Implant-guided supracrestal alveolar bone growth using scaffolds, BMP-2, and novel scaffold-retaining device

Bo Wen
David Shafer
Peter Schleier
David Pendrys
Liisa Kuhn
Martin Freilich

Authors’ affiliations:
Bo Wen, Department of Oral & Maxillofacial Surgery, Division of Implant Dentistry, Nanjing Stomatological Hospital, Medical School of Nanjing University, Nanjing, China
David Shafer, Department of Craniofacial Sciences, Division of Oral & Maxillofacial Surgery, School of Dental Medicine, University of Connecticut, Farmington, CT, USA
Peter Schleier, Department of head and neck, Stavanger University Hospital, Stavanger, Norway
David Pendrys, Department of Reconstructive Sciences, School of Dental Medicine, University of Connecticut, Farmington, CT, USA
Liisa Kuhn, Martin Freilich, Department of Reconstructive Sciences, Center for Biomaterials, School of Dental Medicine, University of Connecticut, Farmington, CT, USA

Corresponding author:
Dr. Martin Freilich
Department of Reconstructive Sciences
School of Dental Medicine
University of Connecticut
263 Farmington Avenue
Farmington, CT, USA
Tel.: 860 679 2649
Fax: 860 679 1370
e-mail: freilich@uchc.edu

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Abstract

Objective: To evaluate the efficacy of various scaffold systems and a Ti scaffold-retaining device with and without non-glycosylated rhBMP-2 (BMP-2) for increasing the vertical alveolar bone growth in the intra-oral mini-pig model.

Methods: Forty-eight Straumann Bone Level implants with hydrophilic (SLActive) surfaces were partially embedded in mandibles of 12 adult mini-pigs with the shoulder of the implant located 3 mm above the bone crest. Twenty-four implants were placed in conjunction with BMP-2 (50 μg) incorporated within resorbable scaffolds. Twenty-four additional control implants were placed with scaffolds only. Scaffolds were placed around the implant and stabilized with a newly developed Ti “umbrella” scaffold retainer. Scaffolds included (i) HA-coated collagen (Healos); (ii) biphasic HA/TCP crystals (Straumann Bone Ceramic, SBC); and (iii) SBC crystals infused with polyethylene glycol (PEG) hydrogel. Eight test and control pairs for each scaffold group were implanted. At 9 weeks, soft tissue healing was assessed and the extent of new vertical bone was evaluated with microCT and histomorphometry.

Results: microCT analysis revealed a mean of 167 ± 47 mm³ new supracrestal mineralized tissue volume formation around the test sites where BMP-2 was released from the scaffold whereas the control group (no BMP-2) showed a significantly lower mineralized tissue volume of 106 ± 55 mm³. The SBC+BMP-2 group had the highest mineralized tissue volume of 189 ± 36 mm³. Histomorphometry showed bone-to-implant contact of 54.5% for the test groups and 33.3% for the control groups and new vertical bone growth of 2.2 ± 1.0 and 1.0 ± 0.9 mm, respectively. The SBC+BMP-2 group again demonstrated the best outcome (2.7 ± 0.4 mm). The qualitative scoring of soft tissue dehiscence showed that the presence of BMP-2 yielded far superior outcomes, 0.63 vs. 1.75 for all control implant sites (with scores ranging from 0, reflecting no soft dehiscence, to 4, showing a completely exposed umbrella).

Conclusion: The release of BMP-2 from a SBC scaffold adjacent to a hydrophilic, rough Ti implant and scaffold retention umbrella consistently regenerated the greatest volume and height of new vertical bone along the length of the implant.

Bone resorption following tooth loss can lead to a significant decrease in alveolar bone height, thereby compromising subsequent implant placement and long-term stability. Bone regeneration of this vertically resorbed ridge continues to be a most challenging clinical problem. Our research team has been working to develop an implant system with minimal technique sensitivity specifically designed to guide a new layer of bone height. During the past few years, we have developed and tested a custom scaffold retainer (umbrella) as a specific enhanced design feature to successfully grow a new layer of bone in extra-oral rabbit mandibles and intra-oral mini-pig models [Freilich et al. 2008, 2009, 2012; Catros et al. 2013].

