experimental study in the dog. *J Clin Periodontol* **32**: 435-440.

Chappuis, V., Araujo, M. G. & Buser, D. (2017) Clinical relevance of dimensional bone and soft tissue alterations post-extraction in esthetic sites. *Periodontol 2000* **73**: 73-83.

Chappuis, V., Engel, O., Shahim, K., Reyes, M., Katsaros, C. & Buser, D. (2015) Soft tissue alterations in esthetic postextraction sites: A 3-dimensional analysis. *J Dent Res* **94**: 187S-193S.

Chen, S. T. & Buser, D. (2014) Esthetic outcomes following immediate and early implant placement in the anterior maxilla--a systematic review. *Int J Oral Maxillofac Implants* **29 Suppl**: 186-215.

Choi, J. Y., Moon, I. S., Yun, J. H., Park, K. H., Huh, J. K. & Lee, D. W. (2015) Effects of thread size in the implant neck area on peri-implant hard and soft tissues: An animal study. *Clin Oral Implants Res*.

Cosyn, J., Eghbali, A., Hermans, A., Vervaeke, S., De Bruyn, H. & Cleymaet, R. (2016) A 5-year prospective study on single immediate implants in the aesthetic zone. *J Clin Periodontol* **43**: 702-709.

Cosyn, J., Hooghe, N. & De Bruyn, H. (2012) A systematic review on the frequency of advanced recession following single immediate implant treatment. *J Clin Periodontol* **39**: 582-589.

Cuijpers, V. M., Jaroszewicz, J., Anil, S., Al Farraj Aldosari, A., Walboomers, X. F. & Jansen, J. A. (2014) Resolution, sensitivity, and in vivo application of high-resolution computed tomography for titanium-coated polymethyl methacrylate (pmma) dental implants. *Clin Oral Implants Res* **25**: 359-365.

de Barros, R. R., Novaes, A. B., Jr., de Carvalho, J. P. & de Almeida, A. L. (2016) The effect of a flapless alveolar ridge preservation procedure with or without a xenograft on buccal bone crest remodeling compared by histomorphometric and microcomputed tomographic analysis. *Clin Oral Implants Res*.

De Bruyckere, T., Eghbali, A., Younes, F., De Bruyn, H. & Cosyn, J. (2015) Horizontal stability of connective tissue grafts at the buccal aspect of single implants: A 1-year prospective case series. *J Clin Periodontol* **42**: 876-882.

de Faria Vasconcelos, K., Dos Santos Corpas, L., da Silveira, B. M., Laperre, K., Padovan, L. E., Jacobs, R., de Freitas, P. H., Lambrichts, I. & Boscolo, F. N. (2016) Microct assessment of bone microarchitecture in implant sites reconstructed with autogenous and xenogenous grafts: A pilot study. *Clin Oral Implants Res*.

de Sanctis, M., Vignoletti, F., Discepoli, N., Munoz, F. & Sanz, M. (2010) Immediate implants at fresh extraction sockets: An experimental study in the beagle dog comparing four different implant systems. Soft tissue findings. *J Clin Periodontol* **37**: 769-776.

De Santis, E., Lang, N. P., Favero, G., Beolchini, M., Morelli, F. & Botticelli, D. (2015) Healing at mandibular block-grafted sites. An experimental study in dogs. *Clin Oral Implants Res* **26**: 516-522.

Derks, J., Schaller, D., Hakansson, J., Wennstrom, J. L., Tomasi, C. & Berglundh, T. (2016) Peri-implantitis - onset and pattern of progression. *J Clin Periodontol* **43**: 383-388.

Derks, J. & Tomasi, C. (2015) Peri-implant health and disease. A systematic review of current epidemiology. *J Clin Periodontol* **42 Suppl 16**: S158-171.

Donath, K. & Breuner, G. (1982) A method for the study of undecalcified bones and teeth with attached soft tissues. The sage-schliff (sawing and grinding) technique. *J Oral Pathol* **11**: 318-326.

