Aus der Poliklinik für Zahn-, Mund- und Kieferheilkunde
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Gesteuerte Knochenregeneration und Biomaterialien zur Osseointegration zahnärztlicher Implantate im defizitären Kieferbereich

Habilitationsschrift

zur Erlangung der Lehrbefähigung
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vorgelegt von
Dr. med. dent. Ilja Mihatovic

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Meiner Familie gewidmet.

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Ich versichere an Eides statt, dass ich die vorliegende Habilitationsschrift ohne unerlaubte Hilfe angefertigt habe. Weiterhin versichere ich an Eides statt, dass das benutzte Schrifttum vollständig erwähnt wurde. Zuletzt versichere ich an Eides statt, dass ethische Grundsatze sowie die Empfehlungen zur Sicherung guter wissenschaftlicher Praxis eingehalten wurden und dass diese Habilitationsschrift bislang keiner anderen Fakultät vorgelegt wurde.

Düsseldorf, den 29. Mai 2020 Dr. med. dent. Ilja Mihatovic

Inhaltsverzeichnis

1. Vorbemerkung - Publikationen

Diese Habilitationsschrift ist eine Zusammenfassung mehrerer Publikationen zur Untersuchung der gesteuerten Knochenregeneration und Biomaterialien zur Osseointegration zahnärztlicher Implantate im defizitären Kieferbereich. Zunächst erfolgt eine Einführung in die Thematik der gesteuerten Knochenregeneration und die dabei verwendeten Knochenmaterialien. Anschließend werden die Fragestellungen dargelegt, mit denen sich die einzelnen Publikationen befassen. Im Abschnitt Originalarbeiten werden Hintergrund und Methodik der Arbeiten erläutert und im Anschluß die Ergebnisse zusammengefasst und diskutiert. Zuletzt erfolgt eine abschließende Zusammenfassung.
Bei den Publikationen handelt es sich um präklinische experimentelle Studien:

Publikation 1:

Staged implant placement after defect regeneration using biphasic calcium phosphate materials with different surface topographies in a minipig model.

Mihatovic I, Schwarz F, Obreja K, Becker J, Sader R, Dard M, John G

Clin Oral Investig. 2020 Jan 24. doi: 10.1007/s00784-020-03206-7. [Epub ahead of print]

Publikation 2:

Shell technique using a rigid resorbable barrier system for localized alveolar ridge augmentation.

Iglhaut G, Schwarz F, Gründel M, Mihatovic I, Becker J, Schliephake H

Clin Oral Implants Res. 2014 Feb;25(2): e149-54. doi: 10.1111/clr.12078. Epub 2012 Dec 21.

Publikation 3:

Influence of two barrier membranes on staged guided bone regeneration and osseointegration of titanium implants in dogs: part 1. Augmentation using bone graft substitutes and autogenous bone.

Schwarz F, Mihatovic I, Golubovic V, Hegewald A, Becker J

Clin Oral Implants Res. 2012 Jan;23(1):83-9. doi: 10.1111/j.1600-0501.2011.02187.x. Epub 2011 Apr 25.

Publikation 4:

Influence of two barrier membranes on staged guided bone regeneration and osseointegration of titanium implants in dogs. Part 2: augmentation using bone graft substitutes.

Mihatovic I, Becker J, Golubovic V, Hegewald A, Schwarz F.

Clin Oral Implants Res. 2012 Mar;23(3):308-15. doi: 10.1111/j.1600-0501.2011.02238. x. Epub 2011 Sep 15.

Publikation 5:

Immunohistochemical analysis of staged guided bone regeneration and osseointegration of titanium implants using a polyethylene glycol membrane.

Mihatovic I, Golubovic V, Becker J, Schwarz F.

Clin Oral Investig. 2014;18(2):429-35. doi: 10.1007/s00784-013-0995-2. Epub 2013 May 9.

2. Einleitung

Der Ersatz fehlender Zähne durch das Einbringen von Implantaten in den Kieferknochen ist heutzutage eine bewährte Behandlungsmethode zur sicheren Befestigung von herausnehmbarem oder festsitzendem Zahnersatz (Pikner et al. 2009, Mertens et al. 2012, Degidi et al. 2012). Seit der Beschreibung der Osseointegration, dem direkten strukturellen und funktionellen Verbund zwischen Implantat und Knochen (Brånemark et al. 1969, Schroeder et al. 1979) kann mittlerweile auf eine jahrzehntelange Erfahrung mit dentalen Implantaten zurückgeblickt werden. Zahlreiche retrospektive Langzeitstudien sowie systematische Reviews mit Untersuchungszeiträumen von über 10 Jahren konnten Überlebensraten zwischen 82,9% und 95% dokumentieren (Adell et al. 1990, Moraschini et al. 2015, Howe et al. 2019).

Osseointegration von Implantaten

Die Basis für die Verankerung von Implantaten im Alveolarknochen ist die Osseointegration. Der Begriff der Osseointegration beschreibt den direkten strukturellen und funktionellen Verbund zwischen Implantatoberfläche und vitalem Knochen (Brånemark et al. 1969). Die Grundlage einer erfolgreichen Osseointegration und damit für den Langzeiterfolg ist ein ausreichender Implantat-Knochen-Kontakt ohne eine Einlagerung nicht knöcherner oder bindegewebiger Strukturen (Brånemark et al. 1969, Albrektsson et al. 1983, Trisi et al. 2002). Die Knochenheilung um Implantate basiert auf dem Mechanismus der intramembranösen Osteogenese, der Bildung von Geflechtknochen und späterer Reifung zu Lamellenknochen. Während neu gebildeter Knochen ca. 1 Woche nach Implantation an der Implantatoberfläche zu erkennen ist, beginnen die Knochenumbauvorgänge um das Implantat nach 6 bis 12 Wochen und halten lebenslang an. Einen entscheidenden Einfluss auf die Qualität des Implantat-Knochen-Kontaktes hat die Oberflächenbeschaffenheit des Implantates. Zahlreiche Studien konnten belegen, dass mikroraue Implantatoberflächen den Prozess der

Osseointegration von Titanimplantaten beschleunigen und polierten Oberflächen überlegen waren (Cook et al. 1987; Cook et al. 1992, Buser et al. 1991). Auch der prozentuale Implantat-Knochen-Kontakt korreliert positiv mit der Oberflächenrauigkeit (Buser et al. 1991). Bei Implantaten mit polierter Oberfläche wurden im Mittel zwischen 20% und 25% und bei sandgestrahlt und säuregeätzen Implantaten 50% bis 60% Implantat-Knochen-Kontakt dokumentiert (Buser et al. 1991). Sandstrahlen und anschließendes Säureätzen gilt aktuell als Goldstandart zur Erzeugung von mikrorauen Implantatoberflächen. Chemische Oberflächenodifikationen zur Erhöhung der Hydrophilie können die Geschwindigkeit der Osseointegration von Implantaten aus Titan und Titan/Zirkonoxid steigern. Dies konnte im Rahmen präklinischer sowie klinischer Studien nachgeweisen werden (Lang et al. 2011, Schwarz et al. 2007). Implantate mit rauen Oberflächen zeigen eine vorhersehbare Osseointegration, jedoch führt die Einbringung des Implantatkörpers zu mechanischen Veränderungen im Alveolarfortsatz und damit zur Umverteilung der Knochendichte und Remodelling. Dabei pendelt sich ein Implantat-Knochen-Kontakt zwischen 50-80% ein (Lian et al. 2010).

Ein weiterer Faktor für den Grad der Osseointegration ist das Ausdrehmoment, welches die aufzuwendende Kraft, die zur Entfernung eines Implantats notwendig ist, beschreibt. In vergangenen Studien wurden für raue Oberflächen höhere Ausdrehmomente als bei polierten Oberflächen verzeichnet (Carlsson et al. 1988, Klokkevold et al. 2002) Dies bestätigte eine weitere Studie, welche zeigte, dass die Ausdrehmomente bei rauen Implantaten im Vergleich zu hochglanzpolierten Implantaten nach einer Einheilzeit von 4 Wochen 4-fach höher und nach 12 Wochen um den Faktor 10 erhöht waren (Buser et al. 1999).

Dimensionsveränderungen des Alveolarkamms

Die Grundlage für den erfolgreichen Einsatz von Implantaten ist ein ausreichendes Knochen- und Weichgewebsangebot. Jedoch besteht bei vielen Patienten in den unbezahnten Kieferregionen eine ausgeprägte Alveolarkammatrophie. Ursachen sind Traumata, Tumore

oder Entzündungen und die damit verbundenen Resorptionsvorgänge, welche Knochen und Weichgewebe aus funktioneller sowie ästhetischer Sicht kompromittieren (Cheung et al. 2011). Im Bereich des Alveolarkamms kommt es zu einer knöchernen Resorption in horizontaler und vertikaler Dimension (Carlsson et al.1967, Atwood & Coy 1971, Schropp et al. 2003, Tallgren 2003, Cardaropoli et al. 2003, Fickl et al. 2008b). Dabei kann das Resorptionsverhalten zwischen Ober- und Unterkiefer unterschieden werden. Im Oberkiefer erfolgt die Alveolarkammatrophie zentripetal und im Unterkiefer zentrifugal. Die Ursachen für die resorptiven Veränderungen sind bis heute unklar. Nach 2 Jahren ohne funktionelle Belastung kann es zu einem Gesamtverlust von 60% Alveolarkammhöhe kommen (Cawood & Howell 1998). Besonders im Bereich der bukkalen Knochenlamelle findet eine signifikante Resorption innerhalb von 8-12 Wochen statt (Araujo et al. 2005, Schropp et al. 2003). Studien zeigten, dass es zu einem horizontalen Alveolarkammverlust von 29 – 63% und einem vertikalen Verlust von 11 - 22% innerhalb der ersten sechs Monate kommen kann (Tan 2012). Daher besteht in vielen Fällen die Notwendigkeit die betroffenen Alveolarfortsatzregionen zu rekonstruieren, um ausreichend Knochen- und Weichgewebe zu erzeugen. Insbesondere implantologische Therapien setzen ein ausreichendes horizontales und vertikales Alveolarkammangebot voraus, welches eine sichere Implantatsetzung und Langzeitstabilität gewährleisten kann (Zambon et al. 2012).

Augmentative Verfahren

Aufgrund der unvermeidbaren Resorptionsvorgänge nach Zahnverlust sind rekonstruktive Maßnahmen vor implantologischen Versorgungen häufig unumgänglich. Nur bei ausreichendem Alveolarkammvolumen kann eine adäquate Implantatpositionierung erfolgen, so dass eine vorhersehbare Langzeitstabilität unter funktionellen sowie ästhetischen Gesichtspunkten gewährleistet ist. Zur Rekonstruktion von Knochendefekten stehen unterschiedliche Techniken zur Verfügung, welche sich nach der Größe der entsprechenden Defekte richten. Dazu zählen An- und Auflagerungsplastiken (Onlay-Technik, Le-Fort-

Osteotomie- Technik, Distraktionsosteogenese), sowie die gesteuerte Knochenregeneration (GBR – guided bone regeneration). Bei der Onlay-Technik werden vorwiegend autologe Transplantate zum Aufbau von vertikalen, horizontalen oder kombiniert horizontalen/vertikalen Alveolarfortsatzdefekten angewendet. Bei Le-Fort-Osteotomie oder Sandwichtechniken erfolgt die Interposition von Transplantaten zum vertikalen Aufbau und Ausgleich sagittaler Diskrepanzen zwischen Ober- und Unterkiefer. Die Technik der Distraktionsosteogenese wird bei entwicklungsbedingten Dysgnathien oder zur Korrektur atrophischer Veränderungen des Alveolarfortsatzes eingesetzt. Dabei werden Distraktoren gezielt angewendet, um eine Osteogenese auszulösen, welche eine Knochen- und Weichgewebsvermehrung in sagittaler oder/und vertikaler Richtung ermöglicht (Hidding et al. 2000). Einen wichtigen Bereich der Knochenaufbaumethoden stellt klinisch mittlerweile die gesteuerte Knochenregeneration dar.

Gesteuerte Knochenregeneration

Das Konzept der gesteuerten Knochenregeneration beinhaltet die Plazierung einer Membran, welche das Blutkoagulum im Knochendefekt stabilisiert und zugleich eine Barriere zwischen Weichgewebe und Knochengewebe schafft. So können knochenbildende Zellen den Defekt regenerieren, ohne dass deutlich schneller proliferierende Zelltypen des Weichgewebes die Knochenheilung negativ beinflussen (Linde et al. 1993, Dahlin et al. 1988). Im Rahmen der gesteuerten Knochenregeneration werden häufig Membranen mit Knochenmaterialien kombiniert und zeigen klinisch gute Ergebnisse (Jensen & Terheyden 2009). Im folgenden Abschnitt wird detailliert auf gängige Knochenmaterialien und Membrantypen eingegangen.

Membranen

Barrieremembranen müssen gewissen Anforderungen genügen, um bei der gesteuerten Knochenregeneration erfolgreich angewendet zu werden. Dazu gehören Biokompatibilität, Zellokklusivität, Volumenstabilität, Gewebeintegration, Permeabilität für Gefäße sowie

klinische Anwendbarkeit (Hardwick et al. 1994). Es sind nicht resorbierbare und resorbierbare synthetische oder xenogene Membranen erhältlich. Zu den nicht resorbierbaren, synthetischen Membranen zählen vorwiegend Membranen aus Polytetrafluorethylen (e-PTFE) und zeichnen sich durch ihre gute Barrierefunktion sowie Biokompatibilität aus. Ein Vorteil ist die starke Barrierefunktion, jedoch leiden unter der Undurchlässigkeit des ePTFEs Permeabilität und Gewebeintegration. Da ePTFE nicht resorbierbar ist, muss die Membran entfernt werden und sich der Patient einem zweiten Eingriff unterziehen. Darüberhinaus ist die Anwendung chirurgisch anspruchsvoll und ein gezieltes Weichgewebsmanagement notwendig, um die im Vergleich zu resorbierbaren Membranen erschwerte Wundheilung nach Membranexposition zu vermeiden. Daher werden heutzutage vorwiegend resorbierbare Kollagenmembranen eingesetzt. Sie werden rückstandsfrei verstoffwechselt und ein zweiter Eingriff zur Entfernung entfällt (Bunyaratavej & Wang 2001). Vor allem native, porcine Membranen aus Kollagen TypI/III zeigen eine ausgeprägte Gewebeintegration und Semipermeabilität in den ersten Phasen der Wundheilung (Rothamel et al. 2005; Schwarz et al. 2006, 2008). Es konnte histologisch gezeigt werden, dass die Resorption ohne Beteiligung von Entzündungszellen funktioniert und die Barrierefunktion über einen Zeitraum von 4 - 8 Wochen gewährleistet wird (Rothamel et al. 2012). Ein weiterer Vorteil von Kollagenmembranen ist deren Eigenschaft bei auftretenden Wunddehiszenzen nach Augmentationen. Im Gegensatz zu nicht resorbierbaren Membranen ist bei kollagenen Membranen eine sekundäre Epithelialisierung möglich, ohne dass das Regenerationsergebnis nachhaltig kompromittiert wird (Friedmann et al. 2001, Friedmann et al. 2002, Beitlitum et al. 2010). Jedoch ist ein potentieller Nachteil des Kollagens die schnelle Biodegradation und die damit geschwächte Formstabilität, welche den Volumenerhalt des augmentierten Bereichs mindern kann (Sela et al. 2003; Rothamel et al. 2005). Ein weiteres Material für Membranen ist Polyethylenglycol (PEG) (Jung et al. 2006). Es wird als Gel, welches aus zwei Komponenten besteht und nach ca. einer Minute aushärtet, angewendet. Studien zeigten, dass die quervernetzten Polyethylenglycol-Komponenten während der Wundheilung ohne Freisetzung von Säureprodukten vollständig degradierten und es zu keiner begleitenden

Entzündungsreaktion der Gewebestrukturen kam (Wechsler et al. 2008; Herten et al. 2009). Die Hydrolyse erlaubte nach 4 Wochen das Einwachsen von Gefäßen bei einer Biodegradationszeit von 16-24 Wochen im präklinischen Tiermodell (Herten et al. 2009). Experimentelle Daten konnten zeigen, dass das Material hoch biokompatibel und zellokklusiv ist und mit anderen Membranen vergleichbare Mengen neuer Knochensubstanz erzeugen konnte (Jung et al. 2006, 2009b; Thoma et al. 2009; Schwarz et al. 2010).

Membranen werden im Rahmen der gesteuerten Knochenregeneration häufig in Verbindung mit Knochenmaterialien als Füllstoff im Knochendefekt eingesetzt. Das nächste Kapitel gibt einen Überblick über die bei Augmentationen eingesetzten Knochenmaterialien.

Knochenmaterialien

Ein ideales Knochenmaterial sollte folgende Eigenschaften haben, um den Anforderungen bei der gesteuerten Knochenregeneration gerecht zu werden:

- Osteogenese
- Osteokonduktivität
- Osteoinduktivität
- Biokompatibilität
- Porosität

Osteogenese beschreibt die Fähigkeit neuen Knochen bilden zu können. Die Osteokonduktivität eines Knochenmaterials ist das Vorhandensein einer Matrix/Leitstruktur für proliferierende Gefäße als Grundlage für das Einwachsen von neuem Knochen (Zerbo et al. 2005). Unter Osteoinduktion versteht man die Fähigkeit zur Stimulation mesenchymaler Zellen in knochenbildende Osteoblasten. Autologer Knochen vereint alle drei Eigenschaften und stellt daher auch den Goldstandard für Knochenmaterialen dar (Artzi et al. 2005). Entsprechend der Größe des zu behandelnden Kieferdefektes können aus intraoralen Entnahmeregionen (Kinn, Tuberregion, linea obliqua externa) oder extraoralen Regionen (Ilium, Fibula) Transplantate entnommen werden. Nachteile sind die begrenzte Verfügbarkeit, die Morbidität der

Spenderregion und die Resorptionsfreudigkeit autologen Knochens (Nyström et al. 1995, Lundgren et al. 1997, Sbordone et al. 2011). Eine Alternative sind xenogene oder alloplastische Knochenersatzmaterialien. Zu den alloplastischen, also synthetisch hergestellten Materialien zählen Kalziumcarbonat, Trikalziumphosphat, Hydroxylapatit, Biogläser sowie kalziumbeschichtete Polymere. Xenogene Knochenersatzmaterialien sind tierischen Ursprungs (bovin, porcin, equin) und wirken osteokonduktiv. Sie besitzen zwar keine osteogenetische oder osteoinduktive Eigenschaften, jedoch zeigen sie neben Osteokonduktivität durch Porosität eine hohe Biokompatibiliät. Zahlreiche Studien zur gesteuerten Knochenregeneration zeigen vergleichbare Ergebnisse zu autologem Knochen (Strietzel et al. 2007; Hämmerle et al. 2008, Donos et al. 2008). Vorteile sind die steuerbare Resorptionszeit, unlimitierte Verfügbarkeit und die Vermeidung der Morbidität der Entnahmestelle.

3. Fragestellungen

1. Welchen Einfluss hat die Art der Membran und das verwendete Knochenmaterial auf die Regeneration lokaler Defekte des Alveolarkamms?

Eine wichtige Anforderung an eine Barrieremembran bei der gesteuerten Knochenregeneration ist, neben einer guten Gewebeintegration, die Volumenstabilität (Dahlin et al. 1988). Vor allem bei komplexeren Alveolarkammdefekten spielt eine temporäre Rekonturierung des defizitären Alveolarkamms sowie eine Stabilisierung des Blutkoagels und Knochenmaterials im Defektbereich eine wichtige Rolle (Chiapasco et al. 1999). Vier der fünf in dieser Schrift aufgeführten Studien analysierten unterschiedliche Typen von Membranen und deren Einfluss auf die Knochenregeneration. Es wurden eine xenogene Kollagenmembran, eine synthetische Polylactidsäuremembran sowie eine Polyetylenglycolmembran untersucht und verglichen. In allen Studien kamen lokalisierte Alveolarkammdefekte zum Einsatz, so dass eine Vergleichbarkeit gewährleistet war. Neben der Verwendung einer Membran werden bei der gesteuerten Knochenregeneration routinemäßig Knochenmaterialien zur Auffüllung des Defektbereichs eingesetzt (Jensen & Terheyden 2009). Diese erlauben die Stabilisation des Blutkoagels, dienen der Konturgebung des defizitären Alveolarkamms und unterstützen mit ihren osteoinduktiven (autologer Knochen) und osteokonduktiven (Knochenersatzmaterialien) Eigenschaften die knöcherne Regeneration. In den vorliegenden Untersuchungen wurden die angewendeten Barrieremembranen mit unterschiedlichen Knochenmaterialien kombiniert. Dabei kamen autologer Knochen, xenogenes Hydroxylapatit und synthetisches biphasisches Calziumphosphat zum Einsatz.

2. Wie verhält sich die Osseointegration von Titanimplantaten in Abhängigkeit der zuvor verwendeten Membranen und Knochenmaterialien?

Das Ziel der Regeneration des Alveolarkamms ist die Erzeugung eines ausreichend dimensionierten Knochenlagers für eine spätere Einbringung von Implantaten. Dabei ist die

Osseointegration, der strukturelle und funktionelle Verbund zwischen Implantatoberfläche und umgebendem Knochen, Grundvoraussetzung für eine langfristige Stabilität des Implantates. Zwar zeigen Implantate in Bereichen gesteuerter Knochenregeneration vergleichbare Überlebensraten wie in unbehandeltem Knochen, aber der Einfluss der vorangegandenen Methode zur Knochenregeneration und der dabei verwendeten Biomaterialien auf die Osseointegration bedarf weitererführender Untersuchungen. In den vorliegenden Studien wurde die Osseointegration von Titanimplantaten nach gesteuerter Knochenregeneration mittels unterschiedlicher Knochenmaterilaien und Membranen aufgebauten Alveolarkammbereichen untersucht.

4. ORIGINALARBEITEN

Shell technique using a rigid resorbable barrier system for localized alveolar ridge augmentation.

Hintergrund

Die gesteuerte Knochenregeneration mithilfe von Membranen und Knochenmaterialien zeigt vorhersehbare Ergebnisse bei der Behandlung von Alveolarkammdefekten. Ein wichtiger Faktor für den Behandlungserfolg und die Wahl der Membran sowie der Knochenmaterialien ist die Defektgröße und Defektgeometrie. Je ausgedehnter der Knochendefekt ist und je weniger begrenzende Knochenwände vorhanden sind, desto schwieriger sind die Vorraussetzungen für die Knochenneubildung. Die Defektränder sind Ausgangspunkt der Einblutung und Bildung des Blutkoagulums und versorgen den Defektbereich mit Vaskularisation, so dass neue Knochensubstanz gebildet werden kann. In der vorliegenden Studie wurden sattelförmige Alveolarkammdefekte untersucht, bei welchen die bukkale und linguale Alveolarfortsatzwand fehlte. Ziel war es, mithilfe einer starren Membran aus Polylactidsäure die Alveolarfortsatzkontur zu stützen und mittels Knochenmaterial die Knochenneubildung im Inneren des Defektes zu fördern. Dabei sollte der Einfluss der eingesetzten Membranen und Knochenmaterialien auf die Regeneration der Defekte klinisch sowie histologisch untersucht werden.

Methode

In dieser experimentellen, präklinischen Studie wurde eine starre synthetische, resorbierbare Membran aus Polylactidsäure zur Augmentation sattelförmiger Alveolarkammdefekte untersucht. Bei 6 Foxhunden wurden im Unterkiefer sattelförmige Alveolarkammdefekte präpariert (jeweils n=4). Bei einem Defekt wurden Polylactidmembranen mittels Polylactid - Pins zur Stützung der bukkalen und lingualen Konturen in Kombination mit bovinem Hydroxylapatit eingesetzt und einer nativen Kollagenmembran abgedeckt. Der zweite Defekt

wurde mit der gleichen Membrantechnik behandelt, jedoch wurden zusätzlich autologe Knochenpartikel in einem Verhältnis von 1:1 zum bovinen Hydroxylapatit hinzugefügt. Der dritte Defekt wurde mit bovinem Hydroxylapatit in Kombination mit autologen Knochenpartikeln gefüllt und mit der nativen Kollagenmembran abgedeckt. Der vierte Defekt wurde der Spontanheilung ohne den Einsatz von Biomaterialien überlassen. Nach einer Heilzeit von 14 Wochen wurden die Defektbereiche entnommen und histologisch aufbereitet. Die histomorphometrische Analyse umfasste folgende Parameter: augmentiertes Areal, mineralisiertes Gewebe und verbliebendes Knochenmaterial (jeweils in mm^2).

Ergebnisse

Während der 14 - wöchigen Heilzeit zeigte sich eine ungestörte geschlossene Wundheilung ohne allergische Reaktionen, Schwellungen oder Infektionen. Lediglich bei zwei behandelten Defekten lagen kleine Anteile der Polylaktidmembran frei. Histologisch konnte bei diesen Proben eine leichte Entzündungsreaktion im bedeckenden Weichgewebe identifiziert werden. Alle behandelten Defektbereiche zeigten eine homogene Stabilisation der Knochenmaterialpartikel innerhalb der durch die Polylaktid- und Kollagenmembran geschaffenen Räume. In allen Proben konnte eine moderate Deplazierung von Knochenmaterialpartikeln und Einlagerung in das bedeckende subepitheliale Bindegewebe verzeichnet werden. Die mit der Polylaktidmembran behandelten Defekte zeigten eine dichtere Knochenneubildung als die nur mit Kollagenmembran abgedeckten Defekte. Dort konnte häufig eingewachsenes Bindegewebe, vor allem im koronalen Bereich, beobachtet werden. In den unbehandelten Gruppen zeigte sich nur ein geringes Knochenremodelling. Histologisch konnte in den meisten ehemaligen Defekten, welche mit der Polylaktidmembran behandelt wurden, Überreste der Membran identifiziert werden. Die Biodegradation der Polylaktidmembran war jedoch mit keiner Entzündungsreaktion verbunden. Die histomorphometrische Untersuchung zeigte signifikant höhere Mittelwerte für mineralisiertes Gewebe sowie das augmentierte Areal im Vergleich zu den unbehandelten Regionen. Die

höchsten Werte für mineralisiertes Gewebe wurden in folgender Reihenfolge der Versuchsgruppen verzeichnet:
Hydroxylapatit + Polylaktidmembran + Kollagenmembran, Hydroxylapatit + autologer Knochen + Polylaktidmembran + Kollagenmembran, Hydroxylapatit + Kollagenmembran und unbehandelter Defekt. Der Anteil an verbliebenem Knochenmaterial war in allen ehemaligen Defekten vergleichbar ($p>0,05$, t-Test).

Diskussion

Sattelförmige Defekte stellen ein etabliertes Modell zur Untersuchung der gesteuerten Knochenregeneration dar (Vignoletti & Abrahamson 2012, Schwarz et al. 2012, Mihatovic et al. 2012). Die erlangten Daten zeigten, dass die verwendete Technik zur gesteuerten Knochenregeneration mittels Polylactidsäuremembran durch eine gute klinische Anwendbarkeit gekennzeichnet war und als Behandlung für Alveolarkammdefekte erfolgreich eingesetzt werden könnte. Die Polylactidsäuremembran zeigte hohe Biokompatibilität sowie geringes Auftreten von Wundheilungsstörungen oder Fremdkörperreaktionen. Diese Beobachtung wurde in einer zuvor durchgeführten retrospektiven, klinischen Analyse bestätigt (Volkel et al. 2011). Dabei ist hervorzuheben, dass die rigide Polylaktidsäuremembran zusätzlich mit einer nativen Kollagenmembran bedeckt wurden. Native Kollagenmembranen sind durch eine hohe Gewebeverträglichkeit und Förderung der Weichgewebsintegration gekennzeichnet (Rothamel et al. 2005, Schwarz et al. 2006). Die histomorphometrische Analyse der Proben zeigte, dass alle Therapievarianten die Knochenregeneration in den Defekten unterstützten. Die behandelten Bereiche waren durch signifikant mehr Knochenneubildung als die unbehandelten Kontrolldefekte charakterisiert. Die Ergebnisse der Gruppen, welche mit Knochenmaterial und Kollagenmembran behandelt wurden, zeigten mit vergleichbaren Studien ähnliche Werte nach 10 Wochen Heilzeit (Bornstein et al. 2007, Schwarz et al. 2012). Die vorliegenden Daten weisen darauf hin, dass die Anwendung der Polylaktidsäuremembran zu verbesserter Knochenmineralisierung und damit besserer Defektheilung gegenüber den Vergleichsgruppen führte. Die aus der histomorphometrischen

Analyse gewonnen Daten zeigten jedoch keine statistische Signifikanz. Eine wichtige Rolle bei der verbesserten Defektheilung in der Gruppe der Polylactidsäuremembran könnte die Formstabilität gespielt haben. Eine weitere interessante Beobachtung war der Einfluss des hinzugefügten autologen Knochens im Augmentat. Es zeigt sich, dass der Zusatz von autologen Knochen den Anteil mineralisierten Gewebes im Defektbereich nicht steigern konnte. In-vitro Studien identifizierten vitale Zellen in intraoral gewonnenem autologen Knochen, welche zu Osteoblasten differenzierten (Chiriac et al. 2005). Obwohl die Osteoblastendifferenzierung eine verbesserte Knochenheilung verspricht, wurde in der vorliegenden Studie kein Vorteil durch Zusatz von autologem Knochen beobachtet. Jedoch konnten weiterführende Studien zur gesteuerten Knochenregeneration mit identischem Defektmodel und der Anwendung von xenogenem Hydroxylapatit kombiniert mit einer xenogenen Kollagenmembran eine verbesserte Mineralisation nach 10 Wochen Heilung dokumentieren (Mihatovic et al. 2012, Schwarz et al. 2012). Auf Basis der Ergebnisse der vorliegenden Studie konnte geschlossen werden, dass alle Behandlungsvarianten die Knochenregeneration unterstützten, jedoch die Anwendung der Polylactidsäuremembran sowie der Verzicht auf zusätzlichen autologen Knochen zu einem leicht erhöhten Anteil mineralisierten Gewebes führte und damit die Knochenregeneration verbesserte.

Staged implant placement after defect regeneration using biphasic calcium phosphate materials with different surface topographies in a minipig model.

Hintergrund

Ziel der gesteuerten Knochenregeneration im Kieferbereich ist das Einbringen von zahnärztlichen Implantaten zur Befestigung von Zahnersatz. Nur ein ausreichendes Knochenangebot kann die sichere Einheilung und Osseointegration gewährleisten. Daher muss häufig im ersten Schritt der Aufbau des defizitären Kieferbereichs erfolgen, bevor in einem zweiten Schritt die Implantate gesetzt werden können. Studien konnten zeigen, dass

Implantate eine vergleichbare Osseointegration und Überlebensrate in unbehandeltem und mittels gesteuerter Knochenregeneration regeneriertem Knochen aufweisen. Jedoch weisen die eingesetzen Biomaterialien unterschiedliche Charakteristiken auf und können die Zusammensetzung des erzeugten Knochens und damit das Osseointegrationsverhalten der eingebrachten Implantate beeinflussen. Eine wichtige Eigenschaft ist die Porosität des Knochenmaterials.

Ziel dieser experimentellen präklinischen Studie war es, den Einfluss eines Knochenersatzmaterials aus biphasischem Calziumphosphat mit unterschiedlichen Oberflächenporositäten auf die Knochenneubildung und Osseointegration von Titanimplantaten innerhalb von Alveolarkammdefekten zu ermitteln.