To enhance alveolar ridge augmentation, the local delivery of osteogenic bioactive molecules has been investigated as an alternative to the use of bone autograft [Schmitt et al. 1999; Rengachary 2002; Seeherman et al. 2002]. The most widely used osteogenic bioactive molecules are those known as bone morphogenetic proteins (BMP-2s), which have two different molecular structures: non-
glycosylated and glycosylated BMP-2. Non-glycosylated recombinant BMP-2 (ng/rhBMP-2) is created by bacteria, while glycosylated recombinant BMP-2 is produced by mammalian cells (Bessho et al. 2000). For our studies, we elected to use the less soluble ng/rhBMP-2, which leads to better sustained release and avoids rapid release and diffusion of BMP-2 into contralateral controls and the systemic circulatory system relative to glycosylated rhBMP-2 (Sampath et al. 1990; Schmoeckel et al. 2004, 2005; Sachse et al. 2005). As a result, many of our previous studies have illustrated the delivery of ng/rhBMP-2 from implant surfaces or scaffold materials, which have allowed a reduction in BMP-2 doses necessary for efficient induction of a new layer of bone (Freilich et al. 2008, 2009, 2012; Wen et al. 2011, 2016a; Catros et al. 2013).

A spongy hydroxyapatite [HA]-coated collagen [commercially known as Healos™, SBC, DePuy, Raynham, MA, USA] composed of cross-linked bovine type I collagen fibers coated with 30% HA [Magit et al. 2006] was tested. Healos allograft is expected to have osteoconductive qualities and be used to serve as the three-dimensional scaffold. The efficacy of bone regeneration with Healos™ scaffolds has been shown, and it was found that Healos enhanced calvarial bone repair in young rats compared to a collagen sponge alone [Helisat] (Teacencu & Wendel 2008). Our previous studies investigated the bone formation ability of Healos infused with ng/rhBMP-2 using an extra-oral rat mandible model and an intra-oral mini-pig model and reported successful implant-guided vertical bone regeneration (Wen et al. 2011 and Freilich et al. 2012). One of these two studies also demonstrated that the Healos scaffold in conjunction with the infusion of PEG hydrogels can successfully deliver BMP-2 and support new bone growth (Wen et al. 2011).

Over the years, PEG hydrogel, which forms a gel upon chemical cross-linking by Michael-type addition reaction of PEG-acrylates with PEG-thiols at physiological pH, has been shown to be a good candidate material for drug delivery (Iza et al. 1998; Elbert & Hubbell 2001; Lutolf et al. 2003; Jung et al. 2008). But its lack of mechanical integrity has been reported as a disadvantage for application as an independent material in large bone defects (Drury & Mooney 2003; Paxton et al. 2009). Meanwhile, a biphasic calcium phosphate (BCP) SBC has been widely used as a delivery vehicle for growth factors in bone regeneration and is known to be osteoconductive (Daculsi et al. 1999; Schopper et al. 2005; Wen et al. 2016a,b). Recently, a number of studies have focused on the bone augmentation capabilities of calcium phosphates in large animal models as well as humans [Jensen et al. 2007, 2009; Artzi et al. 2008; Friedmann et al. 2009]. These two materials were evaluated separately and in combination with each other as the SBC/PEG hydrogel combination was noted to have excellent handling properties. Combining PEG hydrogels with SBC and rhBMP-2 has been shown to enhance bone regeneration in rabbit calvarial defects by Jung et al. (2008).

The present study builds upon our previous animal (mouse, rat, rabbit, and mini-pig) research where we have applied ng/rhBMP-2 and different scaffolds, including resorbable and non-resorbable biomaterials. It was hypothesized the addition of rhBMP-2 to a biomaterial scaffold increases the quality and quantity of new mineralized tissue guided by the implant/umbrella system as compared to the use of the scaffold without BMP-2. The purpose of this study was to investigate the effect of rhBMP-2 on the formation of a new layer of supracrestal bone with three different biomaterial ng/rhBMP-2 release systems (Healos, SBC, or SBC/PEG) around Ti implants in an intra-oral large animal model.