Evans, C. D. & Chen, S. T. (2008) Esthetic outcomes of immediate implant placements. *Clin Oral Implants Res* **19**: 73-80.

Evian, C. I., Rosenberg, E. S., Coslet, J. G. & Corn, H. (1982) The osteogenic activity of bone removed from healing extraction sockets in humans. *J Periodontol* **53**: 81-85.

Favero, G., Botticelli, D., Garcia, B., Mainetti, T. & Lang, N. P. (2013) Alveolar bony crest preservation at implants installed immediately after tooth extraction: An experimental study in the dog. *Clin Oral Implants Res* **24**: 7-12.

Felice, P., Cannizzaro, G., Barausse, C., Pistilli, R. & Esposito, M. (2014) Short implants versus longer implants in vertically augmented posterior mandibles: A randomised controlled trial with 5-year after loading follow-up. *Eur J Oral Implantol* **7**: 359-369.

Ferrus, J., Cecchinato, D., Pjetursson, E. B., Lang, N. P., Sanz, M. & Lindhe, J. (2010) Factors influencing ridge alterations following immediate implant placement into extraction sockets. *Clin Oral Implants Res* **21**: 22-29.

Fickl, S., Schneider, D., Zuhr, O., Hinze, M., Ender, A., Jung, R. E. & Hurzeler, M. B. (2009) Dimensional changes of the ridge contour after socket preservation and buccal overbuilding: An animal study. *J Clin Periodontol* **36**: 442-448.

Fontana, F., Maschera, E., Rocchietta, I. & Simion, M. (2011) Clinical classification of complications in guided bone regeneration procedures by means of a nonresorbable membrane. *Int J Periodontics Restorative Dent* **31**: 265-273.

Furhauser, R., Florescu, D., Benesch, T., Haas, R., Mailath, G. & Watzek, G. (2005) Evaluation of soft tissue around single-tooth implant crowns: The pink esthetic score. *Clin Oral Implants Res* **16**: 639-644.

Galindo-Moreno, P., Nilsson, P., King, P., Becktor, J., Speroni, S., Schramm, A. & Maiorana, C. (2012) Clinical and radiographic evaluation of early loaded narrow diameter implants - 1-year follow-up. *Clin Oral Implants Res* **23**: 609-616.

Gonzalez-Martin, O., Veltri, M., Moraguez, O. & Belser, U. C. (2014) Quantitative three-dimensional methodology to assess volumetric and profilometric outcome of subepithelial connective tissue grafting at pontic sites: A prospective pilot study. *Int J Periodontics Restorative Dent* **34**: 673-679.

Gotfredsen, K. (2012) A 10-year prospective study of single tooth implants placed in the anterior maxilla. *Clin Implant Dent Relat Res* **14**: 80-87.

Halperin-Sternfeld, M., Zigdon-Giladi, H. & Machtei, E. E. (2016) The association between shallow vestibular depth and peri-implant parameters: A retrospective 6 years longitudinal study. *J Clin Periodontol* **43**: 305-310.

Huynh-Ba, G., Pjetursson, B. E., Sanz, M., Cecchinato, D., Ferrus, J., Lindhe, J. & Lang, N. P. (2010) Analysis of the socket bone wall dimensions in the upper maxilla in relation to immediate implant placement. *Clin Oral Implants Res* **21**: 37-42.

Ioannidis, A., Gallucci, G. O., Jung, R. E., Borzangy, S., Hammerle, C. H. & Benic, G. I. (2015) Titanium-zirconium narrow-diameter versus titanium regular-diameter implants for anterior and premolar single crowns: 3-year results of a randomized controlled clinical study. *J Clin Periodontol* **42**: 1060-1070.

Januario, A. L., Duarte, W. R., Barriviera, M., Mesti, J. C., Araujo, M. G. & Lindhe, J. (2011) Dimension of the facial bone wall in the anterior maxilla: A cone-beam computed tomography study. *Clin Oral Implants Res* **22**: 1168-1171.