Methode

In Rahmen dieser experimentellen präklinischen Studie wurden standardisierte Alveolarkammdefekte mit einer zylindrischen Form und einer Größe von 6mm x 6mm im Unterkiefer von 8 Minischweinen erzeugt. Anschliessend erfolgte die Füllung der Defekte mit drei Varianten eines biphasischen Calziumphosphats: BCP1 (90% Tricalciumphosphat, 10% Hydroxylapatit / Partikelgröße 250-1000 µm), BCP2 (90% Tricalciumphosphat, 10% Hydroxylapatit / Partikelgröße 250-1000 µm, geringere Makro- und Mikroporosität) und BCP3 (90% Tricalciumphosphat, 10% Hydroxylapatit / Partikelgröße 250-1000 µm, gesteigerte Makroporosität und geringer Mikroporosität). Als Kontrolle diente bovines Hydroxylapatit. Nach einer Heilzeit von 12 Wochen wurden Titanimplantate in die augmentierten Defekte eingebracht. Nach einer weiteren Heilungsperiode von 8 Wochen erfolgte die Entnahme der behandelten Kieferregionen und die Aufbereitung der Proben für die histologische Untersuchung. Die histomorphometrische Analyse diente der Erfassung des mineralisierten Gewebes, des verbliebenen Knochenmaterials sowie des Knochen-Implantat Kontaktes.

Ergebnisse

Der postoperative Heilungsverlauf zeigte keine Besonderheiten wie allergische Reaktionen, Schwellung oder Infektionen. Nach Abschluss der beiden Heilungsphasen konnte in allen histologischen Proben eine Knochenregeneration im Bereich des ehemaligen Defektes beobachtet werden. In den augmentierten Bereichen befanden sich Geflechtknochen und Blutgefäße sowie darin eingebettete Knochenmaterialpartikel. Dabei befanden sich die Partikel in engem Kontakt zur neugebildeten Knochensubstanz. Es konnten Unterschiede zwischen den verschiedenen Knochenmaterialien identifiziert werden. Während das bovine Hydroxylapatit meistens in direktem Kontakt zum Knochen war, wurden um die Partikel der unterschiedlichen Varianten des biphasischen Calziumphosphats häufig Zonen nicht mineralisierten Gewebes beobachtet. In diesen Zonen zeigte sich eine Auflösung und Fragmentierung der Partikel. Die Resorptionsvorgänge waren bei allen Calziumphosphatvarianten vergleichbar. Die histomorphometrische Analyse ergab, dass der Anteil mineralisierten Gewebes in allen Gruppen des biphasischen Calziumphosphats ebenfalls vergleichbar war, während die ehemaligen Defekte mit bovinem Hydroxylapatit leicht geringer mineralisiert waren. Die Unterschiede zeigten jedoch keine statistische Signifikanz ($p > 0.05$). Den größten Anteil an residuellem Knochenmaterial konnte für das bovine Hydroxylapatit identifiziert werden. Den geringsten Anteil zeigte die Calzuimphosphatvariante mit der höchsten Mikroporosität und war im Vergleich zum bovinen Hydroxylapatit statistisch signifikant. Die Mittelwerte für mineralisiertes Gewebe im ehemaligen Defektbereichs waren bei allen Knochenmaterialien vergleichbar. Der Knochen-Implantat Kontakt erreichte in den Versuchsgruppen Mittelwerte von 73,3% - 84,1% und unterschied sich statistisch nicht signifikant ($p > 0,05$).

Diskussion

Ziel dieser Studie war die histologische Untersuchung des Einflusses von drei Knochenmaterialien aus biphasischem Calziumphosphat mit unterschiedlichen Oberflächentopographien auf die Knochenneubildung und die Osseointegration von

Titanimplantaten bei Minischweinen. Das ausgewählte experimentelle Defektmodell stellt einen etablierten Standard zur Untersuchung von Knochenumbauprozessen in der Implantologie dar (Mardas et al. 2014). Ähnliche zylindrische Defekte wurden auch in früheren experimentellen Studien beschrieben und als unkritisch eingestuft, da sie eine gewisse spontane Heilung ermöglichten (Jensen & Terheyden 2009, Dahlin et al. 2015). Die histologische Analyse der Defektregionen zeigte, dass alle Gruppen, die 3 Varianten des biphasischen Calziumphosphats sowie das bovine Hydroxylapatit, nach 20 Wochen gleichermaßen und homogen in ein neu gebildetes Gerüst aus spongiösen Knochen integriert waren. Dies bestätigten auch die vergleichbaren Mittelwerte für neugebildeten Knochen. Obwohl das bovine Hydroxylapatit niedrigere Werte zeigte als alle Varianten des biphasischen Calzuimphosphats, konnten keine statistisch signifikanten Unterschiede festgestellt werden. Die stärkere Knochenneubildung in den mit biphasischen Calziumphosphaten behandelten Defektregionen könnte durch die höhere Resorptionrate im Vergleich zu bovinem Hydroxylapatit erklärt werden. Alle Varianten des biphasischen Calziumphosphats zeigten niedrigere Anteile an verbliebenem Knochenmaterial, verglichen mit den mit bovinem Hydroxylapatit behandelten Regionen, in der Gruppe BCP1 mit statistischer Signifikanz. Auch die histologische Analyse konnte die Resorption der Calziumphosphatpartikel bestätigen. Eine frühere experimentelle Untersuchung zu einer ähnlichen Variante von mikrostrukturiertem biphasischen Calziumphosphat (BCP1, 10% Hydroxylapatit / 90% biphasisches Calziumphosphat, Partikelgröße 500–1000µm) dokumentierte vergleichbare Beobachtungen (Dahlin et al. 2015). In dieser Studie wurden zylindrische Alveolarkammdefekte mit biphasischem Calziumphosphat des Typs BCP1, einer weiteren Variante mit einem anderem Mischungverhältnis und Partikelgröße (60% Hydroxylapatit / 40% biphasisches Calziumphosphat, Partikelgröße 500-1000 µm) sowie bovinem Hydroxylapatit gefüllt. Nach 8 Wochen zeigten die mit BCP1 behandelten Defekte signifikant höhere Werte für mineralisiertes Knochengewebe. Beim Vergleich der histomorphometrischen Ergebnisse nach 3 und 8 Wochen konnte eine Resorptionsdynamik von BCP1 dokumentiert werden. Dies zeigte sich durch stetig sinkende Mittelwerte für das verbliebende Knochenersatzmaterial (45,5%

nach 3 Wochen und 38,3% nach 8 Wochen) (Dahlin et al. 2015). Obwohl die Beobachtungen der von Dahlin et al. durchgeführten Studie und der vorliegenden Analyse ähnlich sind, muss beachtet werden, dass die Heilungsperiode in dieser Untersuchung mit 20 Wochen deutlich länger war und die ermittelten Werte auf einer fortgeschritteren Knochenreifung basiert. Darüber hinaus kann man annehmen, dass die auf das erste Heilungsintervall gefolgte Implantatinsertion einen Einfluss auf die Knochenumbauvorgänge hatte. Die Implantatinsertion und die damit verbundene Osseointegration könnte einen erneuten Stimulus auf die Knochenremodellationsprozesse ausgeübt und zu einer weiteren Resorption des biphasischen Calziumphosphats beigetragen haben. Die vorliegende Studie zeigte die ersten histologischen Daten zu den hier untersuchten Varianten des biphasischen Calziumphosphats (BCP1-3) nach zweizeitiger Augmentation und Implantation in zylinderförmigen Alveolarkammdefekten. In früheren präklinischen Untersuchungen im Hundemodell wurde biphasisches Calziumphosphat (60% Hydroxylapatit / 40% biphasisches Calziumphosphat, Partikelgröße 500-1000µm) bei Dehiszenz- sowie Satteldefekten zusammen mit einer simultanen oder zweistufigen Implantation analysiert (Schwarz 2012, Mihatovic 2012, Sager et al. 2012). Es wurde eine fortschreitende Resorption der Materialpartikel zwischen 9 und 10 Wochen festgestellt und deutlich niedrigere Werte für verbleibendes Knochenersatzmaterial im Vergleich zu bovinem Hydroxylapatit ermittelt (Schwarz et al. 2012, Mihatovic et al. 2012, Sager et al. 2012). Das ausgeprägte Knochenremodelling in den mit biphasischen Calziumphosphat behandelten Defektregionen wurde auch durch eine erhöhte Reaktivität von Kollagen Typ I, Osteocalcin und Transglutaminase-Antigen im angrenzenden nicht mineralisierten Gewebe belegt (Sager et al. 2012). In der vorliegenden Studie konnten ähnliche Beobachtungen gemacht werden. Obwohl keine immunhistochemische Analyse durchgeführt wurde, zeigte die histologische Analyse gut in die Knochenmatrix integrierte Calzumphosphatpartikel, welche jedoch von einer angrenzenden Zone nicht mineralisierten Gewebes umgeben waren. In dieser Zone befanden sich häufig kleine Knochenmaterialpartikel. Auch Dahlin et al. (Dahlin et al. 2015) berichtete über dieses Phänomen und vermutete, dass dies mit einer Dissolution von Ionen aus dem

Calziumphosphat in das angrenzende Gewebe zusammenhängt. Tatsächlich zeigten vergangene Studien, dass die Knochenumbauvorgänge und die anschließende Resorption von Calziumphosphat durch seine Mikrostruktur beinflusst wird. Dabei wurde eine stärkere Resorptionsaktivität für Materialien im Submikrometerbereich gegenüber Materialien im Mikrometerbereich festgestellt (Davison et al. 2014a, Davison et al. 2014b). Jedoch bedarf es weiterführende Studien zu den spezifischen Resorptionscharakteristika der unterschiedlichen Calziumphosphatvarianten, um fundierte Aussagen treffen zu können.

Ein weiterer Fokus der vorliegenden Studie war die Osseointegration der eingebrachten Implantate. Es konnten nach einer Einheilzeit von 8 Wochen in allen Versuchsgruppen vergleichbare Knochen-Implantat Kontakte ermittelt werden. Die Werte lagen zwischen 74,3% ± 20,5%) und 84,1% ± 7,8%) und sind vergleichbar mit Knochen-Implantat Kontaktenwerten in unbehandelten Knochen. Vorangegangene Studien zeigten für die gleiche Implantatoberfläche ähnliche Werte nach einer Einheilzeit von 2 Wochen (71,9% ± 4,0% bis 74,9% ± 7,8%) (Schwarz et al. 2007a, Schwarz et al. 2007b). Auch frühere Untersuchungen, welche auch die Augmentation mittels biphasichem Calziumphosphat und bovinem Hydroxylapatit sowie späterer Implantation untersuchten, dokumentierten vergleichbare Werte 2 Wochen nach Implantation (54,1% ± 22,6% bis 67,7% ± 16,9%) (Mihatovic et al. 2012). Im Rahmen der Grenzen der vorliegenden Studie wurde geschlussfolgert, dass alle untersuchten Knochenmaterialien die Knochenbildung sowie die Osseointegration von Titanimplantaten unterstützen.

Influence of two barrier membranes on staged guided bone regeneration and osseointegration of titanium implants in dogs: part 1. Augmentation using bone graft substitutes and autogenous bone.

Hintergrund

Die Ergebnisse der ersten in dieser Schrift beschriebenen Studie zeigten, dass komplexe Alveolarkammdefekte mithilfe gesteuerter Knochenregeneration und dem Einsatz von Kollagen- und Polylaktidsäuremembranen in Kombination mit Knochenmaterialien erfolgreich regeneriert werden konnten. Die zweite Studie doukumentierte eine gute Osseointegration von Implantaten in zuvor augmentierten Defektbereichen. Die vorliegende Untersuchung kombinierte Aspekte beider vorangegangenen Untersuchungen. Zum einen die Behandlung von anspruchsvollen sattelförmigen Alveolarkammdefekten mittels gesteuerter Knochenregeneration unter Verwendung von Membranen und Knochenmaterial, zum anderen die Osseointegration von Titanimplantaten innerhalb der ehemaligen Defektbereiche. Ein neuer Ansatzpunkt dieser Studie war die Verwendung einer neu entwickelten Polyethylenglykolmembran, welche durch ihre gute klinische Anwendbarkeit, Formstabiliät und Resorptionsverhalten, Vorteile bei der Regeneration größerer Defekte gegenüber Kollagenmembranen versprach.

Methode

In dieser Studie wurden 4 sattelförmige Defekte im Unterkiefer von 6 Foxhunden präpariert und randomisiert mit bovinem Hydroxylapatit oder biphasischem Calziumphosphat gefüllt. Beiden Knochenmaterialien wurde autologer Knochen im Verhältnis 1:1 beigemischt. Anschließend erfolgte die Abdeckung des augmentierten Bereichs entweder mit einer Polyethylenglykolmembran oder einer nativen Kollagenmembran. Nach einer Heilungsperiode von 8 Wochen wurden Titanimplantate eingebracht und für weitere 2 Wochen zur Einheilung belassen. Darauf folgte die Entnahme der behandelten Kieferbereiche für die histologische Aufbereitung. Gegenstand der histomorphometrischen Analyse war der regenerierte Defektbereich, mineralisieres Gewebe, verbliebenes Knochenmaterial sowie der Knochen-Implantat Kontakt.

Ergebnisse

Die klinische Heilung verlief komplikationsfrei und keine der behandelten Defekte zeigte Wundheilungsstörungen. Histologisch waren die ehemaligen Wundbereiche in allen Versuchgruppen volumenstabil und durch eine gute knöcherne Kontur bukkalen sowie lingualen charakterisiert. Die Partikel beider Knochenmaterialien waren gut in die neugebildete Knochenstruktur integriert. Die Anwendung der Polyethylenglykolmembran war häufig mit einer verbesserten Kortikalisation des Alveolarknochens im Vergleich zur Kollagenmembran verbunden. Die Titanimplantate konnten mit Primärstabilität in die augmentierten Bereiche inseriert werden. Bei der histologischen Untersuchung ließ sich Geflechtknochen mit zahlreichen Blutgefäßen in engem Kontakt zu den Partikeln der beiden verwendeten Knochenmaterialien innerhalb der mittels Polyethylenglykolmembran und Kollagenmembran behandelten Defekte zu erkennen. Es fiel auf, dass die Calziumphosphatpartikel häufiger von nicht mineralisiertem Gewebe umgeben waren als das Hydroxylapatit und in diesen Zonen eine Fragmentierung des Knochenmaterials entstand. In allen Versuchsgruppen waren die Titanimplantate in engem Kontakt zu maturiertem Geflechtknochen, teilweise auch in Kontakt zu den Knochenmaterialien, jedoch ohne Interposition von nicht mineralisiertem Gewebe. Die Mittelwerte der analysierten Parameter waren in allen Versuchsgruppen vergleichbar und ohne statistische Signifikanz. Die mit der Polyethylenglykolmembran behandelten Bereiche zeigten leicht verbesserte Mittelwerte für den regenerierten Bereich, den Anteil mineralisierten Gewebes und Knochen-Implantat Kontakt. Diese waren jedoch statistisch nicht signifikant. Lediglich die Mittelwerte für den regenerierten Defektbereich der Gruppe Calziumphosphat + autologer Knochen + Polyethylenglykolmembran waren statistisch signifikant höher als die Gruppe Calziumphosphat + autologer Knochen + Kollagenmembran.

Diskussion

Diese experimentelle Studie diente der histologischen Bewertung der gesteuerten Knochenregeneration sattelförmiger Alveolarfortsatzdefekte und der anschliessenden Implantation von Titanimplantaten. Die Augmentation erfolgte mit zwei unterschiedlichen Knochenersatzmaterialien, einem biphasischen Calziumphosphat und einem xenogenen

Hydroxylapatit, jeweils gemischt mit autologen Knochenpartikeln (Verhältnis 1:1). Als Barrieremembranen wurden eine Polyethylenglykolmembran oder einer porcinen Kollagenmembran verwendet. Das ausgewählte Defektmodel ist zur Untersuchung der gesteuerten Knochenregeneration etabliert (Schenk et al. 1994; Simion et al. 1999; Bornstein et al. 2007). Die gewonnenen Daten zeigten, dass nach einer Heilunsperiode von 10 Wochen alle Augmentationsverfahren eine homogene Knochenformation und Osseointegration von Titanimplantaten im Defektbereich begünstigten. Beide Arten von Barrieremembranen stabilisierten den Defektbereich aller Gruppen während der gesamten Heilungsperiode suffizient. Die histologische Analyse erfasste folgende Parameter: Regenerierter Defektbereich, mineralisiertes Gewebe, nicht mineralisiertes Gewebe sowie Knochen-Implantat Kontakt. Die Auswertung der erhobenen Daten zeigte leicht erhöhte Werte für den regenerierten Defektbereich in den mit der Polyethylenglycolmembran behandelten Gruppen. Die Kombination von Polyethylenglycolmembran und biphasischem Calziumphosphat mit autologem Knochen zeigte die statististisch signifikant höchsten Werte. Der erfolgreiche Einsatz porciner Kollagenmembranen mit bovinem Hydroxylapatit zur Regeneration von Alveolarkammdefekten ist wissenschaftlich gut belegt (Bornstein et al. 2007). Bornstein et al. untersuchten die Knochenregeneration mittels eines Gemisches aus bovinem Hydroxylapatit und autologem Knochen mit oder ohne Abdeckung durch eine porcine Kollagenmembran. Nach 8 und 16 Wochen Heilung wurde eine kuppelförmige Knochenregeneration in den zuvor erzeugten sattelförmigen Defekten beobachtet. Nach 8 Wochen lagen die Mittelwerte für den regenerierten Bereich in der Gruppe ohne Membran bei 28.8mm^2 und in der Gruppe mit Membran bei 30.2mm^2. Diese Werte blieben bis zum Heilungszeitpunkt 16 Wochen nahezu unverändert (Bornstein et al. 2007). Die höheren Werte für den regenerierten Defektbereich dieser Studie im Vergleich zu der vorliegenden Studie sind wahrscheinlich darin begründet, dass in der vorliegenden Studie nach 8 Wochen Implantate gesetzt wurden und diese den behandelten Defektbereich im Bezug auf die histomorphometrisch bewertete Fläche reduzierten. Die Beobachtung, dass die Verwendung der Kollagenmembran keinen positiven Effekt auf das Regenerationsergebnis hatte (Bornstein et al. 2007) spiegelte sich in den

Ergebnissen der vorliegenden Studie wider. Dieser Umstand stützt die These, dass die verlängerte Barrierefunktion der Polyethylenglycolmembran im Vergleich zur Kollagenmembran für die bessere Regeneration verantwortlich gewesen sein könnte. Die verlängerte Barrierefunktion wurde in vorangegangenen Untersuchungen nachgewiesen (Herten et al. 2009; Thoma et al. 2009). Weiterhin wurde bei unterschiedlichen Indikationen festgestellt, dass sich trotz der verlängerten Biodegradation kein signifikant erhöhtes Risiko für Entzündungen im Vergleich zu porcinen Kollagenmembranen zeigte (Jung et al. 2006, 2009b, 2009a; Thoma et al. 2009). Auch für Kollagenmembranen kann die Biodegradationszeit durch Techniken wie Quervernetzung verlängert werden. Verbesserte Membranstabilität und Knochenregeneration durch chemische Quervernetzung wurde in experimentellen sowie klinischen Untersuchungen demonstriert (Bornstein et al. 2007; Schwarz et al. 2008; Becker et al. 2009). Jedoch zeigte sich, dass eine chemische Quervernetzung im Falle einer vorzeitigen Membranexposition eindeutig zu einer Verschlechterung der Wundheilung sowie Wundinfektionen führte (Bornstein et al. 2007; Becker et al. 2009). Die histologische Untersuchung der vorliegenden Studie dokumentierte eine homogene Integration beider Knochenmaterialien in neuen Geflechtknochen innerhalb der Defekte nach 10 Wochen. Die osteokonduktiven Eigenschaften beider Knochenersatzmaterialien wurden bis dato nur für die humane Sinusbodenaugmentation untersucht (Cordaro et al. 2008; Froum et al. 2008; Lindgren et al. 2010). Dort konnte gezeigt werden, dass beide Materialien vergleichbare Mengen an Knochenneubildung erzielten und ein ähnliches histologisches Erscheinungsbild aufwiesen. Jedoch war das biphasische Calziumphosphat häufiger von nicht mineralisiertem Gewebe umgeben (Cordaro et al. 2008; Lindgren et al. 2009). Diese Beobachtung wurde in der vorliegenden Studie nicht gemacht, da in allen Gruppen ähnliche Mengen an nicht-mineralisierten Gewebe ermittelt wurden. Möglicherweise hat der Anteil autologen Knochens mit seinen osteogenetischen und osteoinduktiven Eigenschaften zu einer Steigerung des mineralisierten Gewebeanteils beigesteuert (Chiriac et al. 2005). Dennoch deuten die histologischen Befunde, gemeinsam mit vorangegangenen Beobachtungen, auf unterschiedliche Resorptionscharakteristika der untersuchten Knochenmaterialien hin

(Cordaro et al. 2008). Jensen et al. beobachtete eine beginnende Auflösung der Partikel des biphasischen Calziumphosphats nach 8 Wochen in Unterkieferdefekten von Minischweinen (Jensen et al. 2007). Die histologische Analyse zeigte mehrkernige Riesenzellen auf der Oberfläche der Partikel, die nicht von mineralisiertem Gewebe bedeckt waren. Obwohl keine Resorptionslakunen auf der Oberfläche des Knochenersatzmaterials zu erkennen waren, penetrierte der histologische Farbstoff in die Partikeloberfläche als Hinweis auf eine beginnende Auflösung (Jensen et al. 2007).

Ein wichtiger Bestandteil der vorliegenden Studie war die Untersuchung des Osseointegrationsverhaltens von Titanimplantaten im Bereich der zuvor augmentierten Alveolarkammareale. Trotz des unterschiedlichen Resorptionsverhaltens der beiden verwendeten Knochenersatzmaterialen und nachgewiesenen Anteilen nicht mineralisierten Gewebes, wurde eine gute Osseointegration der Titanimplantate im Defektbereich nach 10 Wochen in allen Gruppen dokumentiert. Die ermittelten Werte für den Knochen-Implantat Kontakt waren in allen Versuchsgruppen vergleichbar und mit Mittelwerten zwischen 71,3% - 72,4% im Einklang mit vorangegangenen Studien, welche die Osseointegration von Titanimplantaten nach 2 Wochen Einheilung untersuchten (Schwarz et al. 2007a, 2007b). Auch das sehr langsam resorbierbierende Hydroxylapatit erlaubte eine ungestörte Osseointegration (Mordenfeld et al. 2010). Die moderat besseren Knochen-Implantat Kontakte in den Gruppen der Polyethylenglycolmembran im Vergleich zur den mit der Kollagenmembran behandelten Defekte waren statistisch nicht signifikant. Die guten Knochen-Implantat Kontakte in allen Versuchsgruppen verdeutlichten, dass durch gesteuerte Knochenregeneration erzeugter Knochen und unbehandelter Knochen ein ähnliches Potential für die Einheilung von Implantaten haben. Diese Annahme wurde durch eine aktuelle experimentelle Studie unterstützt (Artzi et al. 2010). Sie konnte eine gleichwertige Integration von Titanimplantaten in mit bovinem Hydroxylapatit regenerierten Defekten und unbehandeltem Knochen dokumentieren.

Trotz vielversprechender Daten sind weitere Untersuchungen notwendig, um den Einfluss unterschiedlicher Kombinationen aus biphasischem Calziumphosphat oder Hydroxylapatit mit

einer Polyethylenglycolmembran zur Regeneration von Alveolarkammdefekten zu analysieren. Im Rahmen der Grenzen der vorliegenden Studie konnte gezeigt werden, dass alle verwendeten Augmentationsverfahren die Knochenregeneration und Osseointegration von Titanimplantaten förderten.

Influence of two barrier membranes on staged guided bone regeneration and osseointegration of titanium implants in dogs. Part 2: augmentation using bone graft substitutes.

Hintergrund

Die folgende Studie wurde als Erweiterung der zuvor beschriebenen Untersuchung durchgeführt. Es wurde das gleiche Studienmodel verwendet und die gleichen Materialkombinationen eingesetzt. Hier lag der Fokus auf den eingesetzten Knochenmaterialien ohne Zusatz von autologer Knochenspäne und dem damit verbundenen Effekt auf die Knochenneubildung und Osseointegration der Titanimplantate.

Methode

Es wurden sattelförmige Alveolarkammdefekte im Unterkiefer von 6 Foxhunden erzeugt und mit bovinem Hydroxylapatit und biphasischem Calziumphosphat gefüllt. Anschließend erfolgte die Abdeckung der augmentierte Bereich mit einer Polyethylenglycolmembran oder einer nativen Kollagenmembran. Nach acht Wochen Heilzeit wurden Titanimplantate in die ehemaligen Defektbereiche eingesetzt. Nach einer weiteren Heilungsperiode von 2 Wochen erfolgte die histomorphometrische Analyse und Ermittlung der Anteile mineralisierten Gewebes sowie der Knochen -Implantat Kontakte.

Ergebnisse

Im Rahmen der gesamten Wundheilungsphasen konnten keine Besonderheiten wie Wundheilungsstörungen oder Infektion verzeichnet werden. Nach der ersten Heilungsperiode von 8 Wochen stellte sich ein gut regenerierter Alveolarkamm dar und erkennbare Partikel der beiden Knochenmaterialien waren in die kortikale Knochenstruktur eingebaut. Es konnten in allen augmentierten Bereichen Titanimplantate mit Primärstabilität eingesetzt werden. Histologisch war in allen Versuchsgruppen eine homogene Knochenstruktur im ehemaligen Defektbereich erkennbar. Vereinzelt konnten Partikel beider Knochenmaterialien im bedeckenden Bindegewebe identifiziert werden. Die Knochendichte erschien in den mit Polyethylenglykolmembran behandelten Bereichen höher, während die Präparate mit Kollagenmembran gehäuft eine Invasion von Weichgewebe in den Defektbereichen aufwiesen. Die Calziumphosphatpartikel zeigten verstärkt Kontakt zu nicht mineralisiertem Gewebe und Zeichen einer Fragmentierung. Die Titanimplantate waren in allen suffizient verknöcherten Defekten in direktem Kontakt zu umgebender Knochenstruktur, während sie in den Präparaten mit weichgewebiger Invasion von dichtem Bindewebe umhüllt waren. Der Vergleich der Mittelwerte aller Parameter brachte ähnliche Ergebnisse in allen Versuchsgruppen hervor. Die mit Polyethylenmembran behandelten ehemaligen Defekte zeigten die höchsten Werte für den regenerierten Bereich, mineralisiertes Gewebe sowie den Knochen-Implantat Kontakt.

Diskussion

Diese Analyse wurde als Erweiterung der zuvor beschriebenen Studie durchgeführt. Die Untersuchung konzentrierte sich auf die eingesetzten Knochenmaterialien ohne Zusatz von autologer Knochenspäne und dem damit verbundenen Effekt auf die Knochenneubildung und Osseointegration der Titanimplantate. Es wurden wieder eine Polyethylenglykolmembran oder eine Kollagenmembran, in Kombination mit einem biphasischen Calziumphosphat oder bovinem Hydroxylapatit, zur gesteuerten Knochenregeneration und Osseointegration von Titanimplantaten in sattelförmigen Alveolarfortsatzdefekten angewendet. Das verwendete Defektmodell ist zur Bewertung der gesteuerten Knochenregeneration gut etabliert (Schenk et

al. 1994; Bornstein et al. 2007; Schwarz et al. 2011). Im Verlauf der Studie zeigte sich bei beiden verwendeten Membranen und Knochenmaterialien eine unaufffällige Wundheilung. Diese Beobachtung kann durch vorangegangene experimentelle Tierstudien bestätigt werden (Bornstein et al. 2007; Jung et al. 2009; Schwarz et al. 2011). Innerhalb ihrer Grenzen bewies die histologische Analyse der Studie, dass alle Behandlungskombinationen zu einer vergleichbaren Knochenregeneration und Osseointegration der Titanimplantate führt. Dies zeigte sich durch gesteigerte Werte für mineralisiertes Gewebe sowie vergleichbare Werte für den Implantat-Knochen-Kontakt. Obwohl die Verwendung der Polyethylenglykolmembran leicht erhöhte Werte für mineralisiertes Gewebe und Knochen-Implantat-Kontakt im Vergleich zur Kollagenmembran erkennen ließ, waren die Unterschiede statistisch nicht signifikant. Im Rahmen der histologischen Untersuchung wurden auch Differenzen zwischen den Membranen festgestellt. Während die Polyethylenglykolmembran durch eine homogenere Knochenregeneration im Defektbereich charakterisiert war, zeigten die mit der Kollagenmembran behandelten Areale häufiger Anteile nicht mineralisierten Gewebes um die Partikel des Knochenersatzmaterials. Trotz der eindeutigen Stabilisation der Knochenmaterialien im Defekt durch beide Membranen, schien die schnellere Biodegradation der Kollagenmembran ein Einwachsen von Bindegewebe in den Defektbereich zuzulassen (Herten et al. 2009, Thoma et al. 2009). Aktuelle Studien zeigten eine homogene Knochenregeneration mit einer Kollagenmembran zusammen mit bovinem Hydroxylapatit oder biphasischem Calzuimphosphat und Beimischung von autologen Knochenpartikeln (Bornstein et al. 2007; Schwarz et al. 2011). Es präsentierte sich ein Gefüge von Geflechtknochen mit zahlreichen Blutgefäßen und knochenbildenden Zellen bei der Verwendung von Polyethylenglykol- und Kollagenmembranen in Verbindung mit bovinem Hydroxylapatit oder biphasischem Calziumphosphat und Zusatz von autologen Knochenpartikeln. Die Mittelwerte für mineralisiertes Gewebe und Implantat-Knochen-Kontakt waren in allen Gruppen vergleichbar und statistisch nicht signifikant (Schwarz et al. 2011). Unter Berücksichtigung der Daten der vorliegenden Studie sowie vergleichbarer Untersuchungen lässt sich schließen, dass die mit Kollagenmembran behandelten Defekte

vom Zusatz autologen Knochens im Hinblick auf die Qualität des Regenerats mehr profitieren, als die mit der Polyethylenglycolmembran behandelten Defekte. Die guten osteoinduktive und osteogenetischen Fähigkeiten (Chiriac et al. 2005) des autologen Knochens kompensierten die schnelle Biodegradation des Kollagens und förderten die Knochenregeneration. Diese Annahme muss jedoch durch kontrollierte experimentelle Daten, welche den Einfluss von jeweils Hydroxylapatit und biphasischem Calziumphosphat alleine oder als Mischung mit autologen Knochenpartikeln auf die Knochenregeneration bei einer ähnlichen Defektkonfiguration überprüft werden. Aktuelle klinische Studien verglichen die osteokonduktiven Eigenschaften beider Knochenerstzmaterialien zur Sinusbodenaugmentation (Cordaro et al. 2008; Froum et al. 2008; Lindgren et al. 2010). Es zeigte sich, dass die Calziumphsphatpartikel häufiger von nicht mineralisiertem Gewebe umgeben waren als die mit Hydroxylapatit behandelten Stellen (Cordaro et al. 2008; Lindgren et al. 2009). Obwohl die vorliegende histomorphometrische Analyse vergleichbare Werte für die Anteile nicht mineralisierten Gewebes in beiden Gruppen hervorbrachte, zeigte die semi-quantitative Analyse häufiger Areale nicht mineralisierten Gewebes in Kontakt mit Calziumphosphatpartikeln. Diese Areale waren durch eine Dissolution der Partikel in kleinere Fragmente gekennzeichnet. Diese Beobachtung deckt sich mit experimentellen und klinischen Studien (Jensen et al. 2007; Cordaro et al. 2008; Schwarz et al. 2011). Die Osseointegration der Titanimplantate war in allen Versuchsgruppen suffizient mit Mittelwerten für den Implantat-Knochen-Kontakt zwischen 54,1% und 60%. Die histomorphometrische Analyse zeigte die höchsten Werte in Defekten, welche mit der Polyethylenglycolmembran behandelt wurden, jedoch ohne statistische Signifikanz. Die Osseointegration von Titanimplantaten in Defekten, welche mit einer Polyethylenglycolmembran versorgt wurden, zeigte sich verbessert, jedoch auch ohne statistische Signifikanz (Schwarz et al. 2011). Die erhobenen Daten veranschaulichen, dass die Verwendung von Hydroxylapatit und biphasischem Calziumphosphat in Kombination mit beiden Barrieremembranen zu einer guten Knochenregeneration und späterer Osseointegration von Titanimplantaten führte. Alle Augmentationsvarianten zeigten vergleichbare Ergebnisse.