Methods and materials

Study design

Each mini-pig received two implant constructs including one partially inserted dental implant, adjacent scaffold, and scaffold screw (which fits into the implant), on each side of the posterior mandible. Experimental implant model

The 10 × 4.1 mm Bone Level implants made of commercially pure titanium were obtained from Institut Straumann (Basel, Switzerland). The surfaces of all the implants were modified with rough sandblasted, large-grit, acid-etched surface exhibiting a hydrophilic, positive charge (SLActive) [Straumann AG, Basel, Switzerland] to the head of the implant. The SLActive surface was maintained in saline solution as packaged by Straumann. Custom-designed titanium cover abutments [umbrella] of 9 × 9 mm size and 1 mm thickness with polished surfaces were obtained from Straumann.
Experimental scaffolds

Three different experimental scaffolds were used for this study. They are (i) hydroxyapatite-coated collagen scaffold (Healos/C226; DePuySpine, Raynham, MA, USA); (ii) HA/bTCP particulate bone graft substitute (SBC/C226; Straumann); and (iii) a combination of HA/bTCP particulate graft substitute (SBC/C226) incorporated with PEG-based hydrogel MX10 (PEG; Straumann). The PEG-based hydrogel was prepared by combining a four-arm PEG with acrylate endgroup and a linear PEG with thiol endgroups (Nektar Therapeutics, Huntsville, AL, USA) in an aqueous buffer system (2 mM HCl) (Elbert & Hubbell 2001; Jung et al. 2007). For preparing the activated gels, the PEG-acrylate solution was supplemented by a 9-amino acid cys-RGD peptide (Straumann AG). The thiol groups on the cysteine moieties of this peptide were allowed to react with the PEG-acrylate before the addition of the PEG-dithiol, facilitating their covalent incorporation into the gels (Lutolf et al. 2001; Fittkau et al. 2005).

Healos and SBC/PEG scaffold materials were pre-made in the form of square shaped disks with a length and width of 10 mm, a thickness of 3 mm, and a central opening with a diameter of 5 mm, which allowed the implants to be placed through the scaffolds. The combination scaffold disks were made using the mixture of 195 l/l PEG hydrogel and 162 mg SBC particles (size of 500–1000 l/m) put into a custom-made mold and allowed to set prior to implantation (Fig. 2).

BMP-2 delivery systems

Escherichia coli-expressed non-glycosylated recombinant human bone morphogenetic protein-2 (ng/rhBMP-2) was used as an osteogenic signaling agent (Hans-Knoll-Institut, Jena, Germany). The ng/rhBMP-2 was prepared as described previously [Sachse et al. 2005; Freilich et al. 2009, 2012; Wen et al. 2011]. The three different ng/rhBMP-2 delivery systems tested included three resorbable scaffolds adjacent to SLActive surface Ti implant.

1. BMP-2 released from Healos

Lyophilized ng/rhBMP-2 powder was reconstituted in 50% acetonitrile/0.1% trifluoroacetic acid (ATFA) at a concentration of 1 mg/ml and injected aseptically into Healos scaffold with 50 µl of the ng/rhBMP-2 solution. All samples were dried under laminar airflow in a biological safety cabinet. Each Healos scaffold had an ng/rhBMP-2 dose of 50 µg.

2. BMP-2 released from SBC

An acetic acid buffer was made as lyophilized ng/rhBMP-2 was reconstituted at a concentration of 0.5 mg/ml in 0.04% acetic acid (Weber et al. 2001). The amount of 100 µl of this ng/rhBMP-2 solution was added into a tube containing 162 ± 3 mg of SBC and mixed 100 µl of blood. This resulted in a BMP-2 dosage of 50µg.

3. BMP-2 released from composite of SBC and PEG hydrogel (SBC/PEG)

An acetic acid buffer was made as lyophilized ng/rhBMP-2 was reconstituted at a concentration of 2.57 mg/ml in 0.04% acetic acid (Weber et al. 2001). The

Table 1. Design of the study

<table>
<thead>
<tr>
<th>Group</th>
<th>Test</th>
<th>Contralateral control</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Ti implant</td>
<td>Ti implant</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>Healos Scaffold/BMP-2 (50 µg)</td>
<td>Healos Scaffold</td>
</tr>
<tr>
<td>B</td>
<td>Ti implant</td>
<td>Ti implant</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>SBC/Blood Scaffold/BMP-2 (50 µg)</td>
<td>SBC/Blood Scaffold</td>
</tr>
<tr>
<td>C</td>
<td>Ti implant</td>
<td>Ti implant</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>SBC/PEG Scaffold/BMP-2 (50 µg)</td>
<td>SBC/PEG Umbrella</td>
</tr>
</tbody>
</table>

Eight animals per group, four implants per side per animal.