Jemt, T. (1997) Regeneration of gingival papillae after single-implant treatment. *Int J Periodontics Restorative Dent* **17**: 326-333.

Jemt, T. & Lekholm, U. (2003) Measurements of buccal tissue volumes at single-implant restorations after local bone grafting in maxillas: A 3-year clinical prospective study case series. *Clin Implant Dent Relat Res* **5**: 63-70.

Jemt, T. & Lekholm, U. (2005) Single implants and buccal bone grafts in the anterior maxilla: Measurements of buccal crestal contours in a 6-year prospective clinical study. *Clin Implant Dent Relat Res* **7**: 127-135.

Jeno, L. & Geza, L. (1975) A simple differential staining method for semi-thin sections of ossifying cartilage and bone tissues embedded in epoxy resin. *Mikroskopie* **31**: 1-4.

Jung, R. E., Herzog, M., Wolleb, K., Ramel, C. F., Thoma, D. S. & Hammerle, C. H. (2016) A randomized controlled clinical trial comparing small buccal dehiscence defects around dental implants treated with guided bone regeneration or left for spontaneous healing. *Clin Oral Implants Res*.

Jung, R. E., Holderegger, C., Sailer, I., Khraisat, A., Suter, A. & Hammerle, C. H. (2008) The effect of all-ceramic and porcelain-fused-to-metal restorations on marginal peri-implant soft tissue color: A randomized controlled clinical trial. *Int J Periodontics Restorative Dent* **28**: 357-365.

Jung, R. E., Sailer, I., Hammerle, C. H., Attin, T. & Schmidlin, P. (2007) In vitro color changes of soft tissues caused by restorative materials. *Int J Periodontics Restorative Dent* **27**: 251-257.

Kan, J. Y., Rungcharassaeng, K., Morimoto, T. & Lozada, J. (2009) Facial gingival tissue stability after connective tissue graft with single immediate tooth replacement in the esthetic zone: Consecutive case report. *J Oral Maxillofac Surg* **67**: 40-48.

Khobragade, P., Jain, A., Setlur Nagesh, S. V., Andreana, S., Dziak, R., Sunkara, S. K., Sunkara, S., Bednarek, D. R., Rudin, S. & Ionita, C. N. (2015) Micro-computed tomography (ct) based assessment of dental regenerative therapy in the canine mandible model. *Proc SPIE Int Soc Opt Eng* **9417**.

Kim, Y. J. & Henkin, J. (2015) Micro-computed tomography assessment of human alveolar bone: Bone density and three-dimensional micro-architecture. *Clin Implant Dent Relat Res* **17**: 307-313.

Klein, M. O., Schiegnitz, E. & Al-Nawas, B. (2014) Systematic review on success of narrow-diameter dental implants. *Int J Oral Maxillofac Implants* **29 Suppl**: 43-54.

Lang, N. P. & Jepsen, S. (2009) Implant surfaces and design (working group 4). *Clin Oral Implants Res* **20 Suppl 4**: 228-231.

Lang, N. P., Pun, L., Lau, K. Y., Li, K. Y. & Wong, M. C. (2012) A systematic review on survival and success rates of implants placed immediately into fresh extraction sockets after at least 1 year. *Clin Oral Implants Res* **23 Suppl 5**: 39-66.

Lee, C. T., Tao, C. Y. & Stoupel, J. (2016) The effect of subepithelial connective tissue graft placement on esthetic outcomes after immediate implant placement: Systematic review. *J Periodontol* **87**: 156-167.

Lin, G. H., Chan, H. L. & Wang, H. L. (2013) The significance of keratinized mucosa on implant health: A systematic review. *J Periodontol* **84**: 1755-1767.

Mainetti, T., Lang, N. P., Bengazi, F., Favero, V., Soto Cantero, L. & Botticelli, D. (2016) Sequential healing at implants installed immediately into extraction sockets. An experimental study in dogs. *Clin Oral Implants Res* **27**: 130-138.