Immunohistochemical analysis of staged guided bone regeneration and osseointegration of titanium implants using a polyethylene glycol membrane.

Hintergrund

Auf Basis der beiden zuvor angeführten Studien erfolgte in dieser Untersuchung bei gleichem Versuchsaufbau eine immunhistochemische Analyse der mit Polyethylenglykolmembran und Calziumphosphat oder Hydroxylapatit behandelten Defekte. Als zusätzlicher Heilungszeitpunkt wurde zu 8+2 Wochen noch der Zeitpunkt 10+2 Wochen hinzugefügt. In den mit den unterschiedlichen Materialkombinationen behandelten Defektbereichen wurde die Knochenstoffwechselaktivität bestimmt. Dies erfolgte mittels Bestimmung der Osteocalcinaktivität in den histologischen Präparaten. Die Osetocalcinaktivität dient als Marker für die Knochenneubildung.

Methode

Die aus den vorangegangenen Untersuchungen gewonnenen histologischen Gewebepräparate wurden für die immunhistochemische Analyse vorbereitet. Dazu wurden die Gewebeschnitte mit primären monoklonalen Antikörpern (Maus) gegen Osteocalcin und mit entsprechenden unspezifischen Antikörpern (Maus IgG1) als negative Kontrolle markiert. In den markierten Gewebeschnitten wurde der behandelte Bereich identifiziert und die dortige Osteocalcin-Antigen Aktivität ermittelt.

Ergebnisse

Bei der Analyse der Osteocalcinaktivität nach 8+2 Wochen zeigte sich im regenerierten Defektbereich eine stärkere Osteocalcinaktivität in den nicht mineralisierten Bereichen im Vergleich zu den mineralisierten Arealen. Während die Geflechtknochenbereiche und die zahlreichen Osteozyten in großen Lakunen nur durch leichte Osteocalcinaktivität charakterisiert waren, war das nicht mineralisierte Gewebe in den Randbereichen der Knochentrabekel durch ein stärkeres Osteocalcinsignal gekennzeichnet. Obwohl die Versuchsgruppe Hydroxylapatit + Polyethylenglycolmembran eine etwas intensivere

Osteocalcinaktivität in mineralisierten sowie nicht mineralisierten Bereichen aufwies, konnte keine statistische Signifikanz im Vergleich zur Gruppe Calziumphosphat + Polyethylenglycolmembran festgestellt werden ($p>0,05$). Nach 12+2 Wochen zeigten alle Gruppen nur eine sehr geringe Osteocalcinaktivität in allen Defektbereichen und an der Implantatoberfläche. In der Gruppe Calziumphosphat + Polyethylenglycolmembran schien die Aktivität etwas ausgeprägter als in der Gruppe Hydroxylapatit + Polyethylenglycolmembran zu sein, jedoch ohne statistische Signifikanz ($p>0,05$).

Diskussion

In den beiden vorangegangenen Studien wurden mittels histologischer Analyse Erkenntnisse zur Regeneration der Alveolarkammdefekte und Osseointegration der Titanimplantate gewonnen. Zusätzlich erfolgte bei dieser Studie eine immunhistochemische Analyse der Proben, um weiterführende Informationen zu den Knochenstoffwechselvorgängen in den augmentierten Alveolarkammbereichen zu erhalten. Die Ergebnisse der immunhistochemischen Analyse zeigten, dass alle untersuchten Augmentationsverfahren eine vergleichbare Osteocalcinaktivität während der Knochenregeneration und Osseointegration der Titanimplantate aufwiesen. Die Osteocalcinaktivität wurde jedoch vorwiegend zum früheren Heilungszeitpunkt nach 8+2 Wochen beobachtet, während nach 12+2 Wochen fast keine Aktivität aufgezeichnet wurde. Insbesondere nach 8+2 Wochen waren die Mittelwerte für Osteocalcin in den Defekten mit bovinem Hydroxylapatit höher, als die entsprechenden Defekte mit biphasischem Calziumphosphat (32,7% ± 8,9% vs. 24,4% ± 6,6%). Die Mittelwerte der Osteocalcinaktivität war innerhalb der behandelten Defektregionen heterogen verteilt, jedoch zeigte sich in allen Versuchsgruppen, dass die nicht mineralisierten Bereiche im Vergleich zu den mineralisierten Bereichen eine deutlich höhere Osteocalcinaktivität aufwiesen. Dies könnte auf eine fortschreitende Mineralisierung der extrazellulären Matrix (Owen et al. 1990) oder einen Remodellierungsprozess hindeuten (Hauge et al. 2001). Vor allem in der Nähe der Implantatoberfläche sowie um die Knochenersatzpartikel wurde eine erhöhte Osteocalcinaktivität nachgewiesen. Insbesondere der Heilungszeitpunkt 8+2 Wochen war durch eine hohe Aktivität im Bereich der Knochenmaterialpartikel charakterisiert und weist

auf fortschreitendes Knochenremodelling und Reaktivität beider Knochenmaterialien hin. Das bovine Hydroxylapatit zeigte geringfügig höhere Osteocalcinmittelwerte im Vergleich zum biphasischen Calciumphosphat. Daraus lässt sich auf eine verzögerte Knochenreifung des Hydroxylapatits schliessen. Nach 12+2 Wochen Wundheilung nahmen die Mittelwerte für Osteocalcin bei beiden Knochenamterialien ab (biphasisches Calziumphosphat: 1,6% ± 1,2%, bovines Hydroxylapatit: 2,1% ± 1,4%), ohne statistisch signifikante Unterschiede zu erreichen. Die Abnahme der Osteocalcin-Antigen-Reaktivität nach 12+2 Wochen zeigt deutlich, dass Osteocalcin hauptsächlich während der Anfangsphase der Knochenheilung exprimiert und innerhalb des Reifeprozesses reduziert wird. Auf Basis der geringen Osteocalcinwerte nach 12+2 Wochen könnte man schliessen, dass es innerhalb der behandelten Defekte zu einer fast vollständigen Reifung gekommen ist. Damit ist eine optimale Grundvorraussetzung für eine adäquate Osseointegration von Titanimplantaten geschaffen. Darüber hinaus konnte eine proportionale Verteilung der Osteocalcin-Antigenreaktivität innerhalb des behandelten Bereichs und der Zonen des ursprünglichen Knochens nachgewiesen werden. Daraus lässt sich ableiten, dass der regenerierte Knochen möglicherweise nicht als qualitativ kompromittierter Knochen angesehen werden muss. Die Beobachtung stimmt mit den Ergebnissen neuer tierexperimenteller Studien überein, welche darauf hindeuten, dass bovine Hydroxylapatitpartikel den Prozess der Osseointegration im Vergleich zu ursprünglichen Stellen nicht beeinträchtigten (Carmagnola et al. 2008, Artzi et al. 2010).

Im Rahmen der Grenzen der vorliegenden Studie kann man schliessen, dass alle untersuchten Augmentationsverfahren durch eine vergleichbare Osteocalcinaktivität während des Prozesses der Knochenregeneration und Osseointegration von Titanimplantaten gekennzeichnet waren.

6. Zusammenfassung

Die gesteuerte Knochenregeneration ist ein wichtiger Bestandteil bei der Behandlung von Alveolarkammdefekten, um das Einbringen von Implantaten in ehemals defizitäre Kieferbereiche zu ermöglichen. Dabei werden eine Vielzahl von Membranen und Knochenmaterialien in verschiedenen Indikationen eingesetzt, welche sich in ihren Eigenschaften unterscheiden und so Einfluss auf das Regenerationsergebnis nehmen können. Daher ist die Kenntnis des Regenerationsprozesses sowie die Einheilung anschliessend eingesetzter Implantate von großer Bedeutung. Die vorliegende Habilitationsschrift ist eine systematische Zusammenfassung ausgewählter wissenschaftlicher Arbeiten zur gesteuerten Knochenregeneration lokalisierter Alveolarfortsatzdefekte und Osseointegration von dentalen Implantaten. Im Zentrum der histologischen und immunhistochemischen Untersuchungen stand die Analyse unterschiedlicher Membranen und Knochenmaterialien zur Regeneration verschiedener Knochendefektgeometrien. Dazu wurden eine native Kollagenmembran, eine synthetische Polyethlenglycolmembran sowie eine Polylaktidsäuremembrane in Kombination mit unterschiedlichen Knochenmaterialien untersucht. Die Analyse der Knochenmaterialien umfasste autologen Knochen sowie bovines und synthetisches Knochenmaterial.

Der zweite wichtige Bestandteil war die histomorphometrische und immunhistochemische Untersuchung der Osseointegration von Titanimplantaten in den zuvor augmentierten Knochenbereichen.

Folgende Fragen standen den durchgeführten Studien zu Grunde:

1. Welchen Einfluss hat die Art der Membran und das verwendete Knochenmaterial auf die Regeneration lokalisierter Defekte des Alveolarkamms?
2. Wie verhält sich die Osseointegration von Titanimplantaten in Abhängigkeit der zuvor verwendeten Membranen und Knochenmaterialien?

Ad 1.

Die Ergebnisse der vorliegenden Studien zeigten, dass alle verwendeten Barrieremembranen die Knochenregeneration bei sattelförmigen Defekten unterstützen konnten. Es wurden keine signifikanten Unterschiede im Hinblick auf die Menge neugebildeten Knochens festgestellt. Die synthetischen Polyethylenglycol- und Polylactidmembranen zeigten die höchsten Werte für mineralisiertes Gewebe. Dies könnte in ihrer Formstabilität und verlängerten Barrierefunktion im Vergleich zur Kollagenmembran begründet sein. Bei der Kollagenmembran konnte vereinzelt ein stärkeres Einwachsen von Bindegewebe in den Defektbereich beobachtet werden.

Auch die angewendeten Knochenmaterialien förderten in Kombination mit den unterschiedlichen Barrieremembranen gleichermaßen die Knochenregeneration. Obwohl die statistische Auswertung der verwendeten Knochenmaterialien im Hinblick auf mineralisiertes Knochengewebe keine Signifikanz zeigte, konnten dennoch Unterschiede beobachtet werden. Bei der histologischen Untersuchung zeigte das bovine Hydroxylapatit wenig Resorption, während das biphasische Calziumphosphat im Kontakt zu seinen Partikeln häufig Areale nicht mineralisierten Gewebes als Anzeichen für eine fortschreitende Resorption aufwies. Der Effekt von autologem Knochen als Zusatz zum jeweiligen Knochenersatzmaterial blieb unklar. Während eine der in dieser Schrift verwendeten Untersuchungen verbesserte Werte im Hinblick auf Knochenregeneration und Knochen-Implantat-Kontakt dokumentierte, konnte eine andere keinen positiven Effekt zusätzlichen autologen Knochens zeigen.

Ad 2.

Die Osseointegration der Titanimplantate in den zuvor augmentierten Bereichen war vergleichbar mit der Osseointegration im unbehandelten Knochen. Die ermittelten Mittelwerte für den Knochen-Implantat Kontakt in den Untersuchungen betrugen 54,1% - 74.9% nach einer Einheilzeit von 2 Wochen und 74,3% - 84,1% nach 8 Wochen. Damit lagen die Werte im Einklang mit den Daten vergleichbarer Studien zur Osseointegration von Titanimplantaten nach vorheriger Augmentation mittels gesteuerter Knochenregeneration. Die

histomorphometrische Analyse zeigte die besten Werte in Defekten, welche mit der Polyethylenglycaolmembran behandelt wurden, jedoch ohne statistische Signifikanz. Die Osseointegration der Titanimplantate war in allen in dieser Schrift aufgeführten Studien vergleichbar und wurde nicht signifikant durch die bei der zuvor angewendeten Augmentation verwendeten Membranen oder Knochenmaterialien beinflusst. Ferner konnten alle Techniken die Osseointegration der Titanimpantate unterstützen.

7. Literatur

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10. ANHANG ORIGINALARBEITEN

CLINICAL ORAL IMPLANTS RESEARCH

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Shell technique using a rigid resorbable barrier system for localized alveolar ridge augmentation

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Key words: alveolar ridge defect, animal study, barrier membranes, bone substitutes, guided bone regeneration

Abstract

Objectives: To assess the safety and efficacy of a rigid synthetic barrier system in a shell technique for localized alveolar ridge augmentation.

Materials and methods: Saddle-type defects (n = 4 each) were prepared in the lower jaws of six fox hounds. At two defects, the outer contours were reconstructed using polylactic acid (D and L isomers) (PDDL) pins welded to PDDL plates by ultrasound vibration and the defect area filled using either a natural bone mineral (NBM) or NBM + autogenous bone (AB) and covered by a native collagen membrane (CM). While the third defect was augmented using NBM+AB+CM, the fourth site was left untreated. At 14 weeks, dissected blocks were processed for histomorphometrical analysis [e.g., augmented area (AA)].

Results: AA values (median in mm^2) were significantly increased in all guided bone regeneration (GBR) groups [NBM+PDDL+CM (19.74) > NBM+AB+PDDL+CM (16.98) > NBM+AB+CM (16.66)] when compared with the untreated control sites (7.34). Histological analysis has pointed, in the absence of any foreign-body reactions, to biodegradation of both PDDL plates/pins and CM.

Conclusions: (i) All GBR procedures investigated equally supported bone regeneration, (ii) the application of PDDL+CM may be associated with increased mineralized tissue MT and subsequently AA values than CM alone, and (iii) AB may not improve healing at NBM+PDDL+CM-treated sites.

Volume stability has been defined as a basic requirement of a barrier membrane to support guided bone regeneration (GBR) for localized alveolar ridge augmentation (Dahlin et al. 1988). The temporary creation and maintenance of a secluded space may be of particular importance at non-contained defect sites, thus necessitating a contour-forming GBR prior to implant placement (Chiapasco et al. 1999). For these two-stage horizontal ridge augmentation procedures, intraoral autogenous bone (AB) blocks applied either with or without a barrier membrane may be favoured over bone substitute materials alone (Jensen & Terheyden 2009; Klein & Al-Nawas 2011). However, due to a pronounced remodeling particularly of cancellous AB, additional grafting procedures simultaneous with implant placement may be required in about 15–20% of the cases (Parodi et al. 1998; Chiapasco et al. 1999).

To overcome this potential limitation and ease lateral ridge augmentation in the daily clinical routine, a rigid synthetic barrier system has recently been introduced (Volkel et al. 2011). In particular, resorbable synthetic plates consisting of pure poly-D, L-lactic acid (PDLLA) are used to reconstruct the outer contour of the bone defect and fixed using ultrasound-aided resorbable pins (Schneider et al. 2011), thus creating a stable and secluded space for the application of particulate AB and/or bone graft substitutes. Preliminary clinical data evaluating traumatologic and osteoplastic reconstructions in head and neck surgery suggest that this device was characterized by an easy clinical handling, stable mechanical properties, and low complication rates (Volkel et al. 2011). Despite some promising clinical experiences (unpublished data), the efficacy and safety of this device has not been systematically assessed for localized alveolar ridge augmentation.

Therefore, the aim of this proof-of-concept study was to assess the histological outcome

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of a GBR procedure employing PDLLA plates/pins in a shell technique and combinations of particulate AB and a bone graft substitute at saddle-type defects in a dog model.

Material and methods

Animals

The study was conducted in a total of six adult fox hounds (age 20.0 ± 1.3 months, weight 39.2 ± 2.0 kg) exhibiting a fully erupted permanent dentition. During the experiment, the dogs were fed once per day with soft-food diet and water *ad libitum*. Animal selection, management, and surgery protocol were approved by the Animal Care and Use Committee of the Heinrich Heine University and the local government Düsseldorf. The experimental segment of the study started after an adaption period of 4 weeks.

Study design and randomization

The study was performed in two surgical phases including (1) tooth extraction and (2) surgical creation of standardized alveolar ridge defects (i.e., $n = 4$ defects per animal) and simultaneous grafting employing a rigid resorbable barrier system.

To account for a potential difference between anterior and posterior defect sites, group allocation was based on a computer-generated balanced randomization protocol (RandList®, DatInf GmbH, Tübingen, Germany).

Surgical procedure

Prior to each surgical intervention, intramuscular sedation was accomplished with 0.17 mg/kg acepromazine (Vetranquil 1%, Ceva Tiergesundheit, Düsseldorf, Germany). Subsequently, anesthesia was initiated using 21.5 mg/kg thiopental sodium (Trapanal 2.5%, Altana GmbH, Konstanz, Germany). During all surgical procedures, inhalation anesthesia was performed by the use of oxygen and nitrous oxide and isoflurane. To maintain hydration, all animals received a constant rate infusion of lactated Ringer's solution while anesthetized. Intraoperative analgesia was performed by intravenous injection of 0.1 mg/kg piritramide (Dipidolor®, Janssen-Cilag GmbH, Neuss, Germany) and 4.5 mg/kg carprofen (Rimadyl®, Pfitzer Pharma GmbH, Karlsruhe, Germany). For postoperative treatment, carprofen (days 1–7) was applied subcutaneously in the same dose as described before.

Surgical phase 1 (tooth extraction)

In the first surgery, mucoperiosteal flaps were reflected bilaterally in both jaws, and the mandibular and maxillary first, second, third, fourth premolar as well as first and second molar were carefully removed after tooth separation. Wound closure was accomplished by means of mattress sutures (Resorba®, Nürnberg, Germany), and the sites were allowed to heal for 10 weeks. Prophylactic administration of clindamycin (11.0 mg/kg body weight, Cleorobe®, Pharmacia Tiergesundheit, Erlangen, Germany) was performed intra- and postoperatively for 10 days.

Surgical phase 2 (defect creation and localized ridge augmentation)

After 10 weeks of healing, mid-crestal incisions were made and mucoperiosteal flaps reflected to expose the alveolar bone in the lower jaws. Vertical releasing incisions were placed about 4–5 mm distant to the designated experimental sites. A total of four standardized saddle-type defects including the vestibular and oral aspect of the alveolar ridge were subsequently prepared bilaterally at a distance of at least 5 mm with a straight fissure carbide bur. After bone block removal, the final dimension of all defects revealed a mesiodistal width of 10 mm and an apicocoronal height, as measured from the crestal bone, of 8 mm. The defect sizes were standardized by the use of a periodontal probe (PCP12, Hu-Friedy Co., Chicago, Illinois, USA). All osteotomy procedures were performed under copious irrigation with sterile 0.9% physiological saline. Finally, each defect site was thoroughly rinsed with sterile saline to completely remove any residual debris.

Subsequently, at two experimental sites, resorbable PDDL pins (SonicPin RX, KLS Martin, Mühlheim, Germany) were inserted by active penetration into four pre-drilled holes each and welded to the resorbable PDDL plates (Resorb-X 0.1 mm, KLS Martin) using ultrasound vibration (SonicWelder, KLS Martin). Subsequently, the pins were allowed to return to a solid, thus completing the application process (Fig. 1a,b). The resulting self-contained defect areas were homogeneously filled with either a particulate NBM (Geistlich BioOss® spongiosa granules, particle size 0.25–1 mm, Geistlich Biomaterials, Wolhusen, Switzerland) or NBM homogeneously mixed (ratio 1 : 1) with particulated AB (particle size 0.5–1 mm) collected during defect creation (Fig. 1c). At defect site 3, NBM+AB was homogeneously applied in the non-self-contained defect area, while defect site four was left untreated (Fig. 1d). At defect sites 1–3, particular care was taken that the graft particles did not exceed the contour of the bordering bone walls in either cranial or lateral directions.

Following grafting, CM (Geistlich BioGide®, Geistlich Biomaterials) was adapted over the respective defect areas so as to cover 1–2 mm of the surrounding alveolar bone. Neither sutures nor pins were used for membrane fixation or stabilization (Fig. 1d).

Following periosteal-releasing incisions, the mucoperiosteal flaps were advanced, repositioned tension-free in a coronal position, and fixed with vertical or horizontal mattress sutures (Resorba®, Nürnberg, Germany) in a way to ensure a submerged healing condition (Fig. 1e,f).

Animal sacrifice and retrieval of specimens

At 14 weeks, the animals were killed by an overdose of sodium pentobarbital 3%, respectively. The oral tissues were fixed by perfusion with 10% buffered formalin administered through the carotid arteries. The jaws were dissected, and blocks containing the experimental specimens were obtained. All specimens were fixed in 10% neutral buffered formalin solution for 4–7 days.

Histological preparation

The block biopsies containing the implants were processed for the undecalcified ground sections. Briefly, the specimens were trimmed, rinsed in running tap water, dehydrated in ascending concentrations of ethanol, and embedded in methyl methacrylate. Each defect site was cut in the buccooral direction using a diamond band saw (Exakt®, Apparatebau, Norderstedt, Germany). Four to five serial sections approximately 500 μm in thickness were prepared from the most central aspect of each defect area, mounted on opaque acrylic plates with cyanoacrylate glue (Technovit 7210 VLC, Heraeus Kulzer, Wehrheim, Germany), and ground to a final thickness of approximately 80 μm (Donath 1985). All sections were stained with toluidine blue (TB) to evaluate new bone formation.

Histomorphometrical analysis

Histomorphometrical analyses as well as microscopic observations were performed by one experienced investigator masked to the specific experimental conditions. For image acquisition, a digital camera (Nikon D90, Nikon, Japan) was mounted on a binocular light microscope (Olympus CX40, Olympus, Hamburg, Germany). Digital images were evaluated using software programs (Camera Pro, Apple, USA and Image J 64, National Institute of Health, USA).

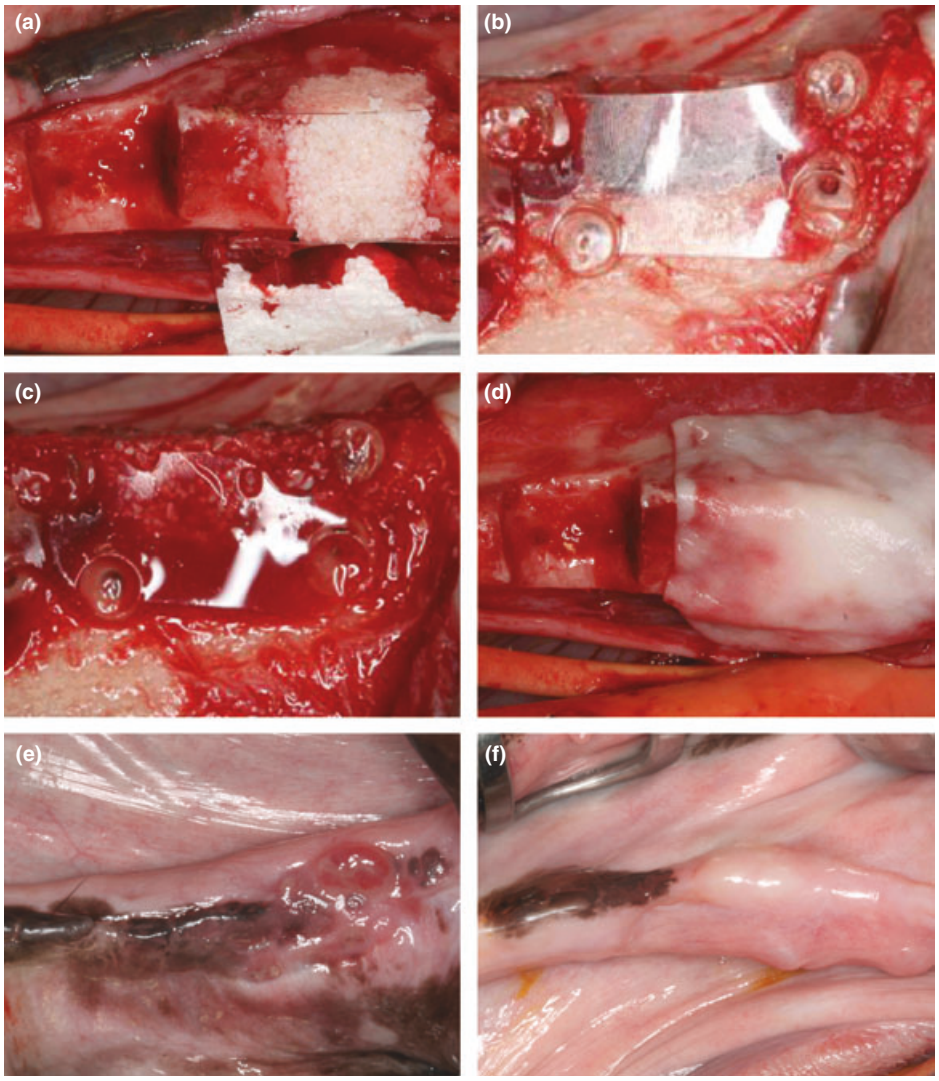

Fig. 1. (a) A total of $n = 4$ standardized saddle-type defects (width: 10 mm; height: 8 mm) were created at a distance of at least 5 mm in each lower jaw of 6 dogs. At two defects, the outer contours were reconstructed using resorbable polylactic acid (D and L isomers) (PDDL) pins welded to PDDL plates by ultrasound vibration. (b) Four pins were positioned at the border area of each defect site. (c) The resulting self-contained defects were homogeneously filled with NBM and NBM+AB. (d) While the third non-self-contained defect was homogeneously augmented using NBM+AB, the fourth xperimental site was left untreated. All augmented defect sites were covered by CM, which was adapted in a way as to cover 1–2 mm of the surrounding alveolar bone. (e) After 14 weeks of submerged healing, a slight exposure of PDDL plates was observed at two defect sites and associated with a slight inflammatory reaction in the adjacent mucosa. (f) At the remaining defect sites, submerged healing was commonly considered as uneventful.

In each specimen, the augmented area (AA) (mm^2) was demarcated using the bottom of the former defect as apical and the newly formed bone crest as coronal reference points. Within AA, the surface area of mineralized tissue (MT) and residual NBM particles was assessed (mm^2) (Fig. 2).

Statistical analysis

The statistical analysis was performed using a commercially available software program (PASW Statistics 20.0, SPSS Inc., Chicago, IL, USA). Mean values and standard deviations among animals were calculated for each variable and group. The data rows were examined with the Kolmogorov–Smirnov test and proven to be normally distributed. Between-group comparisons at 14 weeks were performed using the unpaired *t*-test. The alpha error was set at 0.05.

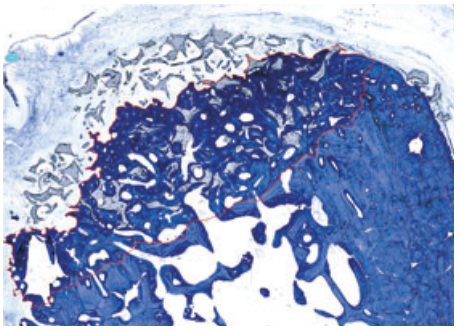

Fig. 2. Within the augmented area (AA) (red demarcated area), the surface fraction (μm^2) of mineralized tissue and NBM was assessed (NBM+AB+ +CM) (original magnification ×25).

Results

Clinical healing

After 14 weeks of submerged healing, a minor exposure of PDDL plates was observed at two defect sites (i.e., groups NBM+AB+ PDDL+CM and NBM+PDDL+CM) and histologically associated with a mixed chronic inflammatory cell infiltrate located in the adjacent mucosa without showing any signs of bone resorption (Fig. 1e). At the remaining sites, the postoperative healing was considered as generally uneventful. No complications such as allergic reactions, swellings, abscesses, or infections were observed throughout the whole study period (Fig. 1f).

Histologic findings

Representative histological views of wound healing in different groups at 14 weeks are presented in Figs. 3 and 4.

Basically, all GBR-treated sites were characterized by a homogeneous stabilization of the NBM particles within the confines of the space provided by both PDDL sheets and CMs. However, all specimens investigated also revealed a slight displacement of the bone filler mainly in a coronal direction, thus resulting in a dispersion of NBM particles to the adjacent subepithelial connective tissue, but also the defect area of one control site (Fig. 3).

While bony filling appeared to be more consistent in both PDDL groups, CM-treated sites were characterized by a partial or an incomplete hard tissue invasion within the former defect area. In these areas, NBM particles were frequently dislocated in coronal direction and mainly surrounded by connective tissue. In contrast, the untreated control defects commonly revealed only minor to moderate signs of bone remodeling (Fig. 3a,b).

Histological analysis of both PDDL groups revealed residues of either PDDL plates/pins in most specimens (Fig. 3c,d and 4). This process of biodegradation was not associated with any inflammatory cell infiltrates in the adjacent tissues. Partially, the membrane structure was stretched (Fig. 3c and d, Fig. 4b and d) and occasionally rolled on top (Fig. 4a, c) and vestibular of the augmented area (Fig. 3d and Fig. 4a). In the NBM+PDDL+CM group, one defect site showed PDDL plate

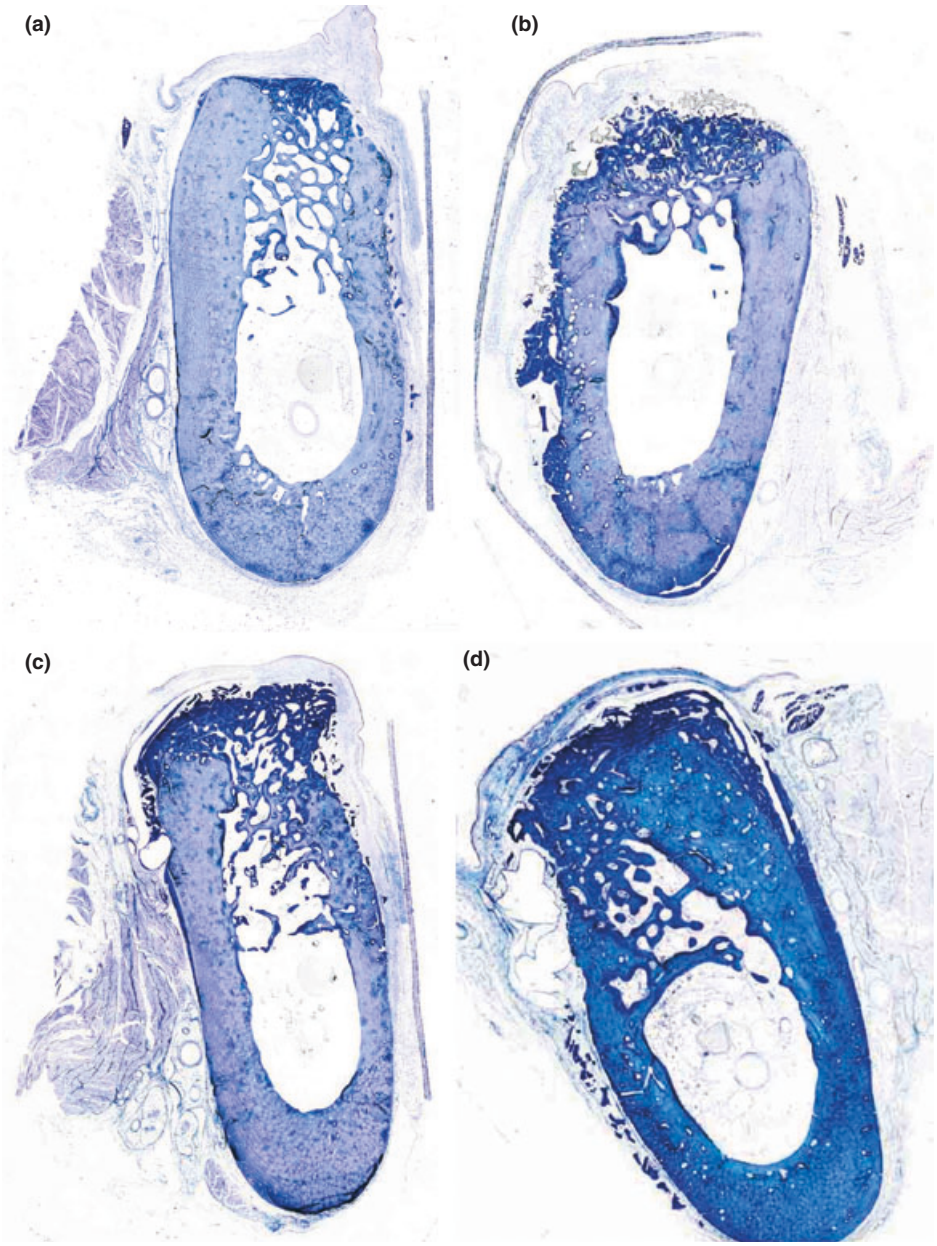

Fig. 3. Representative histological views (vestibulo-oral sections, toluidine blue stain) of wound healing at 14 weeks (original magnification ×12.5). a. Control; b. NBM+AB+CM; c. NBM+PDDL+CM; d. NBM+AB+PDDL+CM.

fragments (Fig. 4b) were well integrated and completely surrounded by newly formed bone tissue. On the surface area of the regenerated alveolar ridge, intact collagen membrane structures were visible. In the NBM+AB+PDDL+CM group, the PDDL plate was partially fragmented on and beside the alveolar ridge (Fig. 4d). The pins revealed poor signs of resorption, and most of them were integrated in hard tissue. Besides pin heads, periosteal new bone formation was obvious and integrated the pins in bone tissue (Fig. 4d). One pin was fractured (Fig. 3c), and a second pin was surrounded by connective tissue (Fig. 3d).