Ti, titanium; Healos, hydroxyapatite-coated collagen; PEG, polyethylene glycol hydrogel; SBC, HA/TCP particulate graft substitute (Straumann Bone Ceramic®).
A total of 195 μl PEG hydrogel containing 22 μl of this ng/rhBMP-2 solution was mixed evenly into 162 ± 3 mg of SBC particles to make a combination scaffold which had an ng/rhBMP-2 dose of 50 μg.

Animal and anesthesia

Twelve adult Göttingen mini-pigs weighing from 30 to 50 kg, 2 years old, were used for the study (Magneten, Malmo, Sweden). This study protocol was approved by the ethics committee for animal research at Malmo University in Sweden. The animals were housed under closed and controlled conditions and were fed with sterile and soft diet food during the whole study. The animals were kept in stables in groups of four animals, and on the day of surgery, each mini-pig was pre-medicated with an intramuscular injection of atropine [Atropinum sulfuricum, 0.05 mg/kg IM]. A combination of ketamine [10 mg/kg, Ketalar Vet 50 mg/ml], Pfizer AB, Sollentuna, Sweden] and midazolam [0.5 mg/kg Dormicum 5 mg/ml, Roche, Basel, Switzerland] was administered IM to anesthetize. During the surgery, ketamine [10 mg/kg] and midazolam [0.5 mg/kg] were re-injected when needed. All animals received 1.8 ml local anesthesia [Xylocain Dental adrenalin 20 + 12.5 mg/ml, Astra AB, Södertälje, Sweden] at each surgical site just prior to making incisions.

Surgical procedures

The surgery was performed on both sides of the mandible as reported previously [Freilich et al. 2012 and Catros et al. 2013]. The surgical procedures are shown in Fig. 2. After extraction of the second, third, and fourth premolars and the first molar. Forty-four Straumann Bone Level implants with SLActive surfaces were partially embedded in mandibles of 12 adult mini-pigs with the shoulder of the implant located 3 mm above the bone crest. Twenty-four implants were placed in conjunction with ng/rhBMP-2 (50 μg), and other 24 were placed without BMP-2. BMP-2 was incorporated within resorbable scaffolds. Eight test and control pairs for each scaffold group were implanted. The scaffolds were placed to direct contact with the underlying partially decorticated bone and maintained in position by a custom-designed scaffold retention screw fastened to the screw channel at the head of each implant. Once all elements of the implant system construct were placed, they were submerged beneath a mucoperiosteal flap closed with 4.0 suture material [Vicryl; Ethicon, Inc., Johnson & Johnson, Somerville, NJ, USA]. All throughout the surgery, a veterinary technician monitored all vital signs of each animal.

Terminal procedures and soft tissue evaluation

All animals were painlessly sacrificed 9 weeks after dental implant placement by inducing cardiac arrest with an intracardiac injection of a 20% solution of pentobarbital (Pentobarbitaltävratrum, Apoteket AB, Stockholm, Sweden, 60 mg/ml) after general anesthesia. After sacrificing the animals, the mandibles were excised and the left and right hemi-mandibles were separated with a hand saw and fixed (4% formaldehyde) for 2 weeks and prepared for micro-computed tomography imaging and histological processing by transfer into an ethanol solution. Soft tissue healing was quantified with scores of 0 (no dehiscence) to 4 (complete dehiscence). This semiquantitative scoring system is shown in Fig. 3.

Micro-computed tomography evaluation (microCT)

microCT analyses were performed by b-cube AG [Schlieren, Switzerland]. Following fixation, all samples were imaged three-dimensionally using cone-beam X-ray computed microtomography (CT80; Scanco Medical AG, Bassersdorf, Switzerland). A trilinear interpolation method was implemented to rotate the microCT images. This resulted in aligning the implant with the z-axis in voxel space and acted as a smoothing filter. Serial tomographic images were acquired transversely to the implant longitudinal axis at 85 kVp and 82 μA, collecting 1000 projections per rotation at 400-ms integration time.

Three-dimensional cone-beam images were reconstructed with 36 mm nominal resolution (isotropic). The images were segmented to separate the implant and bone from the background using a global thresholding procedure [Fig. 4]. Newly regenerated supracrestal bone height and volume were measured directly from the segmented images.