Mangano, C., Piattelli, A., Mangano, F., Rustichelli, F., Shibli, J. A., Iezzi, G. & Giuliani, A. (2013) Histological and synchrotron radiation-based computed microtomography study of 2 human-retrieved direct laser metal formed titanium implants. *Implant Dent* **22**: 175-181.

Meloni, S. M., Jovanovic, S. A., Urban, I., Canullo, L., Pisano, M. & Tallarico, M. (2016) Horizontal ridge augmentation using gbr with a native collagen membrane and 1:1 ratio of particulated xenograft and autologous bone: A 1-year prospective clinical study. *Clin Implant Dent Relat Res*.

Mir-Mari, J., Wui, H., Jung, R. E., Hammerle, C. H. & Benic, G. I. (2016) Influence of blinded wound closure on the volume stability of different gbr materials: An in vitro cone-beam computed tomographic examination. *Clin Oral Implants Res* **27**: 258-265.

Misawa, M., Lindhe, J. & Araujo, M. G. (2016) The alveolar process following single-tooth extraction: A study of maxillary incisor and premolar sites in man. *Clin Oral Implants Res* **27**: 884-889.

Molina, A., Sanz-Sanchez, I., Martin, C., Blanco, J. & Sanz, M. (2017) The effect of one-time abutment placement on interproximal bone levels and peri-implant soft tissues: A prospective randomized clinical trial. *Clin Oral Implants Res* **28**: 443-452.

Muller, R. & Ruegsegger, P. (1997) Micro-tomographic imaging for the nondestructive evaluation of trabecular bone architecture. *Stud Health Technol Inform* **40**: 61-79.

Naenni, N., Schneider, D., Jung, R. E., Husler, J., Hammerle, C. H. & Thoma, D. S. (2016) Randomized clinical study assessing two membranes for guided bone regeneration of peri-implant bone defects: Clinical and histological outcomes at 6 months. *Clin Oral Implants Res*.

Neldam, C. A. & Pinholt, E. M. (2014) Synchrotron muct imaging of bone, titanium implants and bone substitutes - a systematic review of the literature. *J Craniomaxillofac Surg* **42**: 801-805.

Osechinskiy, S. & Kruggel, F. (2011) Slice-to-volume nonrigid registration of histological sections to mr images of the human brain. *Anat Res Int* **2011**: 287860.

Ostman, P. O., Hellman, M. & Sennerby, L. (2012) Ten years later. Results from a prospective single-centre clinical study on 121 oxidized (tiunite) branemark implants in 46 patients. *Clin Implant Dent Relat Res* **14**: 852-860.

Park, Y. S., Yi, K. Y., Lee, I. S. & Jung, Y. C. (2005) Correlation between microtomography and histomorphometry for assessment of implant osseointegration. *Clin Oral Implants Res* **16**: 156-160.

Passoni, B. B., Marques de Castro, D. S., de Araujo, M. A., de Araujo, C. D., Piatelli, A. & Benfatti, C. A. (2016) Influence of immediate/delayed implant placement and implant platform on the peri-implant bone formation. *Clin Oral Implants Res* **27**: 1376-1383.

Pessoa, R. S., Sousa, R. M., Pereira, L. M., Neves, F. D., Bezerra, F. J., Jaecques, S. V., Sloten, J. V., Quirynen, M., Teughels, W. & Spin-Neto, R. (2016) Bone remodeling around implants with external hexagon and morse-taper connections: A randomized, controlled, split-mouth, clinical trial. *Clin Implant Dent Relat Res*.

Pietrokovski, J. & Massler, M. (1967) Alveolar ridge resorption following tooth extraction. *J Prosthet Dent* **17**: 21-27.

Pohl, V., Thoma, D. S., Sporniak-Tutak, K., Garcia-Garcia, A., Taylor, T. D., Haas, R. & Hammerle, C. H. (2017) Short dental implants (6 mm) versus long dental implants (11-15 mm) in combination with sinus floor elevation procedures: 3-year results from a multicentre, randomized, controlled clinical trial. *J Clin Periodontol* **44**: 438-445.