Histomorphometry

The histomorphometrical parameters assessed at 14 weeks in different groups are summarized in Table 1. Histomorphometrical analysis commonly revealed higher mean MT and subsequently AA values at all GBR treated over the untreated control sites. In particular, mean MT values were highest at NBM+PDDL+CM, NBM+AB+PDDL+CM, and NBM+AB+CM groups, which was followed by the control sites. However, between-group comparisons merely revealed a significant difference in mean MT values when comparing NBM+AB+PDDL+CM with the control group ($P = 0.032$; unpaired *t*-test) (Table 1).

Mean AA values were significantly higher at NBM+PDDL+CM-, NBM+AB+PDDL+CM-, and NBM+AB+CM-treated sites when compared with the control group ($P = 0.023$, $P = 0.047$, $P = 0.032$; unpaired *t*-test, respectively) (Table 1).

Both PDDL and CMs equally stabilized the amount of bone graft particles within the confines of the former defect area, thus revealing comparable mean NBM values in respective groups ($P > 0.05$; unpaired *t*-test, respectively) (Table 1).

Discussion

The present proof-of-concept study was designed to clinically and histologically evaluate the safety and efficacy of PDDL plates/pins used as GBR device for localized ridge augmentation employing either NBM+AB or NBM alone as bone fillers in a dog model. In this context, it must be emphasized that saddle-type defects are commonly used and well established to evaluate GBR procedures in canines (Vignoletti & Abrahamsson 2012).

Within its limitations, the present data have indicated that this specific shell technique was characterized by an excellent clinical handling and manageability and therefore may represent a safe procedure for localized alveolar ridge augmentation. However, its clinical applicability may be more challenging at complex defect configurations and therefore requires further investigations. The high biocompatibility, as indicated by low exposure rates and the absence of any pronounced foreign-body reactions in the adjacent tissue, is also supported by the findings of a recent retrospective clinical analysis (Volkel et al. 2011). In this context, however, it must be noted that all PDDL plates were also covered by CM. This native barrier membrane is characterized by an undisturbed tissue integration, thus facilitating early stages of soft tissue healing (Rothamel et al. 2005; Schwarz et al. 2006). Moreover, the present histomorphometrical analysis has indicated that all GBR procedures investigated equally supported bone regeneration in the former defect area. Even though the untreated control defects were also characterized by a slight to moderate bone remodeling process, mean AA values were significantly lower when compared with the respective GBR-treated sites.

Basically, the outcomes observed in the NBM+AB+CM group are comparable with those reported in previous experimental studies employing a comparable type of defect

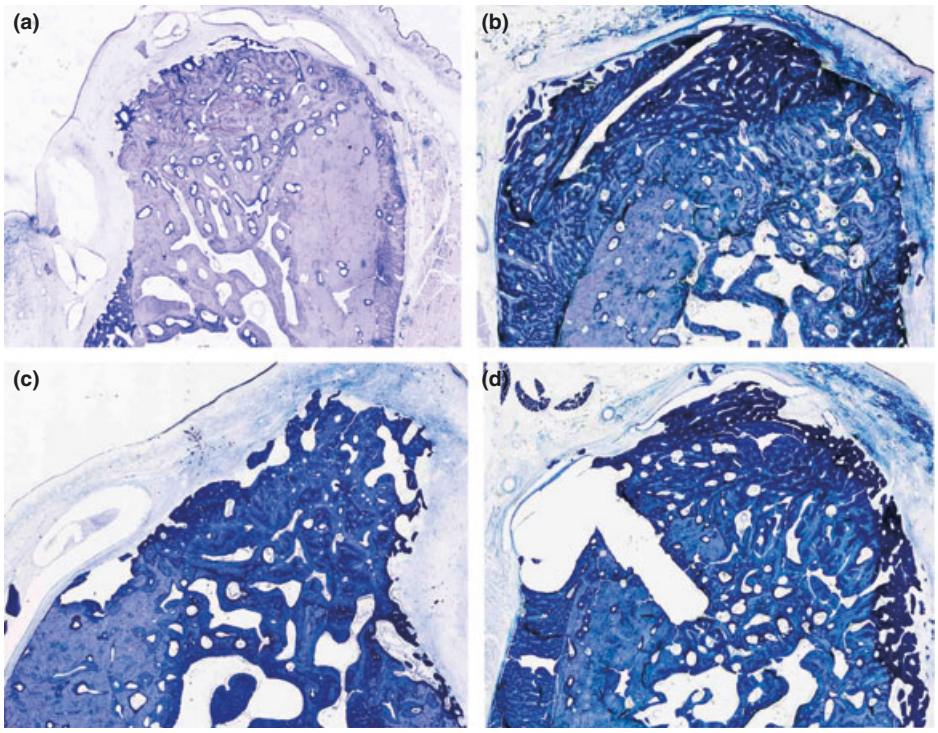

Fig. 4. Representative histological views (vestibulo-oral sections, toluidine blue stain) of wound healing at 14 weeks (original magnification ×25). a. NBM+AB+PDDL+CM; b. NBM+PDDL+CM; c. NBM+AB+PDDL+CM; d. NBM+AB+PDDL+CM.

Table 1. **Mean values (±SD) and medians of AA, MT, and NBM (in mm^2) in different groups after 14 weeks of submerged healing (n = 6 dogs)**

		AA	MT	NBM
NBM+AB+PDDL+CM	Mean	17.33	16.52	0.81
	SD	4.57	4.34	0.54
	MIN	12.47	12.18	0.27
	MAX	23.51	22.16	1.42
	Median	16.98	16.06	0.78
NBM+PDDL+CM	Mean	18.64	17.94	0.70
	SD	8.13	8.17	0.68
	MIN	8.24	8.20	0.00
	MAX	29.29	29.29	1.79
	Median	19.74	18.36	0.60
NBM+AB+CM	Mean	15.66	14.75	0.911
	SD	2.87	2.85	0.89
	MIN	10.58	10.20	0.00
	MAX	18.69	17.14	2.10
	Median	16.66	15.61	0.88
Control	Mean	8.27*	8.25**	0.22
	SD	6.44	6.44	0.49
	MIN	0.20	0.20	0.00
	MAX	17.98	17.98	0.11
	Median	7.34	7.23	0.00

Between-group comparisons: unpaired t- test:
*Significantly lower compared with NBM+AB+PDDL+CM (P = 0.023), NBM+ PDDL+CM (P = 0.047), NBM+ AB+CM (P = 0.032).
**Significantly lower compared with NBM+AB+PDDL+CM (P = 0.032).
AA: augmented area MT: mineralized tissue NBM: bone substitute (*i.e.*, residual NBM particles).

model (Bornstein et al. 2007; Schwarz et al. 2012). In particular, at 16 weeks after defect augmentation using NBM in beagle dogs, Bornstein et al. (2007) assessed mean AA values ranging between 28.59 and 39.0 mm^2 for untreated (i.e., non membrane) and CM (native and cross-linked specimens)-treated sites. Similarly, at 10 weeks after staged GBR (NBM+AB+CM) and osseointegration of titanium implants in the upper jaw of fox hounds, mean AA values varied between 10.2 ± 4.9 mm^2 at the vestibular and 9.1 ± 5.3 mm^2 at the oral aspect of the former defect area (Schwarz et al. 2012).

A further analysis of the present data has also indicated that both PDDL groups tended to be associated with increased MT and subsequently AA values when compared with NBM+AB+CM-treated sites. As CM was applied to all GBR groups, one may speculate that these differences, while not statistically significant, may at least in part be attributed to an increased volume stability of the PDDL plates. In this context, it must also be noted that the addition of AB to NBM (1 : 1) was even associated with a decrease rather than an increase in mean MT values when compared with the application of NBM alone. Even though human AB chips harvested at various intraoral donor sites have been proven to contain vital cells, which differentiated into osteoblasts *in vitro* (Chiriac et al. 2005), the present study failed to reveal a beneficial effect of NBM+AB over NBM when applied in conjunction with this shell technique. In contrast, a historical comparison of recent experimental animal studies may point to an increase in mean MT and subsequently AA values when AB was added to NBM (1 : 1) at CM-treated saddle-type defects. In particular, after 10 weeks of healing, mean MT values at the vestibular and oral aspects varied between 4.1 and 5.3 mm^2 at NBM+AB+CM sites (upper jaws) and tended to be obviously higher when compared with NBM+CM sites (lower jaws) revealing mean MT values ranging between 2.1 and 2.4 mm^2 (Mihatovic et al. 2012; Schwarz et al. 2012).

Within the limits of the present study, it was concluded that (i) all GBR procedures investigated equally supported bone regeneration, (ii) the application of PDDL+CM may be associated with increased MT and subsequently AA values than CM alone, and (iii) AB may not improve healing at NBM+PDDL+CM-treated sites.

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Conflict of interests

The authors declare that they have no conflict of interests related to this study.

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ORIGINAL ARTICLE

Staged implant placement after defect regeneration using biphasic calcium phosphate materials with different surface topographies in a minipig model

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Abstract

Objective To assess the influence of biphasic calcium phosphate materials with different surface topographies on bone formation and osseointegration of titanium implants in standardized alveolar ridge defects.

Materials and methods Standardized alveolar ridge defects (6 × 6 mm) were created in the mandible of 8 minipigs and filled with three biphasic calcium phosphate materials (BCP1–3, 90% tricalcium phosphate/10% hydroxyapatite) with different surface properties (micro- and macroporosities) as well as a bovine-derived natural bone mineral (NBM) as a control. At 12 weeks, implants were placed into the augmented defects. After further 8 weeks of healing, dissected blocks were processed for histological analysis (e.g., mineralized (MT), residual bone graft material (BS), bone-to-implant contact (BIC)).

Results All four biomaterials showed well-integrated graft particles and new bone formation within the defect area. MT values were comparable in all groups. BS values were highest in the NBM group (21.25 ± 13.52%) and markedly reduced in the different BCP groups, reaching statistical significance at BCP1-treated sites (9.2 ± 3.28%). All test and control groups investigated revealed comparable and statistically not significant different BIC values, ranging from 73.38 ± 20.5% (BCP2) to 84.11 ± 7.84% (BCP1), respectively.

Conclusion All bone graft materials facilitated new bone formation and osseointegration after 12 + 8 weeks of healing.

Keywords Bone remodelling · Bone substitute · Histological analysis · Implant osseointegration · Animal model

Introduction

Bone grafting has become an important field in implant dentistry for the treatment of alveolar ridge defects due to trauma, periodontal disease, or infection. Over the years, different biomaterials were used to fill and regenerate bone defects, subsequently allowing an implant-based prosthetic reconstruction. The ideal grafting material should (1) be osteoconductive to serve as a matrix for vascular and cellular migration, (2) feature an osteoinductive potential by stimulating mesenchymal cells to differentiate into bone-forming osteoblasts, and (3) contain osteoprogenitor cells being able to produce new bone matrix. Autogenous bone (AB) comprises all desired properties and represents the gold standard of bone grafts. However, disadvantages of AB like donor site morbidity, limited availability, and graft resorption led to the introduction of alternatives such as allografts, xenografts, and synthetic alloplasts, which include hydroxyapatite, β-tricalcium phosphates, and biphasic calcium phosphates (BCP) [1–4].

Experimental data indicated that the application of either a particulate xenograft or BCP showed predictable bone formation at chronic alveolar ridge defects in the posterior mandible revealing a vertical and horizontal bone deficiency [5, 6]. In particular, these experimental data demonstrated that a

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particulate xenograft or BCP with and without the addition of AB at saddle-type defects were associated with adequate bone formation and osseointegration of titanium implants [5, 6]. Clinical findings could also support the experimental data reporting a clinically important bone gain [1, 3, 7]. While β-tricalcium phosphate is fast resorbing and lacks volume stability over time, hydroxyapatite (HA) is very slowly resorbing and might lead to higher amounts of residual graft material to the disfavor of new bone formation [8]. The ideal bone substitute material should be completely resorbable while having a timeframe of resorption stability to maintain space within the defect [9]. The use of biphasic combinations of HA and β-TCP showed a balance between long-term stability and new bone formation combining the positive features of both materials [10]. Apart from resorption behavior, surface topography of biomaterials seems to play an important role for new bone formation. While macroscale features such as interconnected macropores, particle size and surface concavities have been speculated to be a prerequisite for bone formation, surface microtopography might even influence the osteoinductive capability of certain biomaterials [11–15]. Various experimental models demonstrated that microstructured HA and BCP induced ectopic bone formation [12, 13, 16, 17]. Previously, the dimension of the surface microstructure has also been described to play an important role for osteoinduction. In particular, experimental studies revealed that TCP with a submicron-scale surface structure stimulated de novo bone formation, while TCP with a micron-scale surface did not show any osteoinductive potential [18, 19]. Based on this data, it might be hypothesized that the surface topography of graft materials, especially microporosity, could have a potential to support new bone formation. Therefore, the aim of the present study was to evaluate the influence of three BCP graft materials with different surface topographies on new bone formation and osseointegration of titanium implants in an experimental defect model.

Material and methods

Animals

A total of 8 female Gottingen minipigs (Ellegaard, Dalmose, Denmark) with an age of 20–24 months and a weight of 36–42 kg were recruited for the present study. During the experiment, minipigs were fed using a standard diet (soft food) expanded for minipigs (SDS Standard Service, UK) and water ad libitum. The animals were kept in specially designed areas under supervision of veterinarian staff during the entire study period. Animal selection, management, and surgery protocol were approved by the Malmö-Lund University Animal Experiment Ethics Committee (number M-204-11.7; Malmö-Lund University, Lund, Sweden). The experimental phase of the study started after an adaption period of 1 week. On the day of surgery, animals were fasted and weighed. Premedication was performed by means of an intramuscular injection atropine (0.05 mg/kg) of 10 ml ketamine (Ketalar Vet, Pfizer AB, Sollentuna, Sweden; 50 mg/ml) mixed with 3 ml midazolam (Dormicum 5 mg/ml: Roche, Basel, Switzerland). During surgery, 10 ml of ketamine was additionally injected every 30 to 40 min to maintain sufficient anaesthesia.

Study design and randomization

The study was performed in three surgical phases (Fig. 1). In the first phase, the lower premolars PM2–4 as well as the first molars were extracted and the sites were left to heal for a period of 3 months. In the second phase, a total of three standardized cylindrical-type defects (mesio-distal width, 6 mm; height, 6 mm) were bilaterally prepared in the lower jaws (i.e., $n = 6$ defects per animal).

The following groups were investigated:

- Test 1: BCP1—porous biphasic calcium phosphate (HA 10%, β-TCP 90%/particle size 250–1000 μm (Institut Straumann AG, Basel, Switzerland)
- Test 2: BCP2—porous biphasic calcium phosphate (HA 10%, β-TCP 90%/particle size 250–1000 μm, less macro-/less microporosity (Institut Straumann AG)
- Test 3: BCP3—porous biphasic calcium phosphate (HA 10%, β-TCP 90%), particle size 250–1000 μm, more macro-/less microporosity (Institut Straumann AG)
- Control 1: NBM—natural bovine bone material (HA) (BioOss, Geistlich, Wolhusen, Switzerland)
- Control 2: no defect fill

Macroporosity was defined as pores > 75 μm, and microporosity as pores > 2 nm.

The experimental sites ($n = 48$) were randomized according to a computer-generated list: BCP1 ($n = 10$); BCP2 ($n = 10$); BCP3 ($n = 10$); control 1 ($n = 10$); control 2 ($n = 8$).

The second healing period was 12 weeks. In the third phase, titanium implants were placed at the respective test and control sites. Following another healing period of 8 weeks, animals were terminated, and separated block biopsies prepared for histological processing (Fig. 1).

Surgical phases

All surgeries were performed in an operation room ensuring aseptic conditions. Surgeries were conducted under general anaesthesia utilizing an intramuscular injection of 10 ml ketamine (Ketalar Vet, Pfizer AB, Sollentuna, Sweden, 50 mg/ml) mixed with 3 ml midazolam (Dormicum® 5 mg/ml; Roche, Basel, Switzerland). During surgery, 10 ml of ketamine was

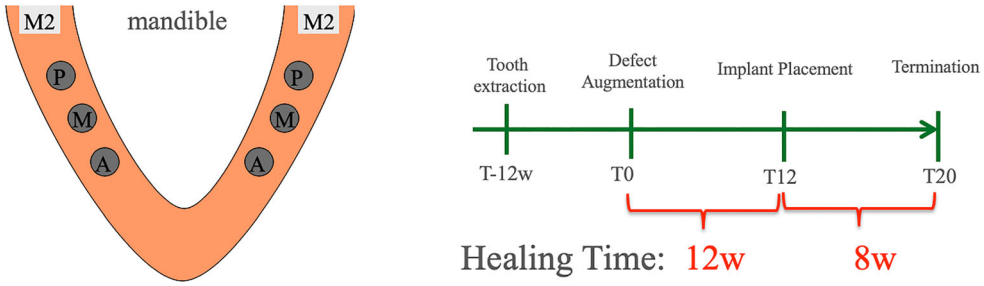

Fig. 1 Study outline including tooth extraction, defect augmentation, and implant placement (P, posterior; M, middle; A, anterior defects)

additionally injected every 30 to 40 min to maintain sufficient anaesthesia. While anaesthetized, all animals received a constant rate infusion of lactated Ringer's solution to maintain hydration. The tooth extraction surgeries were performed by three surgeons, whereas defect creation, augmentation, and implant placement were performed by one blinded surgeon.

Tooth extraction

Following local anaesthesia using 2% xylocain–adrenaline (ASTRA, Södertälje, Sweden) and midcrestal gingival incision, full-thickness mucoperiostal flaps were raised bilaterally in the mandible. Lower PM2–4 and the first molar M1 were carefully extracted (Fig. 2a). After accurate inspection of the sockets, wound closure was achieved using multiple single sutures (Vicryl 4-0, FS2, Ethicon, Somerville, NJ, USA). Postoperatively, radiographs were taken to control the extraction sites.

Defect creation and augmentation

After a healing period of 3 months, edentulous mandibular alveolar ridges were exposed bilaterally using midcrestal incisions and reflection of full-thickness flaps. Subsequently, the crestal area was evened out by means of a carbide bur under continuous cooling with saline to achieve a crestal ridge width of at least 10 mm. Three cylindrical-type defects measuring 6 mm in width and depth were created using spiral burs with ascending diameters. Between each defect and the second molar, a distance of 5 mm was kept. Additionally, titanium miniscrews (1.5 × 6 mm; Medartis AG, Basel, Switzerland) were inserted at the alveolar crest 5 mm mesial of the most anterior defect to ensure orientation and precise implant placement into the defects at re-entry (Fig. 2b). Following adequate rinsing of the drill holes to remove bone debris, respective defects were homogeneously filled with BCP1, BCP2, BCP3, or NBM (Fig.

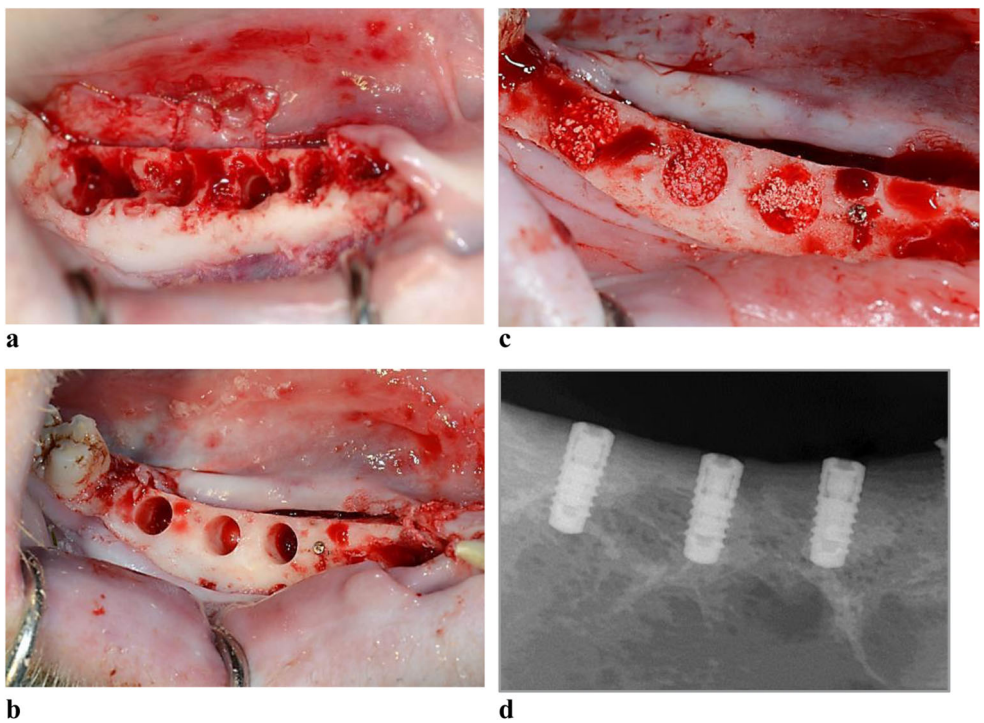

Fig. 2 Surgical procedures. **a** Occlusal view of the alveolar ridge after tooth extraction. **b** Occlusal view of the alveolar ridge after creation of cylindrical-type defects. Mesial of the most anterior defect, a miniscrew is visible. **c** Occlusal view of the alveolar ridge after application of the bone graft material. **d** Radiographic image after implant placement into the previously augmented defects

2c). Particular care was taken that the graft particles did not exceed the contour of adjacent bone walls. After the augmentation procedure, mucoperiostal flaps were repositioned and wound closure was achieved by multiple single sutures (Vicryl 4-0, FS2, Ethicon, Somerville, NJ, USA). Animals received antibiotics for 7 days postoperatively (Streptocillin vet 3–4 ml/animal i.m.; Boehringer Ingelheim, Ingelheim, Germany). Additionally, Temgesic (3–5 ml/animal i.m.; Essex Pharma GmbH, Munich, Germany) was administered for 3 days postoperatively.

Implant placement

Following a healing period of 12 weeks, surgical sites were exposed by midcrestal incisions and elevation of full-thickness flaps. After identification of the augmented defect sites by the help of previously inserted microscrews, titanium implants (Straumann Bone Level SLActive, Straumann AG, Basel, Switzerland) with dimensions of 3.3 × 8 mm were placed in an epicrestal position, according to the manufacturer's surgical protocol. Hereafter, wound closure was obtained by multiple single sutures (Vicryl 4-0, FS2, Ethicon, Somerville, NJ, USA). Postoperatively, digital radiographs were taken to ensure a proper implant installation (Fig. 2d). Animals received antibiotics for 7 days (Streptocillin vet 3–4 ml/animal i.m.; Boehringer Ingelheim, Ingelheim, Germany). Additionally, Temgesic (3–5 ml/animal i.m.; Essex Pharma GmbH, Munich, Germany) was administered for 3 days postoperatively.

Study termination

Eight weeks after implant placement, animals were killed by an intracardiac injection of a 20% solution of pentobarbital (Pentobarbitalnatrium, Apoteket AB; Stockholm, Sweden, 60 mg/ml). Following block resection of the hemi-mandibles, augmented/implanted sites were isolated and fixed in 10% neutral buffered formalin solution for 10 days.

Histological preparation

The collected specimens were dehydrated using ascending grades of alcohol and xylene, infiltrated, and embedded in methylmethacrylate (MMA, Technovit 9100 NEU, Heraeus Kulzer, Wehrheim, Germany) for non-decalcified sectioning. During this procedure, any negative influence of polymerization heat was avoided due to a controlled polymerization in a cold atmosphere (− 4 °C). After 20 h, the specimens were completely polymerized. Each implant site was cut in the buccal-oral direction along with the long axis of the implant using a diamond wire saw (Exakt, Apparatebau, Norderstedt, Germany), resulting in 4 sections of approximately 300 μm in thickness each. Subsequently, all specimens were glued with acrylic cement (Technovit 9100 VLC, Heraeus Kulzer, Wehrheim, Germany) to silanized glass slides (Super Frost, Menzel GmbH, Braunschweig, Germany) and ground to a final thickness of approximately 40 μm. Prepared specimens were employed for histomorphometric analysis.

Histomorphometrical analysis

Histological sections were stained with toluidine blue (TB) to evaluate bone formation. The specific staining technique allows the differentiation of mature and new bone according to the color staining strength. Mature bone can be identified by a light blue staining, whereas new bone stains dark blue due to its higher protein content.

Histomorphometrical analysis was performed by one experienced investigator masked to the specific experimental conditions (I.M.). For image acquisition, a color CCD camera (Color ViewTM III, Olympus, Hamburg, Germany) was mounted on a binocular light microscope (Olympus BX50, Olympus, Hamburg, Germany). Digital images (original magnification × 200) were evaluated using a software program (analySIS FIVE docu, Soft Imaging System, Münster, Germany).

Histomorphometrical analysis concentrated on two aspects: the defect area (DA) and bone-to-implant contact (BIC). DA was identified at the buccal and oral aspects of the inserted implant being confined by the bottom of the defect, the implant surface, the coronal level of bone in contact with the implant surface (CBI), and the demarcation of the defect and pristine bone at the buccal/oral aspect (Fig. 3a). Within DA, the surface areas of mineralized (MT) and residual BCP/NBM particles (BS) were automatically assessed (in %) by the image analysis software. To assess BIC, the implant shoulder (IS) and CBI were identified. Consequently, BIC was assessed as a percentage of the distance from IS at the buccal aspect to IS at the oral aspect (Fig. 3b). In addition, the vertical linear distance from IS to CBI was assessed at both buccal and oral aspects. Before the start of the histomorphometrical analysis, a calibration procedure was initiated for the image analysis software and revealed that repeated measurements of n = 12 different sections were similar at > 95% level.

Statistical analysis

The statistical analysis was performed using a commercially available software program (SPSS Statistics 19.0, SPSS Inc., Chicago, IL, USA). The mean values and standard deviations among animals were calculated for each variable and group. The data rows were examined using the Kolmogorov–Smirnov test for a normal distribution. Between-group

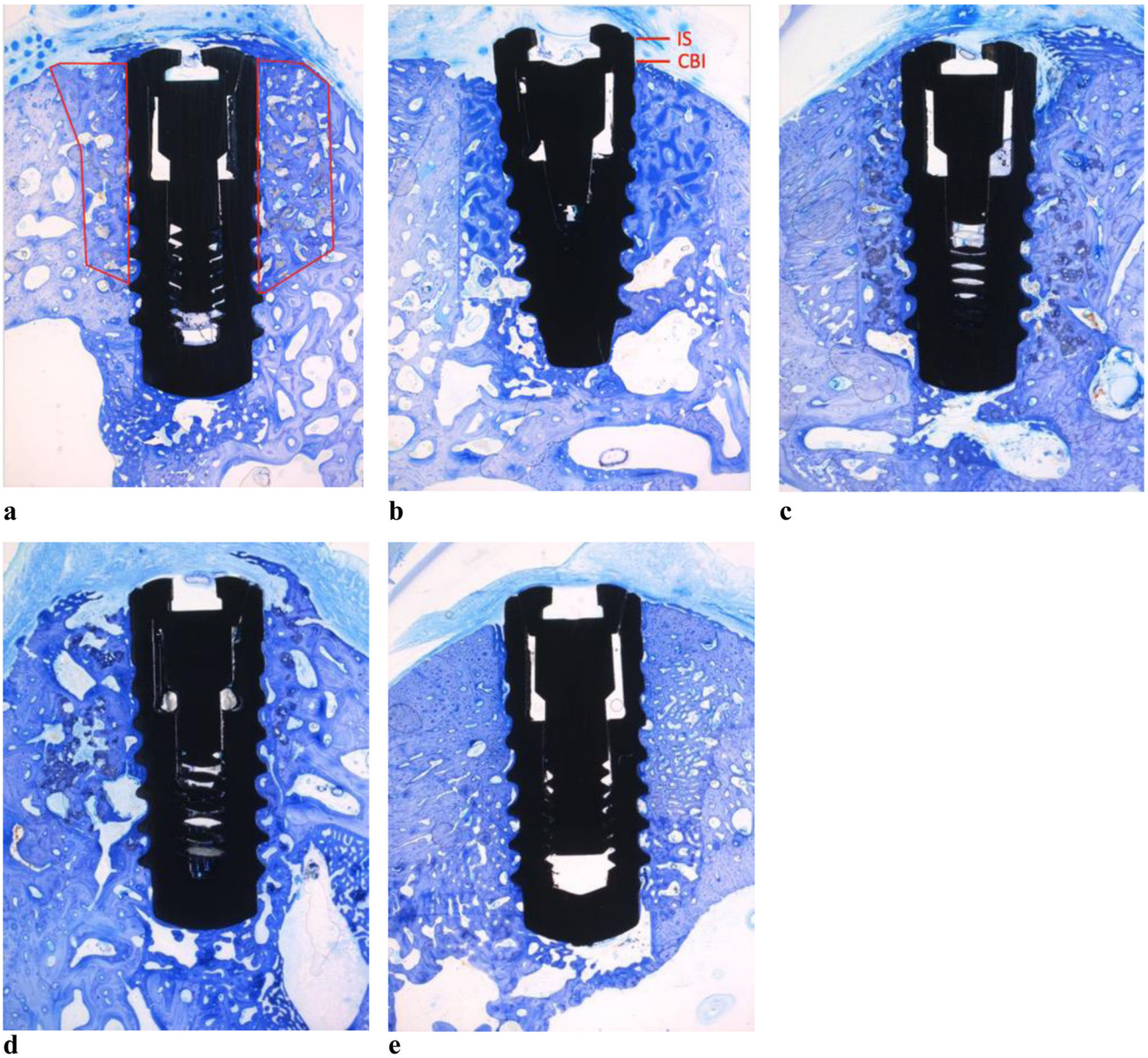

Fig. 3 Representative histological views (TB stain, original magnification × 4) and histological landmarks. **a** Defect area (red line) was defined by the implant (line connecting the implant threads), the alveolar crest, and the defect borders (BCP3 specimen). **b** Red lines marking IS and CBI which served as reference for the assessment of BIC and IS-CBI values (NBM specimen). **c** BCP1 specimen. **d** BCP2 specimen. **e** Untreated control specimen

comparisons were performed using the unpaired *t* test. The alpha error was set at 0.05.