Histopathologic and histomorphometric analyses

Histopathologic and histomorphometric analyses were performed by Biomatech-Namsa (Lyon, France). To identify position of the sites, X-rays were performed before histological preparation to accurately identify the position of the sites. Then, the implanted sites were separated and dehydrated in alcohol solutions of increasing concentration, cleared in xylene, and embedded in PMMA (polyethylmethacrylate) resin. For each site, one sagittal mesio-distal and one frontal buccal histological section were prepared. The ground sections were obtained by a microcutting and grinding technique adapted from Donath & Breuner (1982). The sections were then stained for qualitative and quantitative histology with a modified polychromatic Paragon staining. The histological sections were observed using a NIKON microscope (Eclipse E600) fitted with ×2, ×4, ×10, ×20, and ×40 objectives and equipped with a color image analyzing system SAMBA® (Samba Technologies, Meylan, France). The outcomes adjacent to each implant were assessed by analyzing bone growth, osseointegration, osteoblastic, and osteoclastic, and the presence or absence of neovascularization. Qualitative and semi-quantitative analyses were performed to measure the height of supracrestal bone at the

Fig. 3. Soft tissue summary and scores (groups a, b, and c with and without BMP-2).
implant surface (m), which was measured from just external to cortical surface of the underlying bone to the highest point of bone-to-implant contact % (BIC) to new bone. Additionally, the BIC was determined at both the osteogenic (supracrestal bone) and anchoring (subcrestal) portions of the implant within 1 mm of the implant surface. The regions of interest are shown in Fig. 5.

Data analyses
Data analyses were performed using SPSS software, version 18 [IBM Corporation, Armonk, NY, USA]. Descriptive statistics, including means, standard deviations, medians, and ranges, were generated for all outcome measures. Both within-group and between-group analyses were conducted. All within-group (paired) analyses for both microCT and histological outcomes were conducted using the nonparametric Wilcoxon signed-rank test, the test most appropriate given the structure of the data. Each animal served as its own bilateral control for these within-group analyses. All between-group analyses for both microCT and histological outcomes were conducted using the Mann-Whitney U-test for independent samples. An alpha error of 0.05 was used for all analyses.

Results

Clinical assessment of soft tissue healing
Uneventful healing and recovery of all animals followed the surgical procedures. All outcomes were assessed at 9 weeks post-implant placement. The qualitative scoring of soft tissue dehiscence [0, 1, 2, 3 or 4] from no dehiscence to fully exposed site and the summary of the scores for soft tissue dehiscence comparing BMP-2 and no-BMP-2 treatment is shown in Fig. 3. Groups with BMP-2 showed significant better soft tissue healing than groups without BMP-22 (no dehiscence in 16/24 with BMP-2 vs. 7/24 without BMP-2). If BMP-2 was not used with these three scaffolds, soft tissue healing was inadequate more than 50% of the time with the present design of the umbrella. The combination of SBC with BMP-2 yielded the best outcomes with complete soft tissue closure for seven of eight samples, while the placement of SBC infused with PEG hydrogel without BMP-2 resulted in complete soft tissue closure for one of eight samples. Not surprising, it was also observed that when soft tissue healing did not occur, bone repair was significantly impaired.

Qualitative histological findings
Qualitative histology showed that the newly formed bone showed an alveolar structure and was markedly remodeled in all the study groups. Almost no signs of inflammation of hard tissue were observed. The mucosal inflammation, including a moderate grade of macrophages, a slight grade of lymphocytes, giant cells, and plasma cells, and a slight grade of collagenolysis, and some extent of epithelial erosion were noticed. However, in the Healos group, the bone was markedly remodeled with formation of haversian structure. No residue of Healos material was encountered. In the SBC and SBC/PEG groups, a variable degree of SBC granules together with new bone regeneration was observed. SBC granule dissemination was frequently observed. Signs of enhanced bone regeneration were observed in the groups supplemented with BMP-2 as compared to the controls (Figs 6 and 7).

Bone height
Representative histological bucco-lingual and mesio-distal sections of the three test groups all showing substantial vertical bone regeneration are shown in Figs 6 and 7, respectively. Figure 8b and Table 2 show new bone height as determined via quantitative histology with no differences between the biomaterial
scaffold materials. The pooled BMP-2 test samples yielded significantly higher bone height than the no-BMP-2 control samples \((P < 0.05)\). New mean vertical bone growth in the pooled BMP-2 test sites \((2.2 \pm 1.0 \text{ mm})\) was twice that of the pooled control sites \((1.0 \pm 0.9 \text{ mm})\). The SBC+BMP-2 group exhibited the most consistent positive outcomes with the lowest standard deviation \((2.7 \pm 0.4 \text{ mm})\).