Puisys, A. & Linkevicius, T. (2015) The influence of mucosal tissue thickening on crestal bone stability around bone-level implants. A prospective controlled clinical trial. *Clin Oral Implants Res* **26**: 123-129.

Rocchietta, I., Fontana, F. & Simion, M. (2008) Clinical outcomes of vertical bone augmentation to enable dental implant placement: A systematic review. *J Clin Periodontol* **35**: 203-215.

Roccuzzo, M., Grasso, G. & Dalmasso, P. (2015) Keratinized mucosa around implants in partially edentulous posterior mandible: 10-year results of a prospective comparative study. *Clin Oral Implants Res*.

Roccuzzo, M., Grasso, G. & Dalmasso, P. (2016) Keratinized mucosa around implants in partially edentulous posterior mandible: 10-year results of a prospective comparative study. *Clin Oral Implants Res* **27**: 491-496.

Sanz, M., Cecchinato, D., Ferrus, J., Pjetursson, E. B., Lang, N. P. & Lindhe, J. (2010) A prospective, randomized-controlled clinical trial to evaluate bone preservation using implants with different geometry placed into extraction sockets in the maxilla. *Clin Oral Implants Res* **21**: 13-21.

Sanz, M., Lindhe, J., Alcaraz, J., Sanz-Sanchez, I. & Cecchinato, D. (2016) The effect of placing a bone replacement graft in the gap at immediately placed implants: A randomized clinical trial. *Clin Oral Implants Res*.

Sanz Martin, I., Benic, G. I., Hammerle, C. H. & Thoma, D. S. (2016) Prospective randomized controlled clinical study comparing two dental implant types: Volumetric soft tissue changes at 1 year of loading. *Clin Oral Implants Res* **27**: 406-411.

Sanz-Sanchez, I., Ortiz-Vigon, A., Sanz-Martin, I., Figuero, E. & Sanz, M. (2015) Effectiveness of lateral bone augmentation on the alveolar crest dimension: A systematic review and meta-analysis. *J Dent Res* **94**: 128S-142S.

Sarve, H., Lindblad, J., Borgefors, G. & Johansson, C. B. (2011) Extracting 3d information on bone remodeling in the proximity of titanium implants in srmuct image volumes. *Comput Methods Programs Biomed* **102**: 25-34.

Schenk, R. K., Buser, D., Hardwick, W. R. & Dahlin, C. (1994) Healing pattern of bone regeneration in membrane-protected defects: A histologic study in the canine mandible. *Int J Oral Maxillofac Implants* **9**: 13-29.

Schmitt, C. M., Doering, H., Schmidt, T., Lutz, R., Neukam, F. W. & Schlegel, K. A. (2013) Histological results after maxillary sinus augmentation with straumann(r) boneceramic, bio-oss(r), puros(r), and autologous bone. A randomized controlled clinical trial. *Clin Oral Implants Res* **24**: 576-585.

Schneider, D., Ender, A., Truninger, T., Leutert, C., Sahrmann, P., Roos, M. & Schmidlin, P. (2014) Comparison between clinical and digital soft tissue measurements. *J Esthet Restor Dent* **26**: 191-199.

Schneider, D., Grunder, U., Ender, A., Hammerle, C. H. & Jung, R. E. (2011) Volume gain and stability of peri-implant tissue following bone and soft tissue augmentation: 1-year results from a prospective cohort study. *Clin Oral Implants Res* **22**: 28-37.

Schneider, D., Weber, F. E., Grunder, U., Andreoni, C., Burkhardt, R. & Jung, R. E. (2014) A randomized controlled clinical multicenter trial comparing the clinical and histological performance of a new, modified polylactide-co-glycolide acid membrane to an expanded polytetrafluorethylene membrane in guided bone regeneration procedures. *Clin Oral Implants Res* **25**: 150-158.