Results

Clinical healing

The postoperative healing was uneventful in all animals. No complications such as allergic reactions, swellings, abscesses, or infections could be observed throughout the whole study period.

Descriptive histology

After 12 + 8 weeks of healing, all histological specimens showed a bony filling of the secluded wound area treated with BCP1–3 and NBM (Fig. 4a). A scaffold of cancellous bone accompanied by blood vessels and bone-forming cells homogeneously surrounded all graft particles. Graft particles of all groups appeared to be in close contact with newly formed bone. However, slight differences between different bone graft materials were visible. NBM particles were well integrated into the surrounding new bone tissue, whereas no signs of graft resorption could be detected (Fig. 4b). In groups

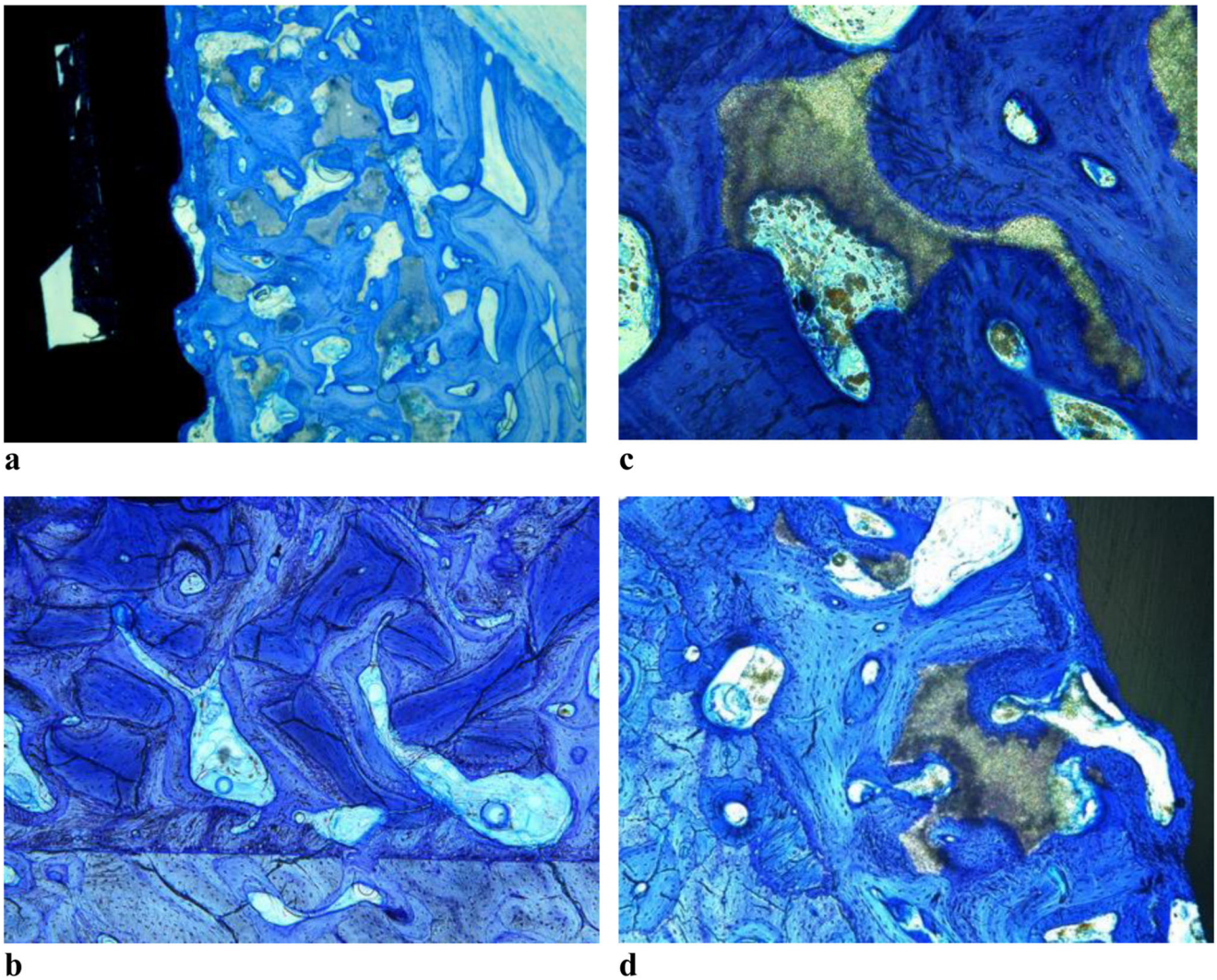

Fig. 4 Descriptive histological findings in different groups. **a** Residual BS could be identified in all groups. These particles were well integrated into the bone matrix (BCP2, TB stain, original magnification × 10). **b** NBM particles were in close contact with adjacent osteoid tissue but without showing signs of resorption. NBM was well incorporated into the newly formed bone (NBM, toluidine stain, original magnification × 20). **c** In all BCP groups, bone graft particles were characterized by an ongoing resorption and dissolution accompanied by osteoclast activity near the graft surface (BCP3, toluidine stain, original magnification × 50). **d** All BCP particles were predominantly surrounded by a compact bone matrix, whereas areas with no contact to matured bone were lined by mineralized tissue (BCP3, toluidine stain, original magnification × 40)

BCP1–3, bone graft particles were more frequently in contact with immature bone revealing increased surface areas lined by a provisional bone matrix. These areas were characterized by a dissolution of BCP, resulting in a superficial disintegration of particles into smaller grains accompanied by osteoclast activity near the graft surface. Disintegrated BCP graft particles were frequently surrounded by newly formed bone (Fig. 4c). Within BCP groups, no pronounced differences regarding resorption pattern and bone remodelling could be noted histologically.

In all groups investigated, titanium implants were lined by a firmly attached mature, parallel-fibered woven bone. Several areas showed implant surfaces being in direct contact with the BCP1–3 or NBM particles (Fig. 4d). However, these areas did not reveal any interposition of NMT between the implant surface and the respective bone graft particles. The maturity of the woven bone was identifiable by the development of

Table 1 Mean values for MT, NMT, and BSM in all groups ($n = 8$ animals)

	BCP1	BCP2	BCP3	NBM	C
Mean BS (%)	9.2*	14.04	13.46	21.25*	7.73
SD	3.28	5.77	2.76	13.52	3.23
Mean MT (%)	67.49	61.75	63.06	57.36	71.63
SD	14.2	9.1	10,36	10.59	14.27
Mean BS + MT (%)	76.69	75.79	76.51	78.64	79.37
SD	13.27	6.52	10.57	13.21	12.31

*Significant between-group difference at $P < 0.05$

Table 2 Mean values for BIC and CBI-IS in all groups ($n = 8$ animals)

	BCP1	BCP2	BCP3	NBM	C
Mean BIC (μm)	18,055.29	15,887.33	17,477.9	17,662.76	16,673.55
SD	2176.29	4467.02	3755.55	1430.12	2581.41
Mean BIC (%)	84.11	74.38	80.26	81.15	77.57
SD	7.84	20.5	16.13	6.23	12.87
Mean CBI-ISb (μm)	− 146.09	− 213.45	− 286.55	246.77	86.37
SD	586.16	472.94	491.34	515.75	308.18
Mean CBI-ISo (μm)	195.91	0	16.09	64.38	139.92
SD	545.13	0	50.87	72.45	258.1

b, buccal aspect; *o*, oral aspect

primary osteons and appeared to be comparable in regenerated and control areas of all treatment groups.

Histomorphometrical analysis

Mean values and percentages of MT and BS values in different groups are presented in Table 1. In particular, mean MT values were equally distributed within the different BCP groups without showing obvious differences between the respective sites (61.75 ± 9.1% (BCP2) to 67.49 ± 14.2% (BCP1)). These percentages were markedly lower in the NBM group (57.63 ± 10.59%), however, without showing any significant differences between the grafted sites ($P > 0.05$, respectively).

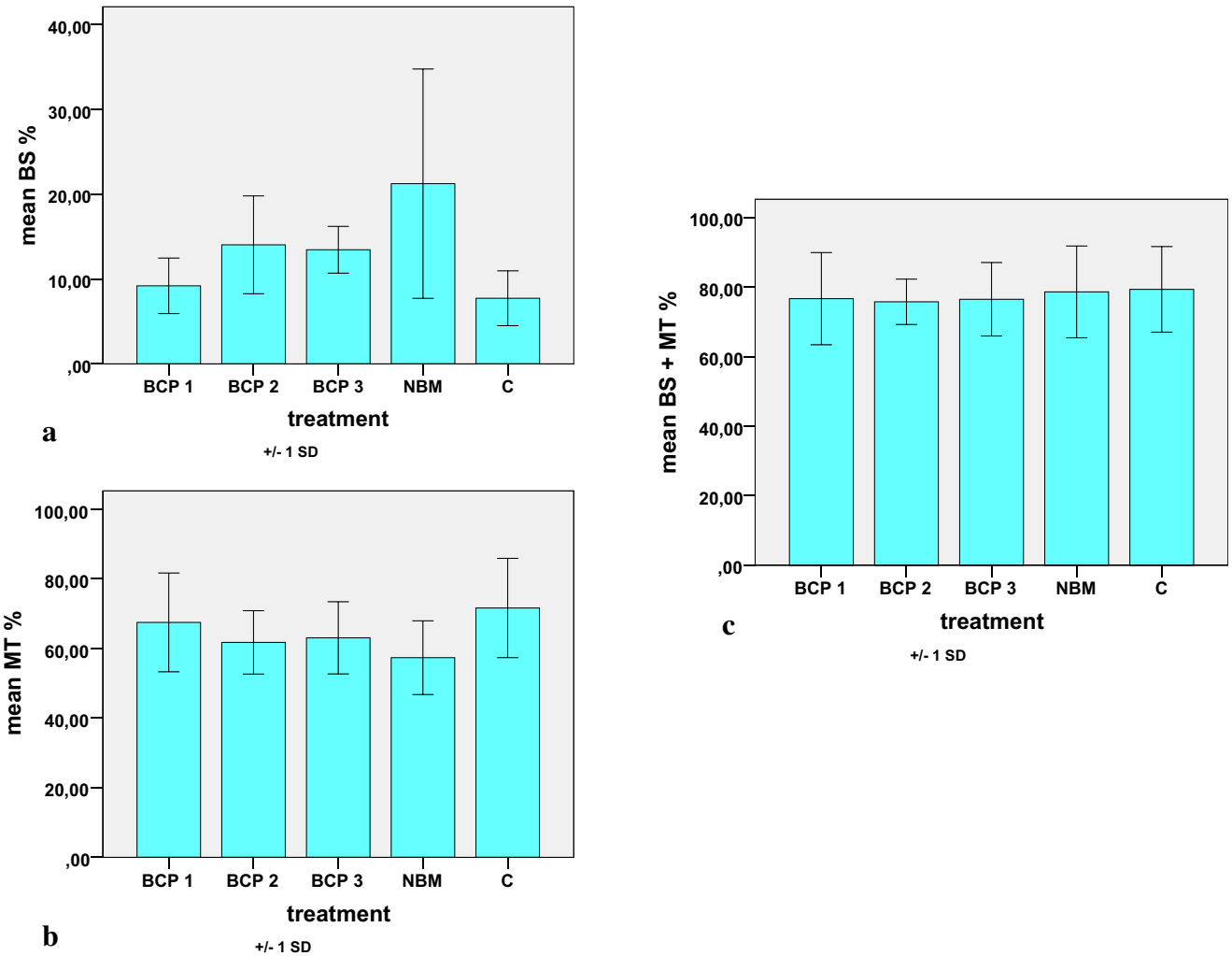

Fig. 5 **a** Mean bone substitute (BS) in all treatment groups (%). **b** Mean mineralized tissue (MT) in all treatment groups (%). **c** Mean bone substitute and mineralized tissue (BS+MT) in all treatment groups (%)

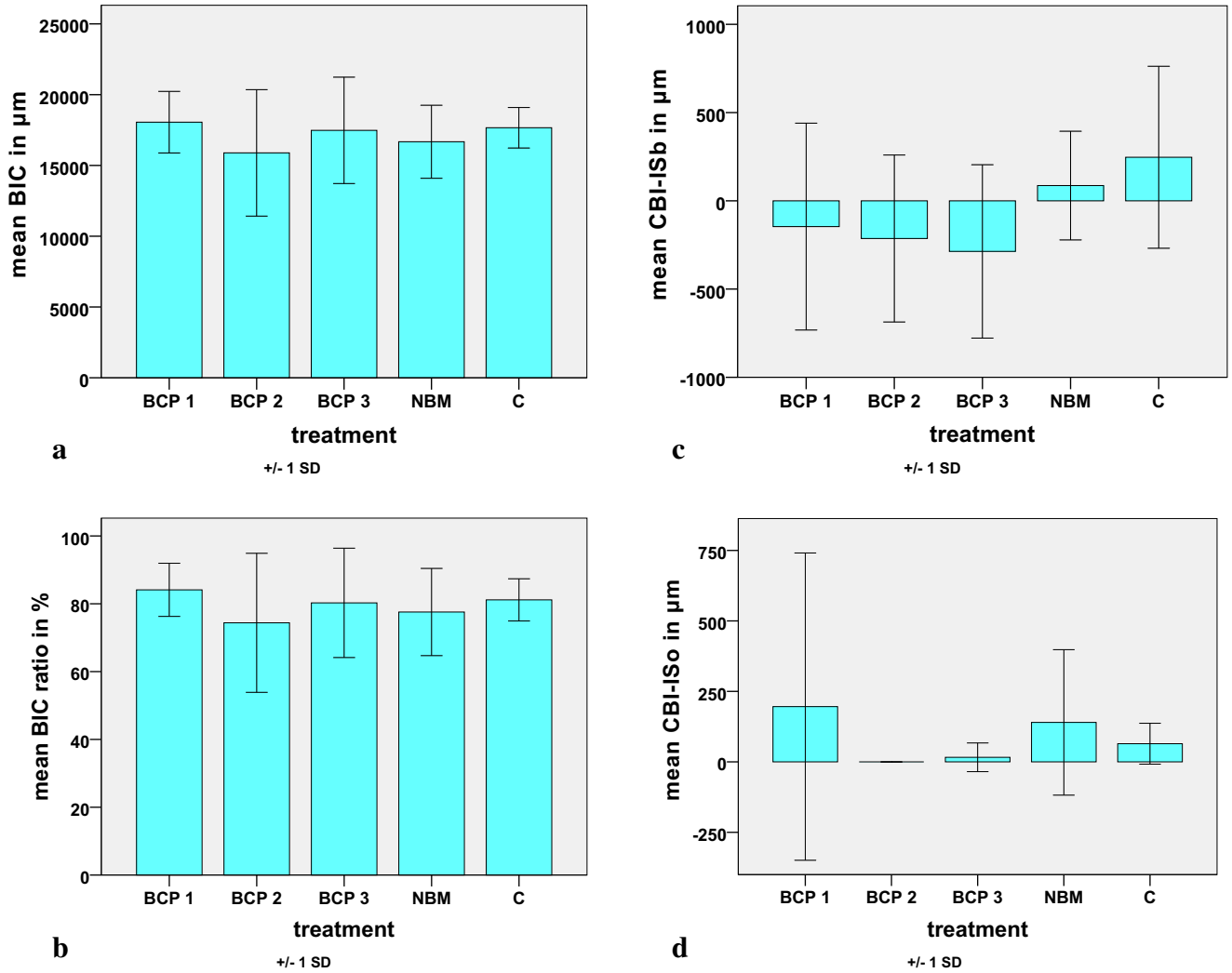

Fig. 6 **a** Mean bone-to-implant contact (BIC) in all treatment groups (µm). **b** Mean bone-to-implant contact ratio (BIC) in all treatment groups (%). **c** Mean distance between crestal bone level (CBL) and the buccal aspect of the implant shoulder (ISb). **d** Mean distance between crestal bone level (CBL) and the oral aspect of the implant shoulder (ISo)

Mean BS values were highest in the NBM group (21.25 ± 13.52%) and markedly reduced in the different BCP groups (Table 1). The lowest BS values were noted in the BCP1 (9.2 ± 3.28%) group, reaching statistical significance when compared with the NBM group. When comparing BS + MT values, all grafted areas revealed similar mean values, ranging between 75.79 ± 6.52% (BCP2) and 78.64% ± 13.21% (NBM), respectively.

Mean values and percentages of BIC and IS-CBI values in different groups are presented in Table 2. All test and control groups investigated revealed comparable and statistically not significant different BIC values, ranging from 73.38 ± 20.5% (BCP2) to 84.11 ± 7.84% (BCP1), respectively. Except for BCP2 grafted sites, all implants investigated were associated with positive IS-CBI values, ranging from 16.09 ± 50.87 µm (BCP3) to 195.91 ± 545.13 µm (BCP1), respectively. These differences, however, failed to reach statistical significance ($P > 0.05$, respectively).

Discussion

The present study aimed at histologically investigating the influence of three BCP graft materials with different surface topographies on new bone formation and staged osseointegration of titanium implants in minipigs Figs 5 and 6.

The selected experimental model represents a well-established standard to investigate bone remodelling processes in implant dentistry [20]. Similar cylindrical-type defects have also been described in previous experimental studies and were reported to be non-critical, as they allowed for a certain spontaneous healing [4, 21].

The present histological analysis has indicated that BCP1–3 and NBM groups were equally and homogeneously integrated into a newly formed scaffold of cancellous bone after 20 weeks. All bone graft materials lead to comparable amounts of new bone formation, as indicated by comparable mean MT values. Even though mean MT values were markedly lower in the NBM group, these differences did not reach statistical significance when compared with BCP1–3 groups. These differences may be attributed to the higher BS values noted at NBM-treated sites, which in turn reflects a higher degradation rate of BCP particles. This was particularly noted in the BCP1 group, reaching statistical significance over NBM-treated sites. The dissolution of BCP particles was clearly depicted in the present descriptive histological analysis and was also confirmed in a previous experimental study employing a similar type of microstructured BCP (10% HA and 90% β-TCP, particle size 500–1000 μm) [21]. The cylindrical-type defects were filled with BCP1, NBM, and another BCP (60% HA and 40% β-TCP, particle size 500–1000 μm) (refers to as SBC). At 8 weeks, BCP was associated with significantly higher MT and BS values when compared with NBM- and SBC-treated defects. However, when comparing the histomorphometrical outcomes at 3 and 8 weeks, this BCP also revealed a marked reduction in mean BS values (median 45.5 at 3 weeks and 38.3% at 8 weeks) [21]. Potential differences between the findings of the latter study and those of the present analysis may be mainly attributed to the prolonged healing period of 20 weeks. Moreover, implant placement and the associated dynamic stages of osseointegration may have also contributed to the dissolution of the various BCP grafts. To the best of our knowledge, these are the first data reporting on the histological outcomes following two-stage implant placement at BCP1–3 grafted sites.

However, previous animal studies performed in the canine tested SBC in dehiscence- and saddle-type defects along with a simultaneous or two-stage grafting procedure [5, 6, 22]. A dissolution of SBC particles was noted between 9 and 10 weeks and resulted in markedly lower BS values than the respective NBM groups, respectively [5, 6, 22]. The pronounced bone remodelling in the SBC group was also evidenced by an elevated collagen type I, osteocalcin, and transglutaminase antigen reactivity in the adjacent non-mineralized tissue [22].

Despite the lack of immunohistochemical analyses, the present analysis also commonly revealed that BCP particles were well integrated and surrounded by bone with an adjacent non-mineralized zone containing multiple dissolved graft particles. Similar findings were made by Dahlin et al. [21] and hypothesized as being linked to a dissolution of ions into the adjacent tissue. In fact, bone remodelling and subsequently resorption of TCP have previously been linked to its microstructure, with a more pronounced activity being noted for submicron-scaled over micron-scaled materials [18, 23]. Accordingly, future studies need to further focus on the specific degradation pattern of BCP1–3.

When further analyzing the present data, it was also noted that BCP1–3 and NBM groups were associated with comparable BIC values after 8 weeks of healing, which were on a level equivalent to those noted at pristine bone sites. Moreover, the BIC values noted in all test and control groups were within the range of those data (71.9 ± 4.0 to 74.9 ± 7.8%) that were reported for the same implant surface after 2 weeks of healing in the mandible of dogs [24, 25].

Previous data also pointed to comparable BIC values following two-stage implant placement at SBC and NBM grafted sites. In particular, after a submerged healing period of 2 weeks, mean BIC values varied between 54.1 ± 22.6 and 67.7 ± 16.9% in the NBM and between 61.0 ± 8.7 and 66.9 ± 17.8% in the SBC group, respectively [6].

Conclusions

Within the limits of the present study, it was concluded that all investigated bone graft materials supported bone formation and the staged osseointegration of titanium implants.

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Compliance with ethical standards

Animal selection, management, and surgery protocol were approved by the Malmö-Lund University Animal Experiment Ethics Committee (number M-204-11.7; Malmö-Lund University, Lund, Sweden).

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval The article does not contain any studies with human participants.

Informed consent Formal consent was not required.

Clinical relevance All graft materials may be equally suited for clinical application.

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CLINICAL ORAL IMPLANTS RESEARCH

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Influence of two barrier membranes on staged guided bone regeneration and osseointegration of titanium implants in dogs: part 1. Augmentation using bone graft substitutes and autogenous bone

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Key words: alveolar ridge defect, animal study, barrier membranes, bone substitutes, guided bone regeneration

Abstract

Objectives: To assess the influence of two barrier membranes and two bone graft substitutes mixed with autogenous bone (AB) on staged guided bone regeneration and osseointegration of titanium implants in dogs.

Materials and methods: Four saddle-type defects each were prepared in the upper jaw of six fox hounds and randomly filled with a natural bone mineral (NBM) + AB and a biphasic calcium phosphate (SBC) + AB and allocated to either an *in situ* gelling polyethylene glycol (PEG) or a collagen membrane (CM). At 8 weeks, modSLA titanium implants were inserted and left to heal in a submerged position. At 8 + 2 weeks, dissected blocks were processed for histomorphometrical analysis (e.g., treated area [TA], bone-to-implant contact [BIC]).

Results: The mean TA values (mm^2) and BIC values (%) tended to be higher in the PEG groups(TA: NBM + AB [10.4 ± 2.5]; SBC + AB [10.4 ± 5.8]/BIC: NBM + AB [86.4 ± 20.1]; SBC + AB [80.1 ± 21.5]) when compared with the corresponding CM groups (TA: NBM + AB [9.7 ± 4.8]; SBC + AB [7.8 ± 4.3]/ BIC: NBM + AB [71.3 ± 20.8]; SBC + AB [72.4 ± 20.3]). A significant difference was observed for the mean TA values in the SBC + AB groups.

Conclusion: It was concluded that all augmentation procedures investigated supported bone regeneration and staged osseointegration of modSLA titanium implants. However, the application of PEG may be associated with increased TA values.

The basic concept of guided bone regeneration (GBR) involves the placement of a barrier membrane to protect the blood clot and create a secluded space around the bone defect (BD), thus enabling access for bone-forming cells without competition from other tissues (Dahlin et al. 1988). Several design requirements such as biocompatibility, cell occlusivity, volume stability, tissue integration, nutrient transfer and also ease of use in the clinic have been proposed for a material that is intended for use as a barrier for GBR procedures (Hardwick et al. 1994). Nowadays, most investigations focus on the use of a porcine-derived native type I and type III collagen membrane (CM), featuring a pronounced tissue integration and semipermeability, thus facilitating nutrient transfer during the early stages of wound healing (Rothamel et al. 2005; Schwarz et al. 2006, 2008). However, a potential drawback particularly of native collagen is the fast biodegradation, resulting in a reduced ability to maintain space, thus compromising the secluded wound area (Sela et al. 2003; Rothamel et al. 2005). Recently, an *in situ* gelling hydrogel composed of two polyethylene glycol (PEG) components was introduced to serve as a new material for barrier membranes (Jung et al. 2006). Upon mixing, PEG is applied as a viscous liquid and gels on the application site within 20–50 s. During wound healing, the cross-linked PEG compounds are completely degraded by hydrolysis without acidic products or causing any foreign body reactions in the adjacent tissues (Wechsler et al. 2008; Herten et al. 2009). The hydrolytic disruption of PEG specimens was associated with an ingrowth of blood vessels at 4 weeks and a prolonged biodegradation at 16–24 weeks after subcutaneous implantation in rats (Herten et al. 2009). Experimental studies have indicated that this material is highly biocompatible and cell-occlusive, and demonstrated at least similar amounts of newly formed bone in the former

defect area when compared with other type of barriers (e.g. expanded polytetrafluorethylene, CM, polylactides, polyglykolides) (Jung et al. 2006, 2009b; Thoma et al. 2009; Schwarz et al. 2010). Because of its liquid consistency during application, it is, however, mandatory to combine the PEG membrane with grafting materials. In recent preclinical studies on localized ridge augmentation simultaneous with implant placement (i.e. one-stage GBR), the PEG membrane was applied over two types of bone fillers including particulated autogenous bone (AB) or a biphasic calcium phosphate (SBC) (Jung et al. 2009b; Schwarz et al. 2010). After 6 months of submerged healing, both groups revealed similar amounts of new bone formation and bone-to-implant contact (BIC), which appeared to be on a level equivalent to that noted when both types of bone fillers were combined with CM (Jung et al. 2009b). In a randomized, controlled clinical trial, a comparable vertical bone fill around dental implants was also noted when both PEG and CM were combined with a bovine-derived natural bone mineral (NBM) (Jung et al. 2009a). Based on these findings, it might be suggested that one-stage GBR using PEG in combination with either AB, NBM or SBC may result in similar histological and/or clinical outcomes as the application of CM. However, when considering the improved volume stability and prolonged barrier function noted for PEG in comparison with CM (Herten et al. 2009), one may speculate that these specific physicochemical properties improve bone regeneration in more advanced defect sites, requiring a staged GBR and implant placement. Even though the survival rates of implants placed in augmented bone have been reported to be comparable to the rates of implants placed in pristine bone, it still remains unknown to what extent a staged GBR procedure may influence the initial process of osseointegration (Jensen & Terheyden 2009). So far, these issues have not been addressed either for PEG or for CM.

Therefore, the aim of the present study was to assess the histological outcome (i.e. new bone formation, BIC) of a staged GBR procedure using a combination of PEG and CM either with NBM + AB or SBC + AB for localized ridge augmentation and subsequent implant placement at saddle-type defects in a dog model.

Material and methods

Animals

In the present study, a total of six fox hounds (age 18–22 months, weight 34–42 kg) were included. All animals exhibited a fully erupted permanent dentition. During the experiment, the dogs were fed once per day with soft-food diet and water *ad libitum*. Animal selection, management and surgery protocol were approved by the Animal Care and Use Committee of the Heinrich Heine University and the local government of Düsseldorf. The experimental segment of the study started after an adaption period of 4 weeks.

Study design and randomization

The study was performed in three surgical phases.

In the first phase, the mandibular and maxillary first, second, third, fourth premolar as well as the first and second molar (P1–M2) were extracted. After a healing period of 10 weeks, a total of four standardized saddle-type defects (mesio-distal width: 10 mm; height: 8 mm) were bilaterally prepared in the upper jaw of each dog (i.e. $n = 4$ defects per animal in the upper jaw).

The defects were filled using NBM + AB and SBC + AB with a random assignment of both treatment procedures to anterior and posterior sites. Subsequently, treated defects were randomly allocated in a split-mouth design to the application of either PEG or CM. Accordingly, all dogs received the following treatment procedures:

NBM + AB + PEG and SBC + AB + PEG vs.
NBM + AB + CM and SBC + AB + CM

At 8 weeks, modSLA titanium implants ($n = 4$ per animal in the upper jaw) were inserted at the respective treated defect sites and left to heal in a submerged position for 2 weeks.

Randomization was performed according to a computer-generated list (RandList®, DatInf GmbH, Tübingen, Germany). The animals were killed after a healing period of 8 + 2 weeks.

Surgical procedure

Before each surgical intervention, intramuscular sedation was accomplished with 0.17 mg/kg acepromazine (Vetranquil 1%, Ceva Tiergesundheit, Düsseldorf, Germany). Subsequently, anesthesia was initiated using 21.5 mg/kg thiopental-sodium (Trapanal 2.5%, Altana GmbH, Konstanz, Germany). During all the surgical procedures, inhalation anesthesia was performed using oxygen and nitrous oxide and isoflurane. To maintain hydration, all animals received a constant-rate infusion of lactated Ringer's solution while anesthetized. Intraoperative analgesia was performed by an intravenous injection of 0.4 mg/kg piritramid (Dipidolor®, Janssen-Cilag GmbH, Neuss, Germany) and 4.5 mg/kg carprofene (Rimadyl®, Pfitzer Pharma GmbH, Karlsruhe, Germany). For postoperative treatment, piritramid and carprofene were applied subcutaneously for 3 days at the same dose as described before.

Surgical phase 1 (tooth extraction)

In the first surgery, mucoperiosteal flaps were reflected bilaterally in both jaws and P1–M2 were carefully removed after tooth separation. Wound closure was accomplished by means of mattress sutures and the sites were allowed to heal for 10 weeks. Prophylactic administration of clindamycine (11 mg/kg body weight, Cleorobe®, Pharmacia Tiergesundheit, Erlangen, Germany) was performed intra- and postoperatively for 10 days.