**Bone-to-Implant Contact (BIC) percentage**

Figure 8b and Table 2 show BIC data within the osteogenic region as determined via histomorphometry. The pooled BMP-2 test groups \((54.5\%)\) yielded significantly higher values of BIC than the pooled no-BMP-2 controls \((33.3\%)\) \((P < 0.05)\). Mean BIC for the SBC group from the BMP-2 test sites \((68.8 \pm 15.7)\) was statistically greater \((P < 0.05)\) than that for the pooled data of the control sites \((32.1 \pm 20.7)\). Mean BIC for the Healos group BMP-2 test sites \((54.0 \pm 21.9)\) was also statistically greater \((P < 0.05)\) than that for the pooled no-BMP-2 controls.

**Mineralized tissue volume**

Figure 8c and Table 2 show new mineralized tissue volume data as determined via microCT. This analysis revealed a mean of \(167.2 \pm 46.8 \text{ mm}^3\) of new supracrestal mineralized tissue volume formation when BMP-2 was released from the scaffolds [all test sites pooled]. Conversely, when the data was pooled for all control sites [without BMP-2 release] there was substantially lower mineralized tissue volume: \(106.4 \pm 55.0 \text{ mm}^3\). However, the SBC with BMP-2 group had the highest mineralized tissue volume of \(189.3 \pm 36.3 \text{ mm}^3\) which was significantly higher than that of either SBC alone \((118.70 \pm 51.58, P < 0.05)\), SBC with PEG.
that the dental implant and umbrella alone could be sufficient to induce new bone formation (Catros et al. 2013).

The outcomes of the present study show that all of the treatment groups displayed new vertical bone formation as determined with microCT and histomorphometry. Scaffold groups with BMP-2 showed significant better soft tissue healing than groups without BMP-2. The histomorphometric analysis showed superior bone growth when BMP-2 was released from the Healos, SBC, or SBC/PEG scaffold as compared to the same scaffolds without BMP-2. The groups with BMP-2 clearly yielded significantly higher measurements of bone height than the control group without BMP-2 ($P < 0.05$). Histomorphometry showed new mean vertical bone growth in the pooled BMP-2 (test) sites (2.2 $\pm$ 1.0 mm) to be twice that of the pooled control sites (1.0 $\pm$ 0.9 mm). The SBC+BMP-2 group exhibited the best performance (2.7 $\pm$ 0.4 mm). microCT assessments confirmed vertical bone regeneration outcomes. As determined via histomorphometry, the BMP-2 scaffold groups yielded significantly higher BIC values than the control groups ($P < 0.05$). Bone-to-implant contact for the SBC+BMP-2 group [mean = 68.8, $SD = 15.7$] was 86% greater than that for the control sites [mean = 32.1, $SD = 20.7$] at $P < 0.05$. The other scaffold groups showed comparable results. microCT analysis revealed $167 \pm 47\text{ mm}^3$ mean volume of new supracrestal mineralized tissue volume formation when BMP-2 was released from the scaffolds (all test sites pooled). When the data were pooled for all control sites (without BMP-2 release), mineralized tissue volume was only $106 \pm 55\text{ mm}^3$. The SBC+BMP-2 group had the highest mineralized tissue volume which was significantly higher than that of SBC alone. SBC/PEG+BMP-2 and Healos+BMP-2 had approximately similar bone volume, respectively, and both had significantly greater bone volume than their controls. Assessment of new mineralized tissue growth showed that the biomaterial/bone ratio was three times higher in the absence of BMP-2. The outcomes of the present study clearly demonstrate that histomorphometric assessments are useful and complimentary tools to microCT evaluation for the field of bone regeneration studies.

The work described here is part of an ongoing series of preclinical studies with the overall aim of developing a reliable method of regenerating alveolar bone height subsequent to significant vertical alveolar bone loss. All of these studies have employed classic tissue engineering methods including the use of scaffolds and inductive factors, but without the use of autogenous grafts. The implant components that have been used for this work are easy to place, and some are commercially available for clinical use. This approach is in contrast to previously described surgical methods that are considerably more technique-sensitive, often utilizing occlusive Ti reinforced non-resorbable membranes or Ti mesh to stabilize large autogenous grafts and/or collagen sponges with high doses of growth factor.