Schropp, L., Wenzel, A., Kostopoulos, L. & Karring, T. (2003) Bone healing and soft tissue contour changes following single-tooth extraction: A clinical and radiographic 12-month prospective study. *Int J Periodontics Restorative Dent* **23**: 313-323.

Schrott, A. R., Jimenez, M., Hwang, J. W., Fiorellini, J. & Weber, H. P. (2009) Five-year evaluation of the influence of keratinized mucosa on peri-implant soft-tissue health and stability around implants supporting full-arch mandibular fixed prostheses. *Clin Oral Implants Res* **20**: 1170-1177.

Schwarz, F., Alcoforado, G., Nelson, K., Schaer, A., Taylor, T., Beuer, F. & Strietzel, F. P. (2014) Impact of implant-abutment connection, positioning of the machined collar/microgap, and platform switching on crestal bone level changes. Camlog foundation consensus report. *Clin Oral Implants Res* **25**: 1301-1303.

Schwarz, F., Sahm, N. & Becker, J. (2012) Impact of the outcome of guided bone regeneration in dehiscence-type defects on the long-term stability of peri-implant health: Clinical observations at 4 years. *Clin Oral Implants Res* **23**: 191-196.

Schwarz, F., Schmucker, A. & Becker, J. (2016) Long-term outcomes of simultaneous guided bone regeneration using native and cross-linked collagen membranes after 8 years. *Clin Oral Implants Res*.

Simion, M., Baldoni, M., Rossi, P. & Zaffe, D. (1994) A comparative study of the effectiveness of e-ptfe membranes with and without early exposure during the healing period. *Int J Periodontics Restorative Dent* **14**: 166-180.

Smeets, R., Stadlinger, B., Schwarz, F., Beck-Broichsitter, B., Jung, O., Precht, C., Kloss, F., Grobe, A., Heiland, M. & Ebker, T. (2016) Impact of dental implant surface modifications on osseointegration. *Biomed Res Int* **2016**: 6285620.

Souza, A. B., Tormena, M., Matarazzo, F. & Araujo, M. G. (2016) The influence of peri-implant keratinized mucosa on brushing discomfort and peri-implant tissue health. *Clin Oral Implants Res* **27**: 650-655.

Spray, J. R., Black, C. G., Morris, H. F. & Ochi, S. (2000) The influence of bone thickness on facial marginal bone response: Stage 1 placement through stage 2 uncovering. *Ann Periodontol* **5**: 119-128.

Thoma, D. S., Jung, R. E., Schneider, D., Cochran, D. L., Ender, A., Jones, A. A., Gorlach, C., Uebersax, L., Graf-Hausner, U. & Hammerle, C. H. (2010) Soft tissue volume augmentation by the use of collagen-based matrices: A volumetric analysis. *J Clin Periodontol* **37**: 659-666.

Thoma, D. S., Muhlemann, S. & Jung, R. E. (2014) Critical soft-tissue dimensions with dental implants and treatment concepts. *Periodontol 2000* **66**: 106-118.

Tonetti, M. S., Cortellini, P., Graziani, F., Cairo, F., Lang, N. P., Abundo, R., Conforti, G. P., Marquardt, S., Rasperini, G., Silvestri, M., Wallkamm, B. & Wetzel, A. (2017) Immediate versus delayed implant placement after anterior single tooth extraction: The timing randomized controlled clinical trial. *J Clin Periodontol* **44**: 215-224.

Trombelli, L., Farina, R., Marzola, A., Bozzi, L., Liljenberg, B. & Lindhe, J. (2008) Modeling and remodeling of human extraction sockets. *J Clin Periodontol* **35**: 630-639.

Urban, I. A., Jovanovic, S. A. & Lozada, J. L. (2009) Vertical ridge augmentation using guided bone regeneration (gbr) in three clinical scenarios prior to implant placement: A retrospective study of 35 patients 12 to 72 months after loading. *Int J Oral Maxillofac Implants* **24**: 502-510.