Surgical phase 2 (defect creation and GBR)

After 3 months of healing, midcrestal incisions were made and mucoperiosteal flaps were reflected to expose the alveolar bone in the upper jaws. Vertical releasing incisions were placed about 4–5 mm distant to the designated experimental sites. Four standardized saddle-type defects including the vestibular and oral aspect of the alveolar ridge were subsequently prepared bilaterally at a distance of at least 5 mm using a straight fissure carbide bur. After bone block removal, the final dimension of all defects revealed a mesio-distal width of 10 mm and an apico-coronal height, as measured from the crestal bone, of 8 mm (Fig. 1a). The defect sizes were standardized using a periodontal probe (PCP12, Hu-Friedy Co., Chicago, IL, USA). All osteotomy procedures were performed under copious irrigation with a sterile 0.9% physiological saline. Finally, each defect site was rinsed thoroughly with sterile saline to completely remove any residual debris. Subsequently, the respective defects were homogeneously filled with a particulate NBM (Geistlich BioOss® spongiosa granules, particle size 0.25–1 mm, Geistlich Biomaterials, Wolhusen, Switzerland) or SBC (60% HA + 40% β-TCP, Straumann Bone Ceramic®, pore diameters: 100–500 μm, Institut Straumann AG, Basel, Switzerland). Particular care was taken that the graft particles did not exceed the contour of the bordering bone walls in both the vestibular/oral and the cranial directions. Before treatment, both NBM and SBC were homogeneously mixed (ratio 1 : 1) with particulated AB (particle size 0.5–1 mm) harvested from the cancellous aspect of the bone blocks. Following treatment, CM (Geistlich BioGide®, Geistlich Biomaterials) was adapted over the respective defect areas so as to cover 1–2 mm of the surrounding alveolar bone. Neither sutures nor pins were used for membrane fixation or stabilization (Fig. 1b). At the contralateral sites, excess of blood was removed from the surrounding bone. Afterwards, the PEG hydrogel (MembraGel®, Institut Straumann AG) was applied in a viscous form, also extending 1–2 mm beyond the margins of the defect walls. After approximately 60 s, the PEG membrane had set to its gelated status (Jung et al. 2009b) (Fig. 1c). Following periosteal-releasing incisions, the mucoperiosteal flaps were advanced, repositioned tension-free in a

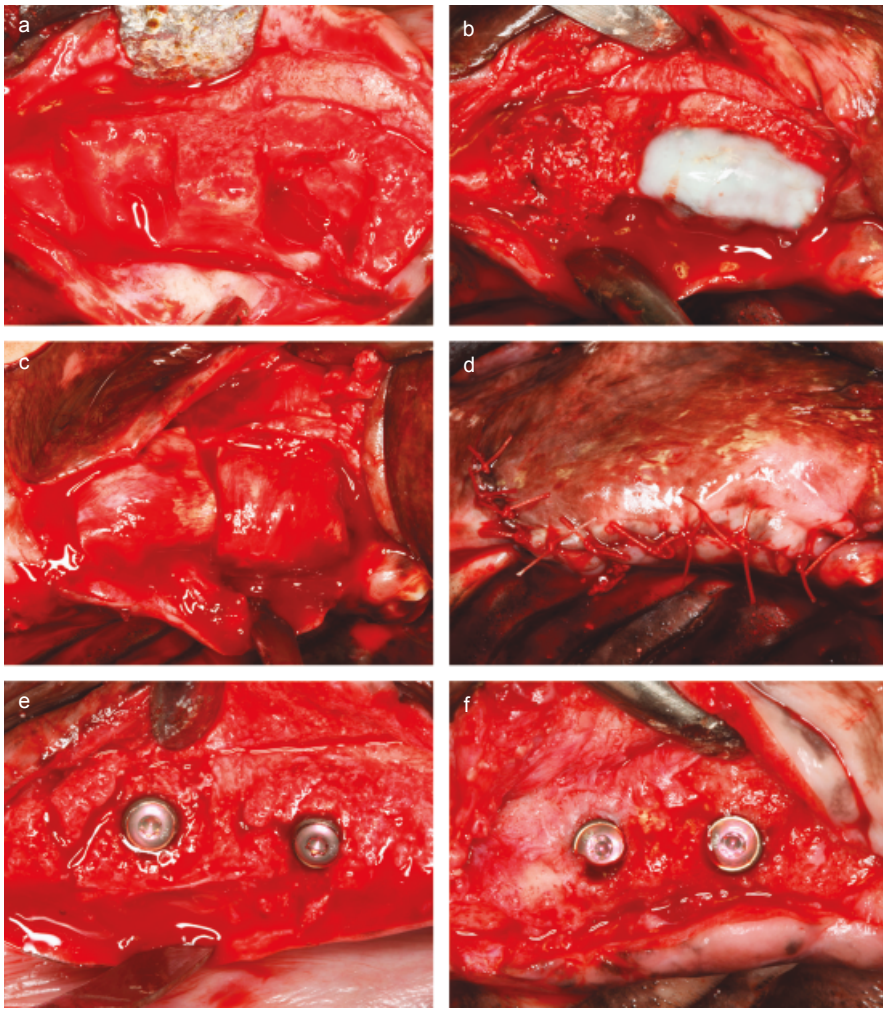

Fig. 1. (a) At 10 weeks after tooth extraction, a total of $n = 4$ standardized saddle-type defects (width: 10 mm; height: 8 mm) were bilaterally created at a distance of at least 5 mm in each upper jaw of 6 dogs. Occlusal view indicating that both vestibular and lingual bone plates were removed. (b) In each hemimandible, the defects were homogeneously filled with NBM + AB and SBC + AB (ratio 1 : 1). Experimental sites receiving the PEG hydrogel after the removal of excess blood from the surrounding bone. Situation at about 60 s after application indicating a gelated status of the material. PEG application at the anterior defect is not shown in this figure. (c) Contralateral sites receiving CM that were adapted in such a way as to cover 1–2 mm of the surrounding alveolar bone. (d) Experimental sites were left to heal in a submerged position for 8 weeks. (e) At reentry, the defect borders were clearly demarcated by residual particles of the respective bone substitutes. Implants were placed at the central aspect of each defect site in such a way that IS at best coincided with the regenerated bone crest at both vestibular and oral aspects (left site: NBM + AB + CM; right site: SBC + AB + CM). (f) A corticalization of the newly formed bone was more frequently observed at PEG-treated sites (left site: SBC + AB + PEG; right site: NBM + AB + PEG). NBM, natural bone mineral; AB, autogenous bone; PEG, polyethylene glycol; CM, collagen membrane; IS, implant shoulder.

coronal position and fixed with vertical or horizontal mattress sutures (Resorba®, Resorba Wundversorgung GmbH & Co. KG, Nürnberg, Germany) in such a way as to ensure a submerged healing condition (Fig. 1d).

Surgical phase 3 (implant placement)

In the third surgery, midcrestal incisions were made and mucoperiosteal flaps were reflected to expose the experimental sites for implant placement. All granulation tissue was carefully removed from the residual defect areas. A total of $n = 4$ implant sites were prepared bilaterally, at the central aspect of each experimental site, using a low-trauma surgical technique under copious irrigation with sterile 0.9% physiological saline (surgery protocol by Institut Straumann AG). Thereafter, screw-type modSLA (Bone Level® SLActive®, ∅4.1 mm, length 10 mm, Institut Straumann AG) titanium implants were inserted with good primary stability (i.e. lack of clinical implant mobility) in such a way that the implant shoulder (IS) at best coincided with the regenerated bone crest at both vestibular and oral aspects (Fig. 1e and f). Following the application of closure screws, the mucoperiosteal flaps were repositioned and fixed with vertical or horizontal mattress sutures (Resorba®) in such a way as to ensure a submerged healing condition.

All surgical procedures were performed by two experienced surgeons (F. S. and I. M.).

Animal sacrifice and retrieval of specimens

After a healing period of 8 + 2 weeks, the animals were killed by an overdose of sodium pentobarbital 3%, respectively. The oral tissues were fixed by perfusion with 10% buffered formalin administered through the carotid arteries. The jaws were dissected and blocks containing the experimental specimens were obtained. All specimens were fixed in a 10% neutral-buffered formalin solution for 4–7 days.

Histological preparation

The specimens were dehydrated using ascending grades of alcohol and xylene, infiltrated and embedded in methylmethacrylate (MMA, Technovit 9100 NEU, Heraeus Kulzer, Wehrheim, Germany) for nondecalcified sectioning. During this procedure, any negative influence of polymerisation heat was avoided due to a controlled polymerization in a cold atmosphere (−4°C). After 20 h, the specimens were completely polymerized. Each implant site was cut in the buccooral direction along with the long axis of the implant using a diamond band saw (Exakt®, Apparatebau, Norderstedt, Germany). Serial sections were prepared from the central defect area, resulting in four sections approximately 300 µm in thickness each (Donath 1985). Only implant sections showing an inner thread were chosen for the histological evaluation. Subsequently, all specimens were glued with acrylic cement (Technovit 7210 VLC, Heraeus Kulzer, Wehrheim, Germany) to silanized glass slides (Super Frost, Menzel GmbH, Braunschweig, Germany) and ground to a final thickness of approximately 40 µm. All sections were stained with toluidine blue to evaluate new bone formation. With this technique, old bone stains light blue, whereas newly formed bone stains dark blue because of its higher protein content (Schenk et al. 1984).

Histomorphometrical analysis

Histomorphometrical analyses as well as microscopic observations were performed by one experienced investigator masked to the specific experimental conditions (A. H.). For image acquisition, a color CCD camera (Color View III, Olympus, Hamburg, Germany) was mounted on a binocular light microscope (Olympus BX50, Olympus). Digital images (original magnification × 200) were evaluated using a software program (Cell D®, Soft Imaging System, Münster, Germany).

The following landmarks were identified in the stained sections at both vestibular and oral aspects: the IS and the bottom of the BD. Defect length (DL) was measured from IS to BD (mm); the amount of new BIC in the defect was measured as percentage of the distance from BD to IS, serving as 100% (Fig. 2). Additionally, the treated

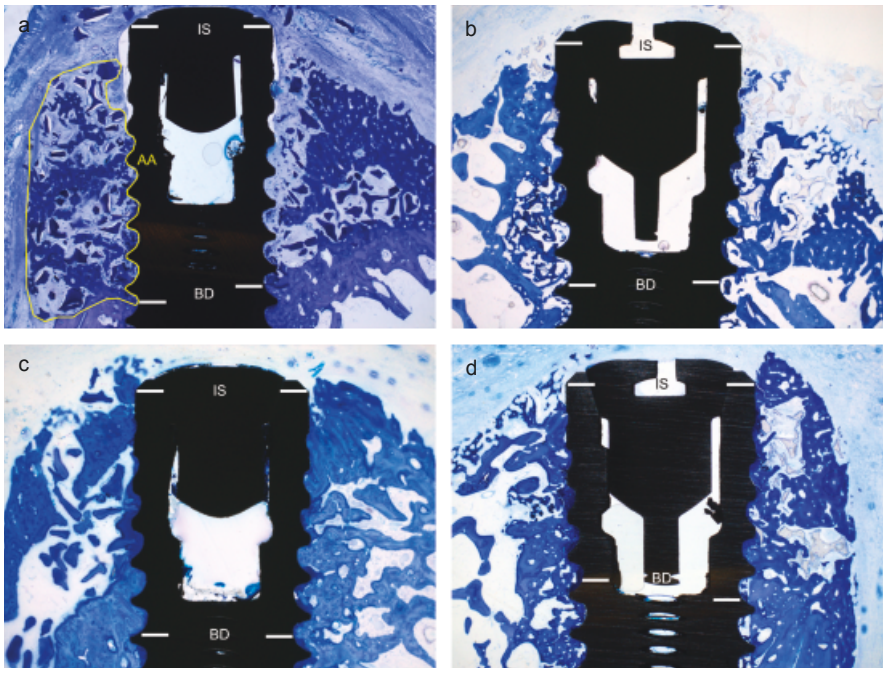

Fig. 2. Representative histological views (vestibulo-oral sections, toluidine blue stain) of wound healing at 8 + 2 weeks (original magnification × 25). Histological observation commonly revealed a homogeneous bone formation and osseointegration within the secluded volume created by both CM and PEG barrier membranes. A corticalization of the cancellous bone was noted in the periphery of the treated area. (a) NBM + AB + CM, (b) SBC + AB + CM, (c) NBM + AB + PEG, (d) SBC + AB + PEG. BD, bottom of the bone defect; TA, treated area; IS, implant shoulder; NBM, natural bone mineral; AB, autogenous bone; PEG, polyethylene glycol; CM, collagen membrane.

area (TA) (mm²) was measured from BD to IS. Within TA, the surface area of mineralized- (MT) and nonmineralized tissue (NMT) as well as residual NBM/SBC particles (BS) were automatically assessed (mm²) by the image analysis software. Before the start of the morphometrical analysis, a calibration procedure was initiated for the image analysis software and revealed that repeated measurements of $n = 12$ different sections were similar at >95% level.

Statistical analysis

The statistical analysis was performed using a commercially available software program (PASW Statistics 19.0, SPSS Inc., Chicago, IL, USA). The mean values and standard deviations among animals were calculated for each variable and group. The data rows were examined using the Kolmogorow–Smirnow test for a normal distribution. Within-group comparisons were performed using the paired *t*-test (i.e. vestibular and oral aspects). For the comparisons between groups at 8 + 2 weeks, the unpaired *t*- test was used. The α error was set at 0.05.

Results

Clinical healing

The postoperative healing was considered as generally uneventful in all dogs. No complications such as allergic reactions, swellings, abscesses or infections were observed throughout the entire study period. None of the defect sites revealed a premature exposure of the wound area (i.e. exposure of barrier membranes or titanium implants). Surgical reentry at 8 weeks indicated that the volume of the treated sites had been fully maintained in all groups investigated, thus resulting in a homogeneous bony contour at either vestibular or oral aspects. Both NBM and SBC particles appeared to be well integrated into a newly formed hard tissue. A corticalization of the newly formed bone was more frequently observed at PEG-treated sites (Fig. 1e and f).

Descriptive histology

Histological observation at 8 + 2 weeks revealed a bony filling of the secluded wound area confined by both CM and PEG membranes. In particular, a scaffold of cancellous bone with numerous blood vessels and bone-forming cells homogeneously invaded the former defect area and appeared to be in close contact with both NBM and SBC as well as residual AB particles. A transformation of the cancellous bone into cortical bone was commonly observed in the periphery of the former defect area (Fig. 2a–d). Moreover, it was observed that SBC particles revealed an increased contact with NMT, while NBM particles were more frequently surrounded by MT (Fig. 3a–c). In general, histological observation failed to demonstrate any osteoclastic activity at the surface of both bone graft particles. However, a dissolution of SBC resulting in a superficial disintegration of particles into individual grains was commonly observed in areas where the bone graft particles were surrounded by NMT (Fig. 3d). In all groups investigated, modSLA implants seemed to be surrounded by a firmly attached mature, parallel-fibered woven bone. Occasionally, the implant surfaces were in direct contact with the NBM and SBC particles. These areas did not reveal any interposition of NMT between the implant surface and the respective bone graft particles (Fig. 3a and c). The maturity of the woven bone was identifiable by the development of primary osteons and appeared to be comparable in both regenerated and pristine areas (Fig. 3a–c).

Histomorphometric analysis

The mean values of DL, TA, MT, NMT, BS and BIC in both groups after 8 + 2 weeks of healing are presented in Tables 1–3. Basically, within-group comparisons in the CM group revealed comparable ($P > 0.05$; paired *t*-test, respectively) mean DL, TA, MT, NMT and BIC values at both NBM + AB- and SBC + AB-treated sites. A significant difference between vestibular and oral aspects was only observed for the mean BS values in the SBC + AB + CM group ($P < 0.05$; paired *t*-test) (Table 1). Similarly, the PEG groups also revealed comparable mean TA, MT, BS and BIC values ($P > 0.05$; paired *t*-test, respectively) at both vestibular and oral aspects. Within-group comparisons only revealed a significant difference for the mean DL and NMT values ($P < 0.01$, $P < 0.05$; paired *t*-test, respectively) in the NBM + AB + PEG group (Table 2). Both NBM + AB + PEG and SBC + AB + PEG groups tended to reveal increased TA, MT and BIC values when compared with the respective CM groups (Fig. 3a–c). However, statistical analysis only revealed a significant difference for the mean TA values between SBC + AB + CM- and SBC + AB + PEG-treated sites (Table 3).

Discussion

The present experimental study was designed to histologically evaluate the outcome of a staged GBR procedure using a combination of PEG and CM either with NBM + AB or SBC + AB for localized ridge augmentation and subsequent implant placement at saddle-type defects in a dog model. In this context, it must be emphasized that this type of defect model is commonly used and well accepted to evaluate GBR procedures in canines (Schenk et al. 1994; Simion et al. 1999; Bornstein et al. 2007). Within its limitations, the present data have indicated that all augmentation procedures investigated resulted in a homoge-

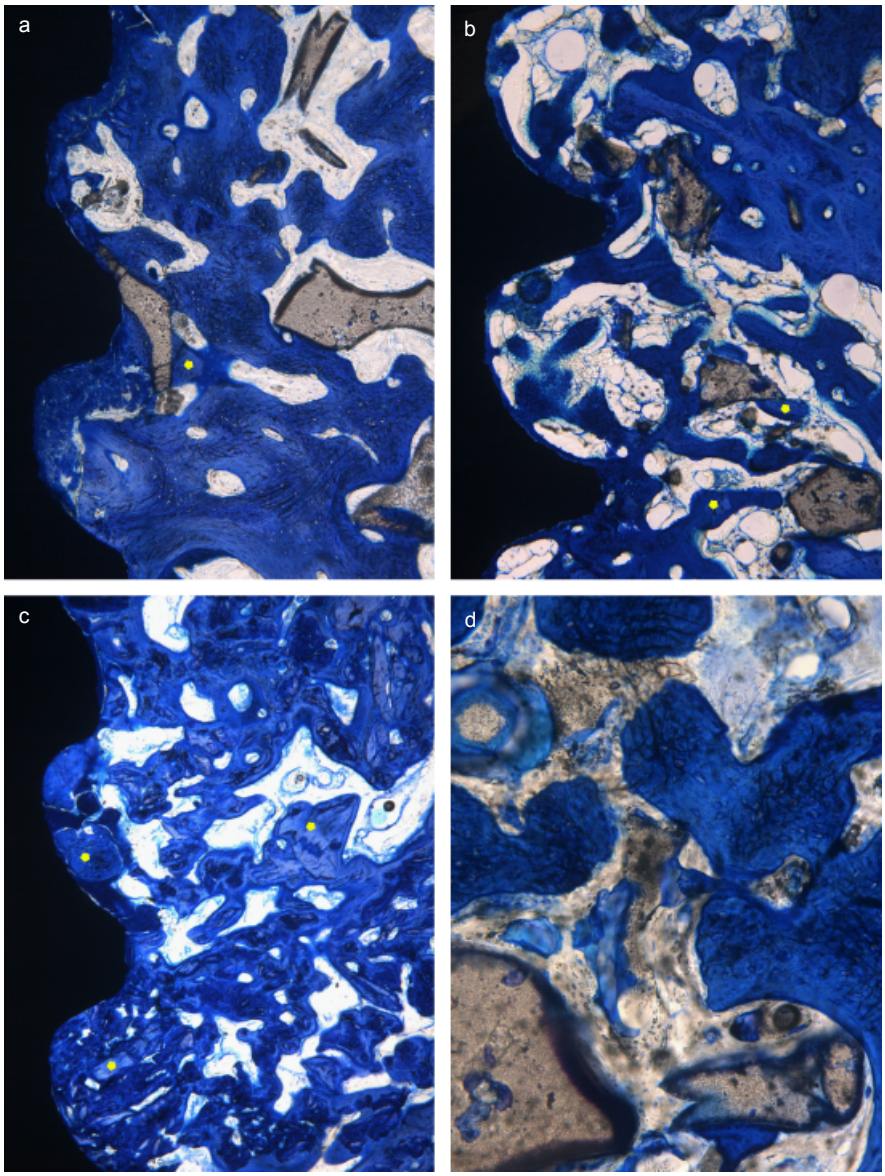

Fig. 3 Both NBM and SBC particles as well as residual AB were homogeneously integrated into a dense network of cancellous bone. The application of PEG appeared to be associated with an increased density of MT within TA (a–c). (a) SBC + AB + PEG (original magnification × 100), (b) SBC + AB + CM (original magnification × 100), (c) NBM + AB + PEG (original magnification × 100). SBC particles were more frequently surrounded by NMT than either NBM or AB particles. In these areas, however, a dissolution of SBC was commonly observed. (d) SBC + AB + PEG (original magnification × 200). Yellow polygons indicate residual autogenous bone particles. NBM, natural bone mineral; AB, autogenous bone; MT, mineralized tissue; PEG, polyethylene glycol; TA, treated area; CM, collagen membrane; NMT, nonmineralized tissue.

Table 1. **Mean values (± SD) of DL (in mm), TA, MT, NMT, BS (in mm^2) and BIC (in %) in the CM group at both vestibular and oral aspects after 8 + 2 weeks of submerged healing ($n = 6$ dogs)**

Group	DL	TA	MT	NMT	BS	BIC
NBM + AB						
Vestibular	5 ± 1.8	10.2 ± 4.8	5.3 ± 3.9	4.1 ± 1.5	0.9 ± 0.4	61.8 ± 26.5
Oral	5.2 ± 2.5	9.1 ± 5.3	4.1 ± 2.2	4.2 ± 3	0.9 ± 0.6	80.8 ± 6.8
P value*	NS	NS	NS	NS	NS	NS
SBC + AB						
Vestibular	3.7 ± 0.6	6.8 ± 2.9	3.9 ± 2.4	2.4 ± 0.9	0.5 ± 0.4	72.4 ± 10.2
Oral	5.5 ± 3	8.7 ± 3.7	3.7 ± 1.3	3.4 ± 2.8	1.5 ± 0.7	72.4 ± 27.2
P value*	NS	NS	NS	NS	<0.05	NS

*Comparisons within groups (paired *t*-test).
DL, defect length; TA, treated area; MT, mineralized tissue; NMT, nonmineralized tissue; BS, bone substitute (i.e residual NBM/SBC particles); BIC, bone to implant contact; NBM, natural bone mineral; AB, autogenous bone.

neous bone formation and subsequently osseointegration of modSLA implants within the confined wound area at 8 + 2 weeks. Within-group comparisons revealed comparable TA values at both vestibular and oral aspects, thus indicating that both types of barrier membranes provided a sufficient stabilization of the wound area over the entire observation period. However, histological analysis has pointed to increased TA values at PEG-treated sites when compared with the corresponding CM sites, even reaching statistical significance in the SBC + AB groups. Basically, the observation that NBM + AB + CM may be associated with a predictable bone formation in saddle-type defects is in agreement with previous experimental data (Bornstein et al. 2007). In particular, after 8 and 16 weeks of submerged healing, a dome-shaped bone regeneration was observed above the bottom of the defects. The mean TA values at 8 weeks ranged from about 28.8 mm^2 in the control group (i.e. NBM + AB without membrane application) to about 30.2 mm^2 at NBM + AB + CM-treated sites (values calculated from the data provided in the publication) and remained almost stable in both groups at 16 weeks (Bornstein et al. 2007). The lower overall mean TA values, as noted in the present study, can mainly be attributed to the surface area occupied by modSLA titanium implants, which was not considered for the histomorphometrical analysis. The observation that the application of CM was not associated with an improved outcome of healing (Bornstein et al. 2007) coupled with the finding of the present study that PEG treated sites revealed increased TA scores in comparison with the corresponding CM groups may point to the beneficial effect of a prolonged barrier function, as noted for the *in situ* gelling hydrogel (Herten et al. 2009; Thoma et al. 2009). In this context, it must be emphasized that chemical cross-linking of CM was also proven to be associated with improved membrane stability and bone regeneration in both animal and human studies (Bornstein et al. 2007; Schwarz et al. 2008; Becker et al. 2009). However, in the case of a premature membrane exposure, cross-linking obviously impaired soft-tissue healing or caused wound infections (Bornstein et al. 2007; Becker et al. 2009). In contrast, the *in situ* formed PEG membrane was safely used in a variety of indications, revealing no biologically significant abnormal soft tissue reactions compared with different GBR membranes (e.g. CM) (Jung et al. 2006, 2009b, 2009a; Thoma et al. 2009). The observation that the application of both CM and PEG may be associated with minimal or even no membrane exposures is also in agreement with previous experimental animal studies (Jung et al. 2006; Bornstein et al. 2007; Schwarz et al. 2010).

Table 2. **Mean values (± SD) of DL (in mm), TA, MT, NMT, BS (in mm²) and BIC (in%) in the PEG group at both vestibular and oral aspects after 8 + 2 weeks of submerged healing (*n* = 6 dogs)**

Group	DL	TA	MT	NMT	BS	BIC
NBM + AB						
Vestibular	5.5 ± 0.8	12.8 ± 4.2	6.1 ± 2.6	5.8 ± 2.6	0.9 ± 0.4	85.7 ± 23.9
Oral	3.9 ± 0.7	7.9 ± 6.5	4.7 ± 3.9	2.3 ± 1.9	0.9 ± 0.8	87 ± 17.7
P value*	<0.01	NS	NS	<0.05	NS	NS
SBC + AB						
Vestibular	6.8 ± 1.9	10.5 ± 2.6	4.9 ± 2.2	4.5 ± 2.2	1.1 ± 0.5	83.9 ± 16.4
Oral	5.2 ± 1.1	10.2 ± 2.5	5.4 ± 1.7	4 ± 1.7	0.8 ± 0.7	76.3 ± 26.7
P value*	NS	NS	NS	NS	NS	NS

*Comparisons within groups (paired *t*-test): $P > 0.05$.

DL, defect length; TA, treated area; MT, mineralizedtissue; NMT, nonmineralized tissue; BS, bone substitute (i.e residual NBM/SBC particles); BIC, bone to implant contact; NBM, natural bone mineral; AB, autogenous bone.

Table 3. **Between-group comparison of mean (± SD) DL (in mm), TA, MT, NMT, BS (in mm²) and BIC (in %) values at 8 + 2 weeks (*n* = 6 dogs)**

Group	DL	TA	MT	NMT	BS	BIC
CM						
NBM + AB	5.1 ± 2.1	9.7 ± 4.8	4.7 ± 3	4.1 ± 2.2	0.9 ± 0.5	71.3 ± 20.8
SBC + AB	4.7 ± 2.4	7.8 ± 3.4*	3.9 ± 1.7	2.9 ± 2.2	1 ± 0.8	72.4 ± 20.3
PEG						
NBM + AB	4.7 ± 1.1	10.4 ± 5.8	5.4 ± 3.3	4.1 ± 2.8	0.9 ± 0.6	86.4 ± 20.1
SBC + AB	5 ± 1.7	10.4 ± 2.5*	5.2 ± 1.9	4.3 ± 1.9	0.9 ± 0.6	80.1 ± 21.5
P value*	NS	<0.05	NS	NS	NS	NS

*Comparisons between groups (unpaired t-test).

DL, defect length; TA, treated area; MT, mineralized tissue; NMT, nonmineralized tissue, BS, Bone substitute (i.e residual NBM/SBC particles); BIC, bone to implant contact; CM, collagen membrane; NBM, natural bone mineral; AB, autogenous bone; PEG, polyethylene glycol.

Histological observation has indicated that both NBM + AB and SBC + AB scaffolds were equally and homogeneously integrated into a newly formed scaffold of cancellous bone at 8 + 2 weeks. Currently, the osteoconductive properties of both bone graft substitutes have only been compared for human maxillary sinus augmentation (Cordaro et al. 2008; Froum et al. 2008; Lindgren et al. 2010). In particular, it was observed that NBM and SBC produced comparable amounts of newly formed bone, exhibiting a similar histologic appearance. However, it was also noted that SBC-treated sites revealed an increased amount of NMT when compared with the NBM sites (Cordaro et al. 2008; Lindgren et al. 2009). This observation was not supported by the present histomorphometrical analysis, as all the groups investigated revealed comparable mean NMT values. One might speculate that the osteoinductive and osteogenetic properties noted for AB (Chiriac et al. 2005) may have increased the mean MT and subsequently reduced the mean NMT values in the SBC group. Nevertheless, the present data clearly support previous findings that both types of bone graft particles reveal different resorption characteristics (Cordaro et al. 2008). An initial dissolution of SBC was also observed after 8 weeks of healing in the mandible of minipigs (Jensen et al. 2007). In particular, histological observation revealed the presence of multinucleated giant cells on the surface of particles that were not covered by MT. Even though there were no signs of any cell-mediated resorption lacunae, these surfaces revealed a higher penetration of the staining agent, thus pointing to an initial dissolution of the graft material (Jensen et al. 2007). In this context, however, it is important to emphasize that the mean BIC values, as noted in the present study, were comparable in both NBM + AB and SBC + AB groups, thus pointing to an undisturbed osseointegration, even in the presence of the almost nonresorbing NBM (Mordenfeld et al. 2010). Even though the application of PEG tended to be associated with an increase in the mean BIC values when compared with the corresponding CM groups, these differences did not reach statistical significance. Basically, the BIC values assessed in all groups investigated are within the range of that noted for modSLA titanium implants after 2 weeks of healing in the canine upper jaw (Schwarz et al. 2007a, 2007b), thus pointing to an equal potential of both regenerated and pristine bone to support osseointegration. This assumption is also supported by the results of a recent experimental study, indicating that BIC at dual-acid etched titanium implants was comparable at both NBM + CM-treated defects and pristine sites (Artzi et al. 2010). At present, the quantitative and qualitative capacities of NBM + PEG-, SBC + CM- and SBC + PEG-treated sites to support the initial process of osseointegration are yet to be determined.

Within the limits of the present study, it was concluded that all augmentation procedures investigated supported bone regeneration and staged osseointegration of modSLA titanium implants. However, the application of PEG may be associated with increased TA values.

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Influence of two barrier membranes on staged guided bone regeneration and osseointegration of titanium implants in dogs. Part 2: augmentation using bone graft substitutes

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Key words: alveolar ridge defect, animal study, barrier membranes, bone substitutes, guided bone regeneration

Abstract

Objectives: To assess the influence of two barrier membranes and two bone graft substitutes on staged guided bone regeneration and osseointegration of titanium implants in dogs.

Materials and methods: Saddle-type defects were prepared in the lower jaws of 6 fox hounds and randomly filled with a natural bone mineral (NBM) and a biphasic calcium phosphate (SBC) and allocated to either an *in situ* gelling polyethylene glycol (PEG) or a collagen membrane (CM). At 8 weeks, modSLA titanium implants were inserted and left to heal in a submerged position. At 8 + 2 weeks, respectively, dissected blocks were processed for histomorphometrical analysis (e.g., mineralized tissue [MT], bone-to-implant contact [BIC]).

Results: The mean MT values (mm^2) and BIC values (%) tended to be higher in the PEG groups (MT: NBM [3.4 ± 1.7]; SBC [4.2 ± 2]/BIC: NBM [67.7 ± 16.9]; SBC [66.9 ± 17.8]) when compared with the corresponding CM groups (MT: NBM [2.5 ± 0.8]; SBC [2.3 ± 1.6]/BIC: NBM [54.1 ± 22.6]; SBC [61 ± 8.7]). These differences, however, did not reach statistical significance.

Conclusion: It was concluded that all augmentation procedures investigated supported bone regeneration and staged osseointegration of modSLA titanium implants.

Staged bone augmentation and implant placement is a clinical routine procedure commonly used at sites showing an extensive bone deficiency in either horizontal and/or vertical dimensions (Chiapasco et al. 2006). For horizontal ridge augmentation, clinical evidence favors intraoral autogenous bone (AB) blocks used alone or in combination with a very slowly resorbing particulate bovine-derived natural bone mineral (NBM), which may be applied either with or without the principle of guided bone regeneration (GBR) (Jensen & Terheyden 2009). The mean gain in ridge width varied between 4.4 mm (with AB block) and 2.6 mm (without AB block). In conjunction with AB blocks, however, the application of a barrier membrane may not necessarily increase the outcome of bone regeneration, as the mean gain in ridge width varied between 2.9 mm (non-resorbable) and 4.2 mm (resorbable) at the GBR-treated sites and 4.5 mm at the control sites (Jensen & Terheyden 2009). Despite these promising outcomes of therapy, harvesting of AB blocks is technically demanding and frequently associated with graft resorption, thus necessitating additional grafting after implant placement in about 15–20% of the cases (Parodi et al. 1998; Chiapasco et al. 1999).

In order to overcome some of the limitations encountered with AB blocks, bone graft substitutes (i.e. NBM or a nanocrystalline hydroxyapatite) were also suggested in combination with GBR for lateral ridge augmentation and proven to be associated with a clinically important horizontal bone gain (Strietzel et al. 2007; Hämmerle et al. 2008). These clinical findings were also supported by experimental data indicating that the application of a particulate NBM or a biphasic calcium phosphate (SBC), which were covered by a porcine-derived native type I and type III collagen membrane (CM), was associated with a predictable bone formation in chronic-type lateral ridge defects (Schwarz et al. 2008, 2009, 2010). Under these experimental conditions, the GBR procedure stabilized the NBM particles and

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increased the regenerated area in comparison with non-protected sites after 3 weeks of healing (Schwarz et al. 2010).