Our previous studies have been conducted in extra-oral models in the mouse, rat, and rabbit, all consistently demonstrating the ability to regenerate a new layer of supracrestal bone (Freilich et al. 2008, 2009; Wen et al. 2011, 2016a,b). These studies have shown some species-specific outcomes, but allowed for the overall assessment of various growth factor delivery vehicles and optimal design of the scaffold, Ti scaffold retainers, and Ti dental implants. Our use

**Discussion**

For the present study, we evaluated the efficacy of an implant system to guide new alveolar bone height in the (intra-oral) mini-pig mandible. Our focus was making direct comparisons between three different scaffold systems that have shown good potential in the above reference studies. The system tested here included hydrophilic rough surface titanium dental implants, one of three different resorbable scaffolds, and a novel scaffold-retaining (umbrella) device. The implants were partly submerged into the alveolar ridge in the posterior mandible on both the right and left sides of each mini-pig. For each of 12 animals, a low dose (50 $\mu$g) of ng/rhBMP-2 (BMP-2) was released from one of the three types of biomaterial scaffolds while the contralateral side utilized the same scaffold, but without BMP-2. Scaffolds included (i) HA-coated collagen (Healos); (ii) biphasic HA/$\beta$-TCP crystals available as SBC, and (iii) SBC crystals infused with PEG hydrogel (SBC/PEG). Eight test and control pairs for each scaffold group were implanted, each pair in a different animal. While this study design allowed the investigators to assess the value of the growth factor, it should be noted that evidence from previous work demonstrated (70.8 $\pm$ 50.8 $\text{ mm}^3$, $P < 0.01$) or SBC with PEG+BMP-2 (146.9 $\pm$ 50.4 $\text{ mm}^3$, $P < 0.05$). Healos+BMP-2 was roughly similar (165.5 $\pm$ 48.1 $\text{ mm}^3$) to SBC with PEG+BMP-2. Meanwhile, regarding new mineralized tissue growth, the biomaterial/bone ratio for SBC was three times as high in the absence of BMP-2 (0.89 $\pm$ 1.70) as when it was present (0.28 $\pm$ 0.18). Representative microCT bucco-lingual (Fig. 9) and mesio-distal (Fig. 10) sections are shown.

**Fig. 8.** Histological and microCT outcomes: (a) bone height as measured by histology; (b) bone-to-implant contact [BIC] percent as measured by histology; (c) mineralized tissue volume as determined by micro-computed tomography.

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Table 2. Descriptive statistics for the measured histomorphometric and microCT outcomes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
<th>n</th>
<th>Mean ± SD (range)</th>
<th>Median (range)</th>
<th>P Value (Wilcoxon)</th>
<th>P Value (Mann-Whitney)</th>
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<tbody>
<tr>
<td>Healos/BMP</td>
<td>Bone height (mm)</td>
<td>8</td>
<td>2.256 ± 0.520</td>
<td>2.674 ± 0.453</td>
<td>2.081</td>
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<td>8</td>
<td>1.99 ± 0.844</td>
<td>2.26 ± 1.097</td>
<td>2.081</td>
<td>0.0078</td>
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<tr>
<td></td>
<td>BIC (%)</td>
<td>8</td>
<td>53.98 ± 21.93</td>
<td>52.38 ± 12.10</td>
<td>51.58</td>
<td>0.078</td>
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<td></td>
<td>8</td>
<td>68.75 ± 15.71</td>
<td>69.53 ± 18.17</td>
<td>69.35</td>
<td>0.078</td>
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<td></td>
<td></td>
<td>8</td>
<td>33.98 ± 23.93</td>
<td>32.64 ± 19.09</td>
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<td>8</td>
<td>26.64 ± 7.02</td>
<td>25.41 ± 11.02</td>
<td>25.93</td>
<td>0.078</td>
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<td>132.20 ± 24.10</td>
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<td>Bone volume (mm³)</td>
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<td>165.50 ± 48.08</td>
<td>146.70 ± 78.76</td>
<td>130.20</td>
<td>0.078</td>
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<td></td>
<td></td>
<td>8</td>
<td>171.19 ± 27.60</td>
<td>169.60 ± 78.76</td>
<td>169.00</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>154.94 ± 38.49</td>
<td>148.60 ± 78.76</td>
<td>148.00</td>
<td>0.078</td>
</tr>
<tr>
<td>SBC/BMP</td>
<td>Bone height (mm)</td>
<td>8</td>
<td>2.23 ± 0.520</td>
<td>2.62 ± 0.453</td>
<td>2.081</td>
<td>0.0078</td>
</tr>
<tr>
<td></td>
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<td>8</td>
<td>1.99 ± 0.844</td>
<td>2.26 ± 1.097</td>
<td>2.081</td>
<td>0.0078</td>
</tr>
<tr>
<td></td>
<td>BIC (%)</td>
<td>8</td>
<td>53.98 ± 21.93</td>
<td>52.38 ± 12.10</td>
<td>51.58</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
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<td>8</td>
<td>68.75 ± 15.71</td>
<td>69.53 ± 18.17</td>
<td>69.35</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
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<td>8</td>
<td>33.98 ± 23.93</td>
<td>32.64 ± 19.09</td>
<td>32.10</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>26.64 ± 7.02</td>
<td>25.41 ± 11.02</td>
<td>25.93</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td>Bone volume (mm³)</td>
<td>8</td>
<td>165.50 ± 48.08</td>
<td>146.70 ± 78.76</td>
<td>130.20</td>
<td>0.078</td>
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<tr>
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<td>8</td>
<td>171.19 ± 27.60</td>
<td>169.60 ± 78.76</td>
<td>169.00</td>
<td>0.078</td>
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<tr>
<td></td>
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<td>8</td>
<td>154.94 ± 38.49</td>
<td>148.60 ± 78.76</td>
<td>148.00</td>
<td>0.078</td>
</tr>
</tbody>
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Range = from minimum to maximum. Bone height, new bone height, BIC, bone-to-implant contact.