Varnavas, A., Carrell, T. & Penney, G. (2013) Increasing the automation of a 2d-3d registration system. *IEEE Trans Med Imaging* **32**: 387-399.

Varnavas, A., Carrell, T. & Penney, G. (2015) Fully automated 2d-3d registration and verification. *Med Image Anal* **26**: 108-119.

Vera, C., De Kok, I. J., Reinhold, D., Limpiphipatanakorn, P., Yap, A. K., Tyndall, D. & Cooper, L. F. (2012) Evaluation of buccal alveolar bone dimension of maxillary anterior and premolar teeth: A cone beam computed tomography investigation. *Int J Oral Maxillofac Implants* **27**: 1514-1519.

Vignoletti, F. & Abrahamsson, I. (2012) Quality of reporting of experimental research in implant dentistry. Critical aspects in design, outcome assessment and model validation. *J Clin Periodontol* **39 Suppl 12**: 6-27.

Vignoletti, F., de Sanctis, M., Berglundh, T., Abrahamsson, I. & Sanz, M. (2009) Early healing of implants placed into fresh extraction sockets: An experimental study in the beagle dog. Ii: Ridge alterations. *J Clin Periodontol* **36**: 688-697.

Vignoletti, F., de Sanctis, M., Berglundh, T., Abrahamsson, I. & Sanz, M. (2009) Early healing of implants placed into fresh extraction sockets: An experimental study in the beagle dog. Iii: Soft tissue findings. *J Clin Periodontol* **36**: 1059-1066.

Vignoletti, F. & Sanz, M. (2014) Immediate implants at fresh extraction sockets: From myth to reality. *Periodontol 2000* **66**: 132-152.

von Arx, T., Cochran, D. L., Hermann, J. S., Schenk, R. K. & Buser, D. (2001) Lateral ridge augmentation using different bone fillers and barrier membrane application. A histologic and histomorphometric pilot study in the canine mandible. *Clin Oral Implants Res* **12**: 260-269.

Wennerberg, A. & Albrektsson, T. (2010) On implant surfaces: A review of current knowledge and opinions. *Int J Oral Maxillofac Implants* **25**: 63-74.

Windisch, S. I., Jung, R. E., Sailer, I., Studer, S. P., Ender, A. & Hammerle, C. H. (2007) A new optical method to evaluate three-dimensional volume changes of alveolar contours: A methodological in vitro study. *Clin Oral Implants Res* **18**: 545-551.

Yi, H. Y., Park, Y. S., Pippenger, B. E., Lee, B., Miron, R. J. & Dard, M. (2016) Dimensional changes following immediate and delayed implant placement: A histomorphometric study in the canine. *Int J Oral Maxillofac Implants*.

Zeltner, M., Jung, R. E., Hammerle, C. H., Husler, J. & Thoma, D. S. (2017) Randomized controlled clinical study comparing a volume-stable collagen matrix to autogenous connective tissue grafts for soft tissue augmentation at implant sites: Linear volumetric soft tissue changes up to 3 months. *J Clin Periodontol* **44**: 446-453.

Zuhr, O., Baumer, D. & Hurzeler, M. (2014) The addition of soft tissue replacement grafts in plastic periodontal and implant surgery: Critical elements in design and execution. *J Clin Periodontol* **41 Suppl 15**: S123-142.

Zuhr, O., Rebele, S. F., Schneider, D., Jung, R. E. & Hurzeler, M. B. (2014) Tunnel technique with connective tissue graft versus coronally advanced flap with enamel matrix derivative for root coverage: A rct using 3d digital measuring methods. Part i. Clinical and patient-centred outcomes. *J Clin Periodontol* **41**: 582-592.

X. ANEXO: RESUMEN EN INGLÉS

ABSTRACT

Title: Soft and hard tissue changes after the use of implants with different geometries and after lateral ridge augmentation procedures.

Introduction

The physiologic changes that occur after tooth extraction may originate alveolar ridge deformities. Depending on the degree of resorption these defects may need hard tissue reconstruction procedures or the use of implants with a reduced diameter or a modified cervical design.