Even though the survival rates of implants placed in augmented bone have been reported to be comparable to the rates of implants placed in pristine bone (Donos et al. 2008), only very few data aimed at investigating the impact of two-stage GBR on the process of osseointegration at grafted implant sites (Carmagnola et al. 2008; Artzi et al. 2010; Schwarz et al. 2011). Basically, it was observed that grafting of self-contained defects using a variety of bone substitutes (e.g. NBM) did not impair the establishment of bone-to-implant contact (BIC) in comparison with non-grafted sites (Carmagnola et al. 2008; Artzi et al. 2010). However, when evaluating a non-contained defect configuration, the process of osseointegration appeared to be positively influenced by the type of barrier membrane applied. In particular, GBR at both NBM + AB and SBC + AB (ratio 1 : 1)-grafted defects using an *in situ* gelling hydrogel composed of two polyethylene glycol (PEG) components was associated with a significant increase of the regenerated area and the subsequent establishment of new BIC at 8 + 2 weeks when compared with CM (Schwarz et al. 2011). This may be attributed to the prolonged biodegradation and subsequently volume stability of PEG membranes (16–24 weeks) over CM (4 weeks), as observed after subcutaneous implantation in rats (Herten et al. 2009). Despite the fact that NBM and SBC reveal interconnected macropores similar to those noted for cancellous human bone, it is, however, yet to be determined whether these results may also be achieved when both types of bone fillers are applied without the addition of particulate AB.

Therefore, the aim of the present study was to assess the histological outcome (i.e. new bone formation, BIC) of a staged GBR procedure using a combination of PEG and CM either with NBM or SBC for localized ridge augmentation and subsequent implant placement at saddle-type defects in a dog model.

Material and methods

Animals

The study was conducted in a total of six adult fox hounds (age 18–22 months, weight 34–42 kg) exhibiting a fully erupted permanent dentition. During the experiment, the dogs were fed once per day with soft-food diet and water ad libitum. Animal selection, management and surgery protocol were approved by the Animal Care and Use Committee of the Heinrich Heine University and the local government of Düsseldorf. The experimental segment of the study started after an adaptation period of 4 weeks.

Study design and randomization

The study design has been described in detail recently (Schwarz et al. 2011).

In brief, the experimental aspect was performed in three surgical phases.

In the first phase, the mandibular and maxillary 1st, 2nd, 3rd, 4th premolar as well as the 1st and 2nd molar (P1–M2) were extracted. After a healing period of 10 weeks, a total of four standardized saddle-type defects (mesio-distal width: 10 mm; height: 8 mm) were bilaterally prepared in the lower jaw of each dog (i.e. $n = 4$ defects per animal in the lower jaw).

The defects were filled using NBM and SBC based on a balanced randomization, which was performed according to a computer-generated protocol (RandList®, DatInf GmbH, Tübingen, Germany) accounting for a potential difference between anterior and posterior defect sites.

Subsequently, treated defects were randomly allocated in a split-mouth design to the application of either PEG or CM. Accordingly, all dogs received the following treatment procedures:

NBM + PEG and SBC + PEG vs.
NBM + CM and SBC + CM

At 8 weeks, modSLA titanium implants ($n = 4$ per animal in the lower jaw) were inserted at the respective treated defect sites and left to heal in a submerged position for 2 weeks. The animals were killed after a healing period of 8 + 2 weeks.

Surgical procedure

The surgical procedure has been described in detail recently (Schwarz et al. 2011).

Before each surgical intervention, intramuscular sedation was accomplished with 0.17 mg/kg acepromazine (Vetranquil 1%, Ceva Tiergesundheit, Düsseldorf, Germany). Subsequently, anesthesia was initiated using 21.5 mg/kg thiopental-sodium (Trapanal 2.5%, Altana GmbH, Konstanz, Germany). During all surgical procedures, inhalation anesthesia was performed using oxygen and nitrous oxide and isoflurane. To maintain hydration, all animals received a constant-rate infusion of lactated Ringer's solution while anesthetized. Intraoperative analgesia was performed by an intravenous injection of 0.4 mg/kg piritramid (Dipidolor®, Janssen-Cilag GmbH, Neuss, Germany) and 4.5 mg/kg carprofene (Rimadyl®, Pfitzer Pharma GmbH, Karlsruhe, Germany). For postoperative treatment, piritramid and carprofene were applied subcutaneously for 3 days at the same dose as described before.

Surgical phase 1 (tooth extraction)

In the first surgery, mucoperiosteal flaps were reflected bilaterally in both the jaws and P1–M2 were carefully removed after tooth separation. Wound closure was accomplished by means of mattress sutures and the sites were allowed to heal for 10 weeks. The prophylactic administration of clindamycin (11 mg/kg body weight, Cleorobe®, Pharmacia Tiergesundheit, Erlangen, Germany) was performed intra- and postoperatively for 10 days.

Surgical phase 2 (defect creation and GBR)

After 3 months of healing, midcrestal incisions were made and mucoperiosteal flaps were reflected to expose the alveolar bone in the lower jaws. Vertical releasing incisions were placed about 4–5 mm distant to the designated experimental sites. A total of four standardized saddle-type defects including the vestibular and oral aspect of the alveolar ridge were subsequently prepared bilaterally at a distance of at least 5 mm with a straight fissure carbide bur. After bone block removal, the final dimension of all defects revealed a mesio-distal width of 10 mm and an apico-coronal height, as measured from the crestal bone, of 8 mm (Fig. 1a). The defect sizes were standardized using a periodontal probe (PCP12; Hu-Friedy Co., Chicago, IL, USA). All osteotomy procedures were performed under copious irrigation with sterile 0.9% physiological saline. Finally, each defect site was thoroughly rinsed with sterile saline to completely remove any residual debris. Subsequently, the respective defects were homogeneously filled with a particulate NBM (Geistlich BioOss® spongiosa granules, particle size 0.25–1 mm, Geistlich Biomaterials, Wolhusen, Switzerland) or SBC (60% HA + 40% β-TCP, Straumann Bone Ceramic®, pore diameters: 100–500 μm, Institute Straumann AG, Basel, Switzerland) (BC). Particular care was exercised to ensure that the graft particles did not exceed the contour of the bordering bone walls in both the vestibular/oral and the cranial directions (Fig. 1b). Following treatment, CM (Geistlich BioGide®, Geistlich Biomaterials) was adapted over the respective defect areas so as to cover 1–2 mm of the surrounding alveolar bone. Neither sutures nor pins were used for membrane fixation or stabilization (Fig. 1c). At the contralateral sites, excess blood was removed from the surrounding bone. Afterwards, the PEG hydrogel (MembraGel®, Institute Straumann AG) was applied in a viscous form, also extending 1–2 mm beyond the margins of the defect walls. After approximately 60 s, the PEG membrane had set to its gelated status (Jung et al. 2009) (Fig. 1d). Following periosteal-releasing incisions, the mucoperiosteal flaps were advanced, repositioned tension-free in a coronal position and fixed with vertical or horizontal mattress sutures (Resorba®, Nürnberg, Germany) in a way to ensure a submerged healing condition (Fig. 1e).

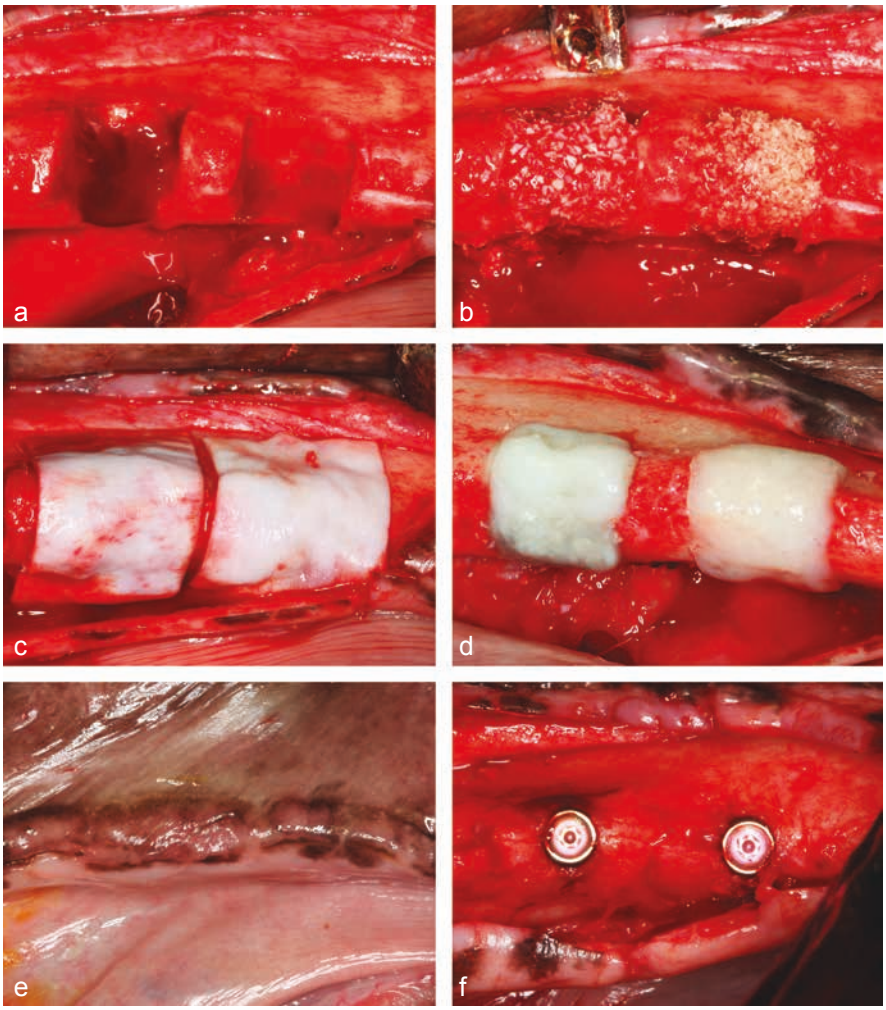

Fig. 1. (a) A total of $n = 4$ standardized saddle-type defects (width: 10 mm; height: 8 mm) were bilaterally created at a distance of at least 5 mm in each lower jaw of six dogs. Occlusal view indicating that both vestibular and lingual bone plates were removed. (b) In each hemimandible, the defects were homogeneously filled with NBM and SBC. (c) Experimental sites receiving CM, which was adapted in a way as to cover 1–2 mm of the surrounding alveolar bone. (d) Experimental sites receiving the PEG hydrogel after the removal of excess blood from the surrounding bone. A gelated status of the material was obtained at about 60 s after its application. (e) Submerged healing at 8 weeks was generally considered as uneventful at both CM- and PEG-treated sites. (f) Implant placement was performed at the central aspect of each defect site in a way so that IS at best coincided with the regenerated bone crest at both vestibular and oral aspects. All titanium implants were successfully inserted with good primary stability. CM, collagen membrane; IS, implant shoulder; NBM, natural bone mineral; PEG, polyethylene glycol; SBC, biphasic calcium phosphate.

Surgical phase 3 (implant placement)

In the third surgery, midcrestal incisions were made and mucoperiosteal flaps were reflected to expose the experimental sites for implant placement. All granulation tissue was carefully removed from the residual defect areas. A total of $n = 4$ implant sites were prepared bilaterally, at the central aspect of each experimental site, using a low-trauma surgical technique under copious irrigation with sterile 0.9% physiological saline (surgery protocol by Institut Straumann AG). Thereafter, screw-type modSLA (Bone Level® SLActive®, ∅4.1 mm, length 10 mm, Institut Straumann AG) titanium implants were inserted with good primary stability (i.e. lack of clinical implant mobility) in a way so that the implant shoulder at best coincided with the regenerated bone crest at both vestibular and oral aspects (Fig. 1f). Following the application of closure screws, the mucoperiosteal flaps were repositioned and fixed with vertical or horizontal mattress sutures (Resorba®) in a way to ensure a submerged healing condition.

All surgical procedures were performed by one experienced surgeon (F. S.).

Animal sacrifice and retrieval of specimens

At 8 + 2 weeks, the animals were killed by an overdose of sodium pentobarbital 3%, respectively. The oral tissues were fixed by perfusion with 10% buffered formalin administered through the carotid arteries. The jaws were dissected and blocks containing the experimental specimens were obtained. All specimens were fixed in a 10% neutral-buffered formalin solution for 4–7 days.

Histological preparation

The specimens were dehydrated using ascending grades of alcohol and xylene, infiltrated and embedded in methylmethacrylate (MMA, Technovit 9100 NEU, Heraeus Kulzer, Wehrheim, Germany) for non-decalcified sectioning. During this procedure, any negative influence of polymerization heat was avoided due to a controlled polymerization in a cold atmosphere (−4°C). After 20 h, the specimens were completely polymerized. Each implant site was cut in the buccooral direction along with the long axis of the implant using a diamond band saw (Exakt®, Apparatebau, Norderstedt, Germany). Serial sections were prepared from the central defect area, resulting in four sections approximately 300 µm in thickness each (Donath 1985). Only implant sections showing an inner thread were chosen for the histological evaluation. Subsequently, all specimens were glued with acrylic cement (Technovit 7210 VLC, Heraeus Kulzer) to silanized glass slides (Super Frost, Menzel GmbH, Braunschweig, Germany) and ground to a final thickness of approximately 40 µm. All sections were stained with toluidine blue to evaluate new bone formation. With this technique, old bone stains light blue, whereas newly formed bone stains dark blue because of its higher protein content (Schenk et al. 1984).

Histomorphometrical analysis

Histomorphometrical analyses as well as microscopic observations were performed by one experienced investigator masked to the specific experimental conditions (A. H.). For image acquisition, a color CCD camera (Color View III, Olympus, Hamburg, Germany) was mounted on a binocular light microscope (Olympus BX50, Olympus). Digital images (original magnification × 200) were evaluated using a software program (Cell D®, Soft Imaging System, Münster, Germany).

The following landmarks were identified in the stained sections at both vestibular and oral aspects: the implant shoulder (IS) and the bottom of the bone defect (BD). Defect length (DL) was measured from IS to BD (mm); the amount of new BIC in the defect was measured as percentage of the distance from BD to IS, serving as 100% (Fig. 2). Additionally, the treated area (TA) (mm^2) was measured from BD to IS. Within TA, the surface area of mineralized (MT) and nonmineralized tissue (NMT) as well as residual

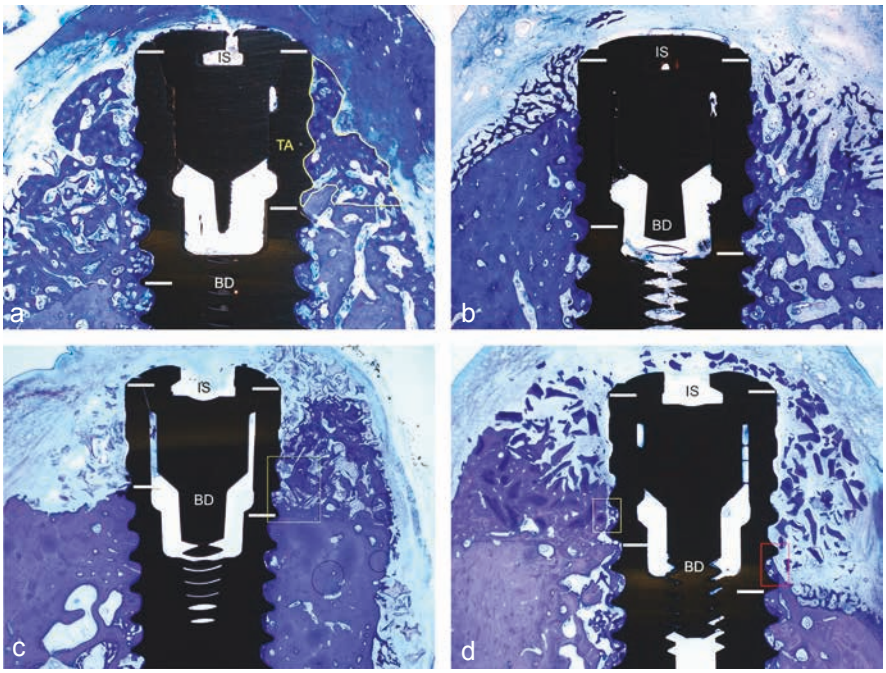

Fig. 2. Representative histological views (vestibulo-oral sections, toluidine blue stain) of wound healing at 8 + 2 weeks (original magnification × 25). While bony filling appeared to be more consistent in the PEG groups, CM-treated sites frequently featured an incomplete hard tissue within the confines of the barrier membrane. (a) SBC + PEG, (b) NBM + PEG, (c) SBC + CM (yellow box area refers to Fig. 3b) and (d) NBM + CM (yellow/red box areas refer to Fig. 3c and d). BD, bottom of the bone defect; TA, treated area; IS, implant shoulder; CM, collagen membrane; NBM, natural bone mineral; PEG, polyethylene glycol; SBC, biphasic calcium phosphate.

NBM/SBC particles (BS) were automatically assessed (mm^2) using the image analysis software. Before the start of the morphometrical analysis, a calibration procedure was initiated for the image analysis software and revealed that repeated measurements of $n = 12$ different sections were similar at >95% level.

Statistical analysis

The statistical analysis was performed using a commercially available software program (PASW Statistics 19.0, SPSS Inc., Chicago, IL, USA). The mean values and standard deviations among animals were calculated for each variable and group. The data rows were examined using the Mann–Whitney test for a normal distribution. Between-group comparisons at 8 + 2 weeks were performed using the non-parametric Kruskal–Wallis test. The α error was set at 0.05.

Results

Clinical healing

The postoperative healing was considered as generally uneventful in all dogs. No complications such as allergic reactions, swellings, abscesses or infections were observed throughout the entire study period (Fig. 1e). A premature exposure of the augmented area (i.e. exposure of barrier membranes, NBM, SBC or titanium implants) was not observed in any of the experimental sites.

During implant placement after 8 weeks of healing, it was observed that all defect sites were homogeneously bridged by a newly formed hard tissue, exhibiting integrated NBM and SBC particles. A corticalization of the bony contour at both vestibular and oral aspects was commonly observed in both CM and PEG groups. Primary implant stability was successfully achieved at each defect site (Fig. 1f).

Descriptive histology

Representative histological views of wound healing at 8 + 2 weeks in different groups are presented in Figs 2 and 3. Basically, all groups investigated were characterized by a homogeneous stabilization of the particulate bone fillers within the confines of the space provided by both CM and PEG membranes. A dispersion of NBM and SBC particles to the adjacent connective tissue was only observed occasionally (Fig. 2a–d). While bony filling appeared to be more consistent in the PEG groups, CM-treated sites commonly featured a partial or an incomplete hard tissue invasion within the former defect area (Fig. 2c and d). In these areas, NBM and SBC particles were mainly surrounded by NMT. New bone formation was limited to BD but tended to follow along to modSLA titanium implant surfaces in the coronal direction (Fig. 3a). Even though both NBM and SBC particles established close contact with newly formed hard tissue in both the groups, it was also apparent that SBC exhibited increased contact with NMT than NBM (Fig. 3b and c). In these areas, dissolution of SBC was frequently observed and distinguishable as a collapse of the particles into smaller fragments (Fig. 3b). Obvious differences were also observed when evaluating and comparing the maturation of newly formed bone between groups. In particular, PEG-treated sites most frequently exhibited a dense cancellous bone with well-defined trabeculae and a regular bone marrow in the center, which was confined by a more compact bone layer in the periphery. However, some specimens also revealed obvious signs of a bone remodelling at the most crestal aspect of the alveolar ridge (Fig. 2b). In contrast, CM specimens most commonly featured the formation of a primary spongework of woven bone, which only occasionally underwent transformation into cancellous and cortical bone (Fig. 2a–d). At all defect sites exhibiting a homogeneous bony filling, modSLA implants were surrounded by a firmly attached mature, parallel-fibered woven bone, similar to that noted for pristine sites (Fig. 3c). In contrast, at sites predominantly showing an ingrowth of NMT, modSLA implants were mainly surrounded by a dense connective tissue. These areas exhibited multiple spots of mineralization also establishing a tiny BIC (Fig. 3d).

Histomorphometric analysis

The mean values of DL, TA, MT, NMT, BS and BIC in both groups at 8 + 2 weeks are presented in Tables 1–3.

Within-group comparisons (i.e. comparison of vestibular and oral aspects) revealed comparable mean DL, TA, MT, NMT, BS and BIC values at both NBM- and SBC-treated sites. In all the groups investigated, the mean TA and NMT values tended to be higher at the vestibular when compared with the corresponding oral aspects (Tables 1 and 2).

Both NBM + PEG and SBC + PEG groups tended to reveal increased DL, MT and BIC values when compared with the NBM + CM and SBC + CM groups, respectively (Fig. 2a–d). However, these differences did not reach statistical significance ($P > 0.05$, Kruskal–Wallis test) (Table 3).

Discussion

The present experimental study was designed to histologically evaluate the outcome of a staged

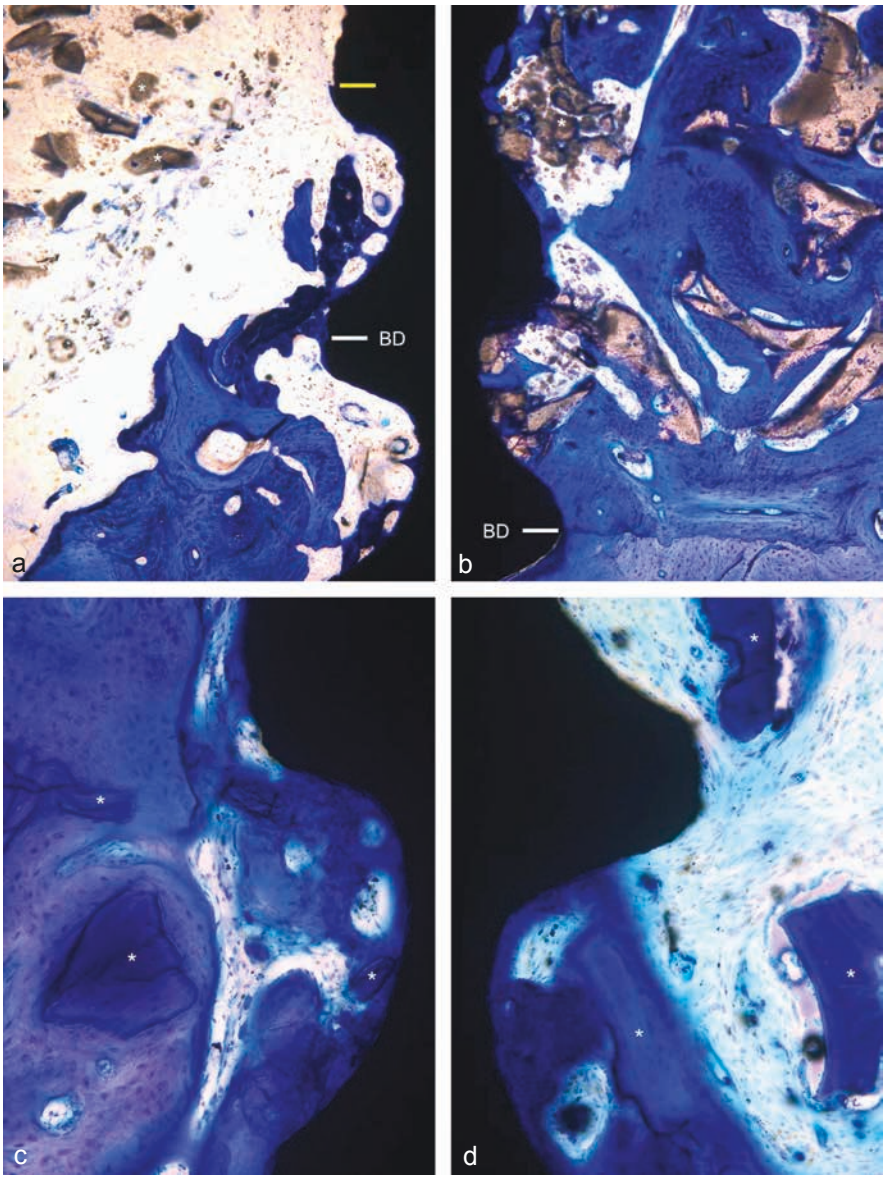

Fig. 3. (a) Wound area showing incomplete hard tissue formation, but coronal extension (yellow line) of trabecular bone along to the surface of modSLA titanium implants (PEG group, original magnification × 100). (b) SBC particles established a close contact with newly formed hard tissue but frequently revealed a higher contact to NMT than NBM particles. A disintegration of SBC into smaller fragments (white asterisks) was apparent (CM group, higher magnification view × 100 of yellow box area shown in Fig. 2c). (c) New bone formation was commonly associated with a close embedding of NBM particles (white asterisks) in MT. In these areas, modSLA surfaces established a close bone-to-implant contact (BIC) (CM group, higher magnification view × 200 of yellow box area shown in Fig. 2d). (d) Multiple spots of mineralization establishing a tiny BIC were observed at defect sites lacking a homogeneous bony filling (white asterisks indicate NBM particles) (CM group, higher magnification view × 200 of red box area shown in Fig. 2d). CM, collagen membrane; IS, implant shoulder; MT, mineralized tissue; NBM, natural bone mineral; NMT, non-mineralized tissue; PEG, polyethylene glycol; SBC, biphasic calcium phosphate.

GBR procedure using either PEG or CM barrier membranes at NBM- and SBC-grafted saddle-type ridge defects in dogs. This type of non-contained defect model is commonly used and well accepted to evaluate GBR procedures in canines (Schenk et al. 1994; Bornstein et al. 2007; Schwarz et al. 2011). From a clinical point of view, it is important to emphasize that the application of both CM and PEG membranes was not associated with any impairment of wound healing, which is basically in agreement with previous experimental animal studies (Bornstein et al. 2007; Jung et al. 2009; Schwarz et al. 2011). Within its limitations, the present histomorphometrical analysis has indicated that all the treatment procedures investigated were associated with comparable improvements as assessed by the mean TA, MT, NMT and BIC values. Even though PEG application tended to be associated with increased mean MT, and BIC values over CM, these differences did not reach statistical significance. However, semi-quantitative histological observations pointed to potential differences between both types of barrier membranes. While PEG application was commonly associated with a more homogeneous bony filling of the former defect area, CM-treated sites more frequently revealed the formation of NMT around NBM and SBC particles. Despite an apparent stabilization of the particulate bone fillers within the confines of the space provided by both barrier membranes, the pronounced biodegradation of CM over PEG may have decreased its barrier function, thus enabling a premature ingrowth of connective tissue (Herten et al. 2009; Thoma et al. 2009).

In this context, it must be realized that these are the first experimental data using either CM or PEG for GBR at NBM- and SBC-grafted saddle-type defects. However, recent studies also reported on a more homogeneous bone regeneration when CM was combined either with NBM + AB or with SBC + AB (Bornstein et al. 2007; Schwarz et al. 2011). In particular, after 8 weeks of submerged healing in the lower jaws of dogs, the mean TA value at NBM + AB + CM-treated sites was about 30.2 mm^2, but tended to be on a level equivalent to that noted for the control group, exhibiting a mean value of about 28.8 mm^2 (i.e. NBM + AB without membrane application) (values calculated from the data provided in the publication) (Bornstein et al. 2007). In a similar study, Schwarz et al. (2011) also reported on the establishment of a homogeneous scaffold of cancellous bone with numerous blood vessels and bone-forming cells when both CM and PEG were applied at NBM + AB- and SBC + AB-grafted defect sites. In particular, the mean TA and MT values ranged from 9.7 ± 4.8 to 4.7 ± 3 mm^2 in the NBM + AB + CM group and from 7.8 ± 3.4 to 3.9 ± 1.7 mm^2 in the SBC + AB + CM group, respectively. Similarly, the mean TA and MT values ranged from 10.4 ± 5.8 to 5.4 ± 3.3 mm^2 in the NBM + AB + PEG group and from 10.4 ± 2.5 to 5.2 ± 1.9 mm^2 in the SBC + AB + PEG group, respectively. A significant difference was only observed for the mean TA values in the SBC + AB groups (Schwarz et al. 2011). All these data, taken together with the results from the present study, seem to indicate that CM-treated non-contained defect sites may benefit from the addition of particulate AB to either NBM or SBC (ratio 1 : 1) rather than PEG-treated sites. Indeed, one might speculate that the osteoinductive and osteogenetic properties noted for AB chips (Chiriac et al. 2005) may have compensated the pronounced biodegradation of CM by promoting new bone formation, thus resulting in almost comparable MT values at CM- and PEG-treated sites (Schwarz et al. 2011). This issue, however, needs to be supported

by controlled experimental data aimed at investigating the impact of both NBM and SBC used either alone or as a composite with AB chips on bone regeneration in a non-contained defect model. Previous clinical studies compared the osteoconductive properties of both bone graft substitutes for human maxillary sinus augmentation (Cordaro et al. 2008; Froum et al. 2008; Lindgren et al. 2010). It was noted that SBC-treated sites more frequently revealed an increased amount of NMT in direct contact with residual graft particles than the NBM group (Cordaro et al. 2008; Lindgren et al. 2009). Even though the present histomorphometrical analysis revealed comparable mean NMT values in both NBM and SBC groups, the semi-quantitative analysis has also pointed to increased NMT areas in direct contact with SBC particles. These areas, however, were characterized by dissolution of this type of bone filler into smaller fragments, which is basically in agreement with previous experimental and clinical data (Jensen et al. 2007; Cordaro et al. 2008; Schwarz et al. 2011).

When interpreting the present histomorphometrical analysis, it was also noted that the mean BIC values at modSLA titanium implants tended to be improved at PEG-treated defects. This observation is in agreement with recent data pointing to an improved osseointegration of modSLA titanium implants inserted at NBM + AB + PEG- and SBC + AB + PEG-treated sites (Schwarz et al. 2011). In particular, at 8 + 2 weeks following implant placement in the upper jaws, the mean BIC values ranged from 71.3 ± 20.8% in the NBM + AB + CM group to 72.4 ± 20.3% in the SBC + AB + CM group. These values tended to be higher in the PEG groups, ranging from 80.1 ± 21.5% at NBM + AB- to 86.4 ± 20.1% at SBC + AB-augmented defects. However, these differences also failed to reach statistical significance. The slight increase in the mean BIC values at NBM + AB and SBC + AB over NBM- and SBC-augmented defects, as observed in the present study, may mainly be attributed to the osteoinductive

Table 1. **Mean values (± SD) and medians of DL (in mm), TA, MT, NMT, BS (in mm²) and BIC (in %) in the CM group at both vestibular and oral aspects after 8 + 2 weeks of submerged healing (*n* = 6 dogs)**

Group	DL	TA	MT	NMT	BS	BIC
NBM						
Vestibular						
Mean (± SD)	3.7 ± 1.3	6.7 ± 2.5	2.4 ± 0.6	3.4 ± 2.5	1 ± 0.5	51.1 ± 26.6
Median	3.2	6.1	2.1	2.9	1	44.5
Oral						
Mean (± SD)	3.4 ± 1.2	6 ± 2.6	2.6 ± 1.3	2.3 ± 1.6	1 ± 0.5	57 ± 24.8
Median	3.3	5.4	2.4	1.8	1	51.8
SBC						
Vestibular						
Mean (± SD)	4 ± 1.8	6.3 ± 3.9	1.4 ± 0.8	3.8 ± 3	1.1 ± 0.6	56.9 ± 19.3
Median	3.2	5.2	1.2	3	1.2	56.8
Oral						
Mean (± SD)	3.7 ± 1.1	6.2 ± 3.5	3.1 ± 2.5	1.7 ± 1	1.4 ± 1.3	65.1 ± 10.9
Median	3.9	6.4	2.5	1.6	1.1	63.5

DL, defect length; TA, treated area; BS, bone substitute (i.e. residual NBM/SBC particles); MT, mineralized tissue; NMT, non-mineralized tissue; BIC, bone-to-implant contact; CM, collagen membrane; NBM, natural bone mineral; SBC, biphasic calcium phosphate.

Table 2. **Mean values (± SD) and medians of DL (in mm), TA, MT, NMT, BS (in mm²) and BIC (in %) in the PEG group at both vestibular and oral aspects after 8 + 2 weeks of submerged healing (*n* = 6 dogs)**

Group	DL	TA	MT	NMT	BS	BIC
NBM						
Vestibular						
Mean (± SD)	4.4 ± 1.9	7.1 ± 4.1	3.8 ± 2.8	2.7 ± 1.8	0.5 ± 0.3	62.9 ± 19.9
Median	4	6.1	3.3	1.7	0.4	65.5
Oral						
Mean (± SD)	4.2 ± 0.9	5.3 ± 1.7	3 ± 1.3	1.6 ± 0.9	0.6 ± 0.5	72.3 ± 19.2
Median	4.4	4.9	2.9	1.4	0.5	75.6
SBC						
Vestibular						
Mean (± SD)	4.1 ± 1.8	5.1 ± 3.7	2.6 ± 2	2.3 ± 2.1	0.3 ± 0.2	71.4 ± 20.3
Median	3.8	4.4	1.9	1.2	0.2	67.8
Oral						
Mean (± SD)	4.3 ± 1.9	10.2 ± 6.2	5.9 ± 4.2	3.3 ± 2.5	1 ± 1.1	62.4 ± 19.1
Median	4	8.4	3.5	2.2	0.5	53.5

DL, defect length; TA, treated area; BS, bone substitute (i.e. residual NBM/SBC particles); MT, mineralized tissue; NMT, non-mineralized tissue; BIC, bone-to-implant contact; NBM, natural bone mineral; SBC, biphasic calcium phosphate.

Table 3. **Mean values (± SD) and medians of DL (in mm), TA, MT, NMT, BS (in mm²) and BIC (in %) in different groups at all aspects after 8 + 2 weeks of submerged healing (*n* = 6 dogs)**

Group	DL	TA	MT	NMT	BS	BIC
CM						
NBM						
Mean (± SD)	3.5 ± 0.7	6.4 ± 2.2	2.5 ± 0.8	2.8 ± 1.5	1 ± 0.4	54.1 ± 22.6
Median	3.3	5.4	2.1	1.8	1	47.8
SBC						
Mean (± SD)	3.8 ± 1.3	6.3 ± 3.4	2.3 ± 1.6	2.7 ± 1.7	1.3 ± 0.9	61 ± 8.7
Median	3.5	5.3	1.5	2.2	1.2	58.7
PEG						
NBM						
Mean (± SD)	4.3 ± 1.4	6.2 ± 2.4	3.4 ± 1.7	2.1 ± 1.3	0.6 ± 0.2	67.7 ± 16.9
Median	4.4	5.6	2.9	1.6	0.4	73.4
SBC						
Mean (± SD)	4.2 ± 1.8	7.7 ± 4.3	4.2 ± 2	2.8 ± 2.3	0.7 ± 0.5	66.9 ± 17.8
Median	3.9	5.4	3	2	0.3	64
P value*	0.588	0.926	0.133	0.882	0.06	0.316

*Between-group comparisons: nonparametric Kruskal–Wallis test.
DL, defect length; TA, treated area; BS, bone substitute (i.e. residual NBM/SBC particles) MT, mineralized tissue; NMT, non-mineralized tissue; BIC, bone-to-implant contact; NBM, natural bone mineral; SBC, biphasic calcium phosphate.

and osteogenetic properties noted for AB (Chiriac et al. 2005). Basically, these values are within the range of the mean BIC values noted for modSLA titanium implants after 2 weeks of healing in the canine lower jaw, ranging from 71.9 ± 4% to 74.9 ± 7.8% (Schwarz et al. 2007a, 2007b). Accordingly, all these data, taken together with the present results, seem to indicate that two-stage GBR using NBM and SBC used either alone or as a composite with AB chips may not impair the osseointegration of modSLA titanium implants, even in the presence of an almost non-resorbing NBM (Mordenfeld et al. 2010). This is in agreement with the results of recent experimental animal studies, indicating that NBM particles did not impair the process of osseointegration in comparison with pristine sites (Carmagnola et al. 2008; Artzi et al. 2010).

Within the limits of the present study, it was concluded that all augmentation procedures investigated equally supported bone regeneration and staged osseointegration of modSLA titanium implants.

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Conflict of interests: The authors report no conflicts of interest related to this study.

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ORIGINAL ARTICLE

Immunohistochemical analysis of staged guided bone regeneration and osseointegration of titanium implants using a polyethylene glycol membrane

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Abstract
Objectives This study aimed to immunohistochemically evaluate staged guided bone regeneration and osseointegration of titanium implants using two bone graft substitutes in combination with a polyethylene glycol (PEG) membrane in a dog model.
Materials and methods Saddle-type alveolar ridge defects were prepared in the lower jaws of 12 foxhounds and randomly filled with a natural bone mineral (NBM) or a biphasic calcium phosphate (SBC) and covered with an in situ gelling PEG membrane. After a healing period of 8 and 12 weeks (six animals each), modSLA titanium implants were inserted to heal in a submerged position. At 8+2 and 12+2 weeks, respectively, dissected blocks were processed for immunohistochemical analysis [osteocalcin (OC)].
Results After 8+2 weeks, mean OC values (%) tended to be higher in the NBM group (NBM, 32.7±8.9 %), but failed to reach statistical significance over the SBC group (SBC, 24.4± 6.6 %). After 12+2 weeks, mean OC values decreased in both groups and was almost identical in both groups (NBM 1.6± 1.2 %/SBC 2.1±1.4 %).
Conclusion It was concluded that all augmentation procedures investigated were characterised by a comparable OC activity during the process of bone regeneration and osseointegration of modSLA titanium implants.

Keywords Guided bone regeneration · Barrier membranes · Bone substitutes · Alveolar ridge defect · Animal study

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Introduction

Titanium implants are a successful and predictable treatment of partially or fully edentulous patients [1, 2]. However, an imperative necessity for an implant placement ensuring long-term stability is a proper osseointegration based on a minimum amount of bone width and height of the recipient site. Therefore the method of guided bone regeneration (GBR) is frequently applied to create adequate bone volume. At sites showing an extensive bone deficiency in either horizontal and/or vertical dimensions, staged bone augmentation and implant placement becomes necessary [3]. A clinical routine procedure is the application of intraoral autogenous bone (AB) blocks used alone or in combination with a very slowly resorbing particulate bovine-derived natural bone mineral (NBM), either with or without utilizing a barrier membrane [4]. However, harvesting autogenous bone blocks is accompanied by clinical drawbacks like technical difficulties, patients' morbidity as well as graft resorption necessitating additional grafting after implant placement in about 15–20 % of the cases [5, 6]. Thus, the principle of GBR frequently serves as an alternative to AB blocks using a combination of bone substitutes (i.e. NBM or a nanocrystalline hydroxyapatite) and barrier membranes for lateral ridge augmentation, resulting in a clinically important horizontal bone gain [7, 8]. These clinical observations were supported by experimental data investigating various defect configurations. At chronic-type lateral ridge defects, the application of a particulate NBM or a biphasic calcium phosphate (SBC), which were covered by a porcine-derived native type I and type III collagen membrane (CM), was associated with a predictable bone formation [9–11]. Under these experimental conditions, the GBR procedure stabilised the NBM particles and increased the regenerated area in comparison to

non-protected sites after 3 weeks of healing [11]. In an experimental study evaluating the outcome of a staged GBR procedure employing either a polyethylene glycol (PEG) or a CM membrane at NBM and SBC grafted saddle-type defects, a homogeneous bony filling could be observed at all defect sites, similar to that noted for pristine sites [12, 13]. In a similar study, adding AB to NBM and SBC, Schwarz et al. also reported on the establishment of a homogeneous scaffold of cancellous bone with numerous blood vessels and bone forming cells in either CM or PEG groups [13].

Even though the survival rates of implants placed in augmented bone have been reported to be comparable to the rates of implants placed in pristine bone [14], only a very few data aimed at investigating the impact of two-stage GBR on the process of osseointegration at grafted implant sites [13, 15, 16]. Basically, it was observed that grafting of self-contained defects using a variety of bone substitutes (e.g. NBM) did not impair the establishment of a bone-to-implant contact (BIC) in comparison to non-grafted sites [15, 16]. At a non-contained defect configuration, recent data pointed out that all augmentation procedures supported osseointegration of chemically modified, hydrophilic sandblasted large grid and acid-etched (modSLA) titanium implants, revealing BIC values ranging between 71.3±20.8 % and 86.4±20.1 % in the upper jaw and 51.1±26.6 and 71.4±20.3 in the lower jaw [12, 13]. These values are within the range of mean BIC values noted for modSLA titanium implants after 2 weeks of healing in the canine lower jaw (i.e. pristine bone), ranging from 71.9±4.0 % to 74.9±7.8 % [17, 18]. Despite these promising outcomes, it must be noted that conventional histology may not be appropriate to assess the complex biological pattern of wound healing associated with both types of bone graft substitutes.

The aim of the present study was to immunohistochemically analyse the outcome of a staged GBR procedure employing NBM or SBC in combination with a PEG membrane for localised ridge augmentation and subsequent implant placement at saddle-type defects in a dog model.

Material and methods

Animals

In the present study a total of 12 adult fox hounds (age 18–22 months, weight 34–42 kg) exhibiting a fully erupted permanent dentition were applied. These animals were allocated in two groups (i.e. healing periods 8+2 and 12+2 weeks) including six animals each. During the experiment, the dogs were fed once per day with soft-food diet and water ad libitum. The study protocol was approved by the appropriate local authority (Landesamt für Natur und Verbraucherschutz, Recklinghausen, Germany). The experimental segment of the study started after an adaption period of 4 weeks.

Study design and randomisation

The study design has been described in detail recently [12]. The experimental aspect was performed in three surgical phases. In the first phase, the mandibular and maxillary first, second, third, fourth premolar as well as first and second molar (P1–M2) was extracted. After a healing period of 10 to 12 weeks, two standardised saddle-type defects (mesio-distal width, 10 mm; height, 8 mm) were prepared in the lower jaw of each dog and randomly filled using NBM or SBC. These defects were either located in one hemimandible (six animals—healing period 8+2 weeks) or on opposite sides (six animals—healing period 12+2 weeks). A balanced randomisation accounted for potential differences in defect location in both healing groups (RandList®, DatInf GmbH, Tübingen, Germany). Subsequently, all defects were covered with a PEG membrane.

After 8 and 12 weeks, modSLA titanium implants (n=2 per animal in the lower jaw) were inserted at the respective sites and left to heal in a submerged position for 2 weeks. Six animals each were killed after a healing period of 8+2 and 12+2 weeks.

Surgical procedure

The surgical procedure has been described in detail recently [12, 19]. Prior to each surgical intervention, intramuscular sedation was accomplished with 0.17 mg/kg acepromazine (Vetranquil 1 %, Ceva Tiergesundheit, Düsseldorf, Germany). Subsequently, anaesthesia was initiated using 21.5 mg/kg thiopental-sodium (Trapanal 2.5 %, Altana GmbH, Konstanz, Germany). During all surgical procedures, inhalation anaesthesia was performed by use of oxygen and nitrous oxide and isoflurane. To maintain hydration, all animals received a constant rate infusion of lactated Ringer's solution while anaesthetised. Intraoperative analgesia was performed by intravenous injection of 0.1 mg/kg piritramid (Dipidolor®, Janssen-Cilag GmbH, Neuss, Germany) and 4.5 mg/kg carprofene (Rimadyl®, Pfitzer Pharma GmbH, Karlsruhe, Germany). For postoperative treatment, carprofene (days 1–7) was applied subcutaneously in the same dose as described before.

Surgical phase 1 (tooth extraction)

In the first surgery, mucoperiosteal flaps were reflected bilaterally in both jaws and P1–M2 were carefully removed after tooth separation. Wound closure was accomplished by means of mattress sutures and the sites were allowed to heal for 10 weeks. Prophylactic administration of clindamycine

(11.0 mg/kg body weight, Cleorobe®, Pharmacia Tiergesundheit, Erlangen, Germany) was performed intra- and postoperatively for 10 days.

Surgical phase 2 (defect creation and GBR)

After 10 to 12 weeks of healing, midcrestal incisions were made and mucoperiosteal flaps reflected to expose the alveolar bone in the lower jaws. Vertical releasing incisions were placed about 4–5 mm distant to the designated experimental sites. Standardised saddle-type defects encompassing both vestibular and oral aspects of the alveolar ridge were prepared with a straight fissure carbide bur under copious irrigation with sterile 0.9 % physiological saline. After bone block removal, the final dimension of all defects revealed a mesio-distal width of 10 mm and an apico-coronal height, as measured from the crestal bone, of 8 mm (Fig. 1a). The defect sizes were standardised by the use of a periodontal probe (PCP12, Hu-Friedy Co., Chicago, Illinois, USA). Finally, each defect site was thoroughly rinsed with sterile saline to completely remove any residual debris. Subsequently, the respective defects were homogeneously filled with a particulate NBM (Geistlich BioOss® spongiosa granules, particle size 0.25–1 mm, Geistlich Biomaterials, Wolhusen, Switzerland) or SBC (60 % HA + 40 % ß-TCP, Straumann Bone Ceramic®, pore diameters: 100–500 µm, Institut Straumann AG). Particular care was taken that the graft particles did not exceed the contour of the bordering bone walls in both vestibular/oral and cranial directions (Fig. 1b). Following treatment, excess of blood was removed from the surrounding bone. Afterwards, the PEG hydrogel (MembraGel®, Institute Straumann AG, Basel, Switzerland) was applied in a viscous form, also extending 1–2 mm beyond the margins of the defect walls. After approximately 60 s, the PEG membrane had set to its gelated status [12] (Fig. 1c). Following periosteal-releasing incisions, the mucoperiosteal flaps were advanced, repositioned tension-free in a coronal position and fixed with vertical or horizontal mattress sutures (Resorba®, Nürnberg, Germany) in a way to ensure a submerged healing condition (Fig. 1d).

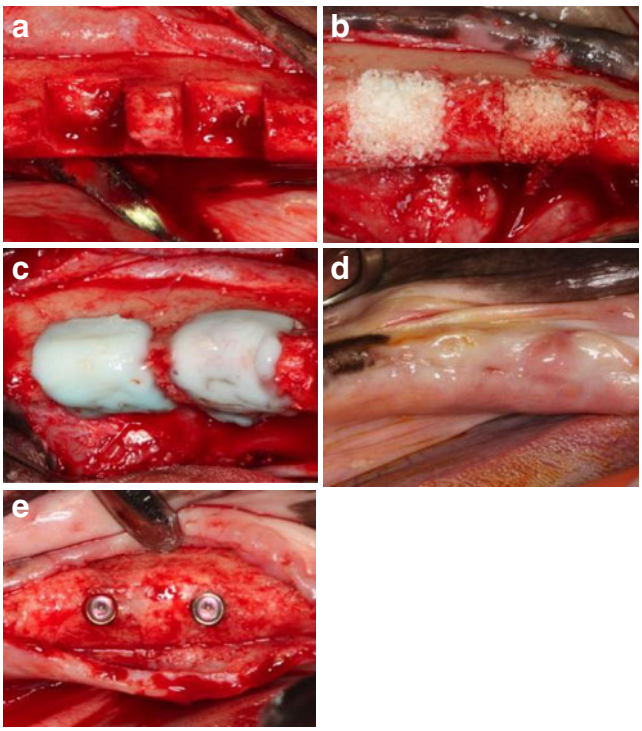

Fig. 1 **a** Two standardised saddle-type defects (width, 10 mm; height, 8 mm) were surgically created in each of the lower jaw of each animal (occlusal view). **b** Homogeneous filling of the defects with NBM (*left*) and SBC (*right*). **c** After removal of excess blood from the surrounding bone, defect sites were covered by the PEG hydrogel which reached its gelated status within 60 s. **d** Clinical healing after 8 and 12 weeks was generally considered as uneventful. **e** Implant placement was performed at the central aspect of each defect site in a way so that implant shoulder at best coincided with the regenerated bone crest at both vestibular and oral aspects. All titanium implants were successfully inserted with good primary stability

Surgical phase 3 (implant placement)

In the third surgery, midcrestal incisions were made and mucoperiosteal flaps reflected to expose the experimental sites for implant placement. All granulation tissue was carefully removed from the residual defect areas. Implant sites were prepared at the central aspect of each defect, using a low-trauma surgical technique under copious irrigation with sterile 0.9 % physiological saline (surgery protocol by Institut Straumann AG, Basel, Switzerland). Thereafter, screw-type modSLA (Bone Level® SLActive®, diameter 4.1 mm, length 10 mm, Institut Straumann AG) titanium implants were inserted with good primary stability (i.e. lack of clinical implant mobility) in a way so that the implant shoulder at best coincided with the regenerated bone crest at both vestibular and oral aspects (Fig. 1e). Following application of closure screws, the mucoperiosteal flaps were repositioned and fixed with vertical or horizontal mattress sutures (Resorba®, Nürnberg, Germany) in a way to ensure a submerged healing condition. All surgical procedures were performed by two experienced surgeons (F.S.).

Animal sacrifice and retrieval of specimens

After 8+2 and 12+2 weeks, six animals each were killed by an overdose of sodium pentobarbital 3 %, respectively. The oral tissues were fixed by perfusion with 10 % buffered formalin administered through the carotid arteries. The jaws were dissected and blocks containing the experimental specimens were obtained. All specimens were fixed in 10 % neutral buffered formalin solution for 4–7 days.

Histological preparation

The specimens were dehydrated using ascending grades of alcohol and xylene, infiltrated and embedded in methylmethacrylate (MMA, Technovit 9100 NEU, Heraeus Kulzer, Wehrheim, Germany) for nondecalcified sectioning. During this procedure, any negative influence of polymerisation heat was avoided due to a controlled polymerisation in a cold atmosphere (−4 °C). After 20 h the specimens were completely polymerised. Each implant site was cut in the bucco-oral direction along with the long axis of the implant using a diamond band saw (Exakt®, Apparatebau, Norderstedt, Germany). Serial sections were prepared from the central defect area, resulting in four sections of approximately 300 μm in thickness each [30]. Only implant sections showing an inner thread were chosen for the histological evaluation. Subsequently, all specimens were glued with acrylic cement (Technovit 7210 VLC, Heraeus Kulzer, Wehrheim, Germany) to silanised glass slides (Super Frost, Menzel GmbH, Braunschweig, Germany) and ground to a final thickness of approximately 40 μm.

Immunohistochemical labelling

Immunohistochemical labelling was performed according to a standardised procedure [20]. In brief, all tissue sections were deplasted in xylol (2×30 min) followed by a treatment in 2-methoxyethylacetate (2×20 min) and acetone (2×5 min). After rehydration in phosphate-buffered saline (PBS), antigen unmasking was performed by incubating the slides for 15 min in trypsin (PAA Laboratories GmbH, Pasching, Austria; 0.05 % in PBS) at 37 °C. After washing with PBS, the activity of endogenous peroxidase was quenched with 0.9 % hydrogen peroxide in PBS for 10 min at room temperature, the specimens were washed and non-specific binding sites were blocked with a blocking solution for 30 min (TDakoCytomation, Hamburg, Germany), the primary mouse monoclonal antibody to osteocalcin (Acris Antibodies GmbH, Hiddenhausen, Germany) (OC) (1:40 dilution) and corresponding unspecific antibodies (mouse IgG1) (DakoCytomation, Hamburg, Germany), respectively as negative controls were applied to tissue sections in a humidified chamber and incubated over night at 8 °C. The slides were washed in PBS and incubated with secondary biotinylated anti-mouse antibody (1:50 dilution) for 90 min at room temperature. After washing in PBS the presence of antibody–antigen complexes was visualised using a streptavidin–peroxidase solution (1:250 dilution) and AEC (3-amino-9-ethylcarbazole) as the chromogen (Dako).

Immunohistochemical analysis

Immunohistochemical analysis was performed by one experienced investigator masked to the specific experimental conditions (I.M.). For image acquisition a colour CCD camera (Color View III, Olympus, Hamburg, Germany) was mounted on a binocular light microscope (Olympus BX50, Olympus, Hamburg, Germany). Digital images (original magnification ×200) were evaluated using a software program (Cell D®, Soft Imaging System, Münster, Germany).

In the immunohistochemically labelled sections the implant shoulder (IS) and the bottom of the bone defect (BD) were identified at both vestibular and oral aspects. The outer contour of the treated area (TA) was demarcated from BD to IS. Within TA, the OC antigen reactivity was automatically assessed (%) by the image analysis software. Prior to the start of the morphometrical analysis, a calibration procedure was initiated for the image analysis software and revealed that repeated measurements of $n=12$ different sections were similar at >95 % level.

Statistical analysis

The statistical analysis was performed using a commercially available software program (PASW Statistics 20.0, SPSS Inc., Chicago, IL, USA). Mean values and standard deviations among animals were calculated for each variable and group. The data rows were examined with the Kolmogorow–Smirnov test and proven to be normally distributed. Between group comparisons at 8+2 and 12+2 weeks were performed using the paired *t* test. The alpha error was set at 0.05.

Results

Clinical healing

The postoperative healing was considered as generally uneventful in all dogs. No complications such as allergic reactions, swellings, abscesses, or infections were observed throughout the whole study period. A premature exposure of the augmented area (i.e. exposure of barrier membrane, NBM, SBC or titanium implants) was not observed in any of the experimental sites.

During implant placement after 8 and 12 weeks of healing, it was observed that all defect sites were homogeneously bridged by a newly formed hard tissue, exhibiting integrated NBM and SBC particles. A corticalisation of the bony contour was commonly observed and primary implant stability was successfully obtained at each defect site (Fig. 1e).

Immunohistochemical analysis

The OC antigen reactivity within TA in NBM and SBC groups at 8+2 and 12+2 weeks is presented in Table 1 as well as in Fig. 2.

Table 1 Mean values and standard deviation of osteocalcin (OC) in NBM + PEG and SBC + PEG groups (in %) after 8+2 as well as 12+2 weeks of submerged healing (n=6 animals per healing period)

Healing period	Group	Mean value (%)	SD (%)	P value[a]
8+2 weeks	NBM + PEG	24.45	6.57	NS
	SBC + PEG	31.18	9.56	
12+2 weeks	NBM + PEG	1.62	0.54	NS
	SBC + PEG	1.37	0.61	

[a] Comparisons between groups: paired t test: $P>0.05$, respectively

8+2 weeks

When evaluating OC values within TA after 8+2 weeks, both groups investigated revealed a more pronounced antigen reactivity at non-mineralised (NMT) when compared with mineralised (MT) tissue areas. While the mineralised matrix and the numerous osteocytes located in large lacunae were mainly characterised by a moderate OC reactivity, the intensity of staining was obviously higher in the bordering NMT (Fig. 3a and b). Even though NBM + PEG groups tended to exhibit a more intense OC antigene reactivity at both MT and NMT areas, mean OC values failed to reach statistical significance in comparison to the corresponding SBC + PEG groups ($p>0.05$; Table 1 and Fig. 2).

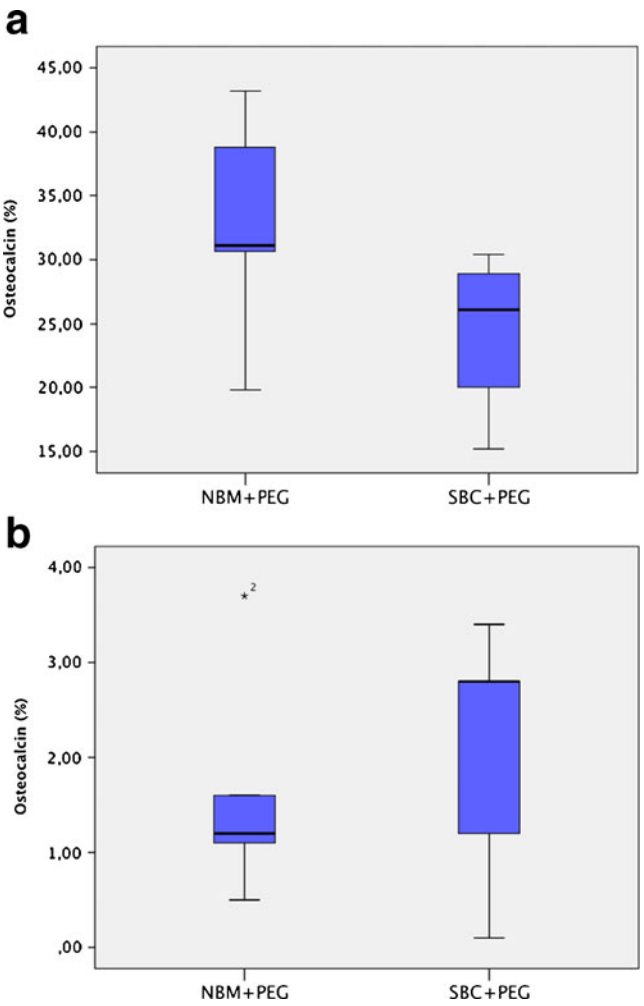

Fig. 2 Box plots for OC antigen reactivity in NBM + PEG and SBC + PEG groups. **a** 8+2 weeks. **b** 12+2 weeks

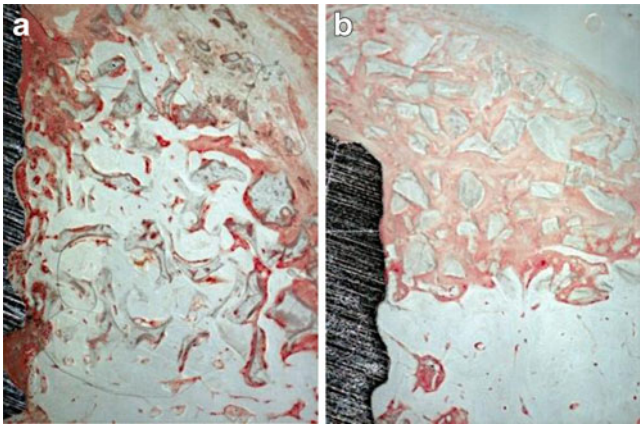

Fig. 3 Representative immunohistochemical views (vestibulo-oral sections, OC labelling) of wound healing at 8+2 weeks (original magnification ×25). OC antigen reactivity was particularly pronounced in close proximity to the implant surface. **a** SBC + PEG. **b** NBM + PEG

12+2 weeks

After a healing period of 12+2 weeks, both NBM + PEG and SBC + PEG groups revealed only a slight OC antigen reactivity within TA. Most of the sections exhibited a homogenous OC signal at either vestibular or oral aspects, which was mainly distributed at NMT areas bordering newly formed bone as well as in the vicinity of the implant surface (Fig. 4a and b). In the SBC + PEG group, OC antigen reactivity tended to be more pronounced in comparison to the corresponding NBM + PEG group, however, this difference did not reach statistical significance ($p>0.05$; Table 1 and Fig. 2).

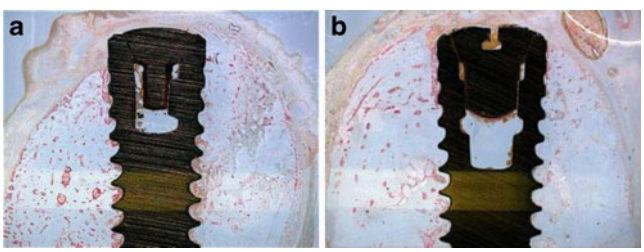

Fig. 4 Representative immunohistochemical views after 12+2 weeks. Only minimal OC antigen reactivity could be detected in both groups (original magnification ×100). **a** SBC + PEG. **b** NBM + PEG

Discussion

The present experimental study was designed to immunohistochemically analyse the outcome of a staged GBR procedure using a polyethylene glycol membrane at NBM or SBC grafted saddle-type alveolar ridge defects in dogs. This non-contained defect model is well-accepted and commonly used to evaluate GBR procedures in canines [12, 13, 21, 22].

The histomorphometrical assessment of the treated area (TA), mineralised tissue (MT), non-mineralised tissue (NMT), residual bone substitute particles (BS) and BIC values of the corresponding PEG groups at 8+2 weeks have been reported previously [12]. All treatment procedures investigated were associated with comparable improvements as assessed by mean TA, MT, NMT and BIC values at 8+2 weeks. In particular, differences between mean values for PEG + SBC (TA, 7.7±4.3; MT, 4.2±2.0; NMT, 2.8±2.3; BIC, 66.9±17.8) and PEG + NBM (TA, 6.2±2.4; MT, 3.4±1.7; NMT, 2.1±1.3; BIC, 67.7±16.9) did not reach statistical significance. However, histological observations pointed to potential differences between both types of grafting materials. Even though the histomorphometrical analysis revealed comparable mean NMT values in both NBM and SBC groups, histological sections also exhibited increased NMT areas in direct contact with SBC particles. These areas, however, were characterised by dissolution of this type of bone filler into smaller fragments, which is basically in agreement with previous experimental and clinical data [13, 23, 24]. These observations are in line with previous clinical studies comparing the osteoconductive properties of both bone graft substitutes for human maxillary sinus augmentation [24–26]. It was noted that SBC-treated sites more frequently revealed an increased amount of NMT in direct contact with residual graft particles than the NBM group [24, 27].

In addition to the already gathered histological data on staged GBR using PEG at SBC or NBM grafted saddle-type defects, the aim of the present investigation was to gain complementary information on the process of bone regeneration by employing an immunohistochemical approach. In this context, it must be realised that these are the first experimental data based on an immunohistochemical analysis of staged GBR employing PEG at NBM and SBC grafted saddle-type defects. Within the limits of the present immunohistochemical analysis it was concluded that all augmentation procedures investigated showed comparable OC activity during the process of bone regeneration and osseointegration of modSLA titanium implants. However, OC activity was predominantly observed at 8+2 weeks whereas at 12+2 weeks almost no activity was recorded. In particular, after 8+2 weeks, mean OC values tended to be higher in NBM groups (NBM, 32.7±8.9 %) when compared with the corresponding SBC groups (SBC, 24.4±6.6 %). In both groups, histological observations pointed to a homogeneous bone formation and subsequently osseointegration of modSLA implants within the confined wound area. Basically, mean OC values within TA revealed a large heterogeneity, but were obviously more pronounced at NMT compared with MT areas in all groups. This may potentially point to an ongoing mineralisation of the extracellular matrix [28], or a remodelling process [29]. Accordingly, OC activity was particularly detected in the vicinity of the implant surface as well as around bone substitute particles at 8+2 weeks, indicating bone remodelling and reactivity of both grafting materials. The marginally higher mean OC values for NBM groups might point to a delayed bone maturation in comparison with SBC.

In addition to the healing period of 8+2 weeks, the present study evaluated OC activity after 12+2 weeks. At 12+2 weeks, mean OC values decreased in both groups (NBM 1.6±1.2 %/SBC 2.1±1.4 %) without reaching statistical significant differences. The decrease of OC antigen reactivity after 12+2 weeks clearly indicates that OC is mainly expressed during initial stages of bone healing and is reduced within the maturation process. Thus, the minor OC values in both groups at 12+2 weeks might imply an almost complete maturation of the treated area allowing an adequate osseointegration of titanium implants.

Within the limits of the present study, it was concluded that all augmentation procedures investigated were characterised by a comparable OC activity during the process of bone regeneration and osseointegration of modSLA titanium implants.

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Conflict of interests The authors report no conflicts of interest related to this study.

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