The success of implant supported restoration and implant therapy is not based anymore solely on the survival of the implant but also on the integration of the restoration with the surrounding tissues. In this context, peri-implant soft tissue plays a critical role in the esthetic appearance of the reconstructions. Despite of its crucial importance the current knowledge of its clinical behavior, the impact of the surgical approach and the mutual interplay that soft and hard tissue have is scarce are poorly understood.

These limitations are mainly originated by the inherent restrictions of the avaialble methodological techniques employed to evaluate them.

Objectives

The main objective of these series of investigations were i) to analyze the soft and hard tissue integration when using implants with a triangular cervical design and implants with a conventional cylindrical design placed in healed sites or in post-extraction sockets (Study 1), ii) to analyze the relative differences in soft and hard

tissue volume utilizing a novel imaging methodology (Study 2) and iii) to study the soft tissue contour changes in lateral ridge augmentation procedures when employing three different treatment alternatives based on the use of xenograft bone substitutes, collagen membranes or the combination of the two (Study 3).

Material and Methods. Results.

Study 1. For the first investigation a preclinical model in the Beagle dog was utilized were implants with a triangular cervical design (Test/T) or implants with a conventional cylindrical (Control/C) design were tested. First and second premolars were extracted together with the mesial root of the first molar. Eight weeks after T and C implants were randomly placed in the healed ridges of PM2 and M1 and immediate implants were placed in the mesial post-extraction sockets of PM3 and PM4. Eight weeks after surgery was performed in the contralateral side of the mandible and sacrifice tooth place 4 weeks after to give two healing times of 4 and 12 weeks. Silicone impressions were taken before tooth extraction and after sacrifice. Samples were processed for Micro CT analysis and later embedded in methacrylate for histologic processing with the ground section technique. The results showed greater values of buccal crestal bone width for test implants and similar vertical histomorphometric values of soft and hard tissue. The percentage of bone to implant contact (BIC), bone volume and soft tissue contours were similar for both implant designs.

Study 2. Based on the samples with 12 weeks healing of the previously mentioned study, a novel methodology was employed which was able to superimpose the Micro CT images reporting on the hard tissue values to the STL images that were obtained by means of optical scanning of the stone casts. Three distinct volumes of interest were calculated; the buccal volume of bone (B-BV), the soft tissue volume bellow the implant shoulder (EC-STV), the soft tissue volume above the implants shoulder (SC-

STV). The results proved that; i) the methodology employed was able to provide information on the relative volume of the soft and hard tissues, ii) buccal bone volume was superior for the test implants in 3 out of the 4 locations while implant geometry appeared to have no influence on the soft tissues and iii) the healing modus (immediate or delayed) affected the soft tissue response.

Study 3. To address the third objective an experimental model in the beagle dog was developed where after tooth extraction three box shape defects were created in each hemimandible. Three months after extraction the augmentation surgeries were performed by employing a xenograft embedded in a collagen matrix (BRG), a porcine derived native collagen membrane (MBG) or the combination of the two (CBG). Silicone impressions were taken before tooth extraction (T1), before the augmentation surgeries (T2) and 3 months after the regenerative surgeries (T3). Stone casts were obtained and were optically scanned. Dedicated software was employed to analyze the contour changes in the three different time-points. The results showed that the BRG and the CBG achieved better results that the membrane alone although there were no difference between BRG and CBG. The values after the regenerative techniques seldom reached the baseline values before tooth extraction.

Conclusions

Implants with a triangular cervical design obtained greater values of buccal crestal bone width and buccal bone volume without differences in the vertical soft and hard tissue histomorphometric measurements. The implant cervical design did not affect the contour of the soft tissues or the soft tissue volumes. The surgical protocol had a clear impact in soft and hard tissue behavior. Regarding the regenerative techniques, the use of bone substitutes and barrier membranes obtained the best results rebuilding the ridge however the values seldom met those before tooth extraction.