Bone height, new bone height, BIC, bone-to-implant contact.

of the intra-oral mini-pig model demonstrated vertical alveolar ridge augmentation when rough (SLA) or rough hydrophilic surface (SLActive) Ti implants delivered 50 µg of ng/rhBMP-2 at each intra-oral test site (Freilich et al. 2012).

RGD-PEG (PEG) hydrogels have been successfully used to release rhBMP-2 to induce new bone regeneration (Jung et al. 2008; Wen et al. 2011). For the present study, PEG hydrogel was used as a BMP-2 carrier as a component of SBC/PEG scaffolds and used in conjunction with partially submerged implants. In this model, SBC/PEG scaffold with BMP-2 demonstrated improved bone volume, height, density, and BIC when compared to the SBC/PEG scaffold alone, but did not perform as well as the scaffolds without PEG. SBC has been used for in vitro delivery of enamel matrix derivative (Emdogain®; Straumann) where there was a stimulatory effect of mesenchymal cells toward osteogenic differentiation (Mrozik et al. 2012). Healos® infused with recombinant human growth and differentiation factor-5 [rhGDF-5] was successfully used in a spinal fusion study in New Zealand white rabbits (Magit et al. 2006). Healos has also been successfully infused with PEG hydrogels releasing non-glycosylated rhBMP-2 (Wen et al. 2011; Freilich et al. 2012).

Other studies have made attempts to use dental implants to guide vertical bone growth. These studies used non-resorbable occlusive membranes such as polytetrafluoroethylene (PTFE) with and without titanium reinforcement to maintain space and prevent soft tissue ingrowth while preventing soft tissue cells from populating the area where bone regeneration is desired [Roos-Jansaker et al., 2002; Polimeni et al. 2005]. Later work in a canine intra-oral model showed formation of a narrow dimension of new vertical bone of low density when a dose of adsorbed glycosylated rhBMP-2 of 200 or 600 µg per implant was coated onto a titanium implant with a porous oxide surface [Leknes et al. 2008; Wikesjo et al. 2008]. The concept and approach used for our work including the study reported here was different than the approach used for the aforementioned dog studies where a dental implant helped to guide new bone formation. We utilized a scaffold-stabilizing device with perforations and the absence of any occlusive membrane to maintain space while allowing for vascular ingrowth providing access to populate the site with autogenous cells having osteogenic potential. This allowed for the formation of new alveolar height composed of thick, dense
positive results of the series of studies utilizing the methodologies described here have been consistent over many experiments carried out in a variety of species and may be applied to a current clinical need. These outcomes are promising, but need to be repeated in human trials to demonstrate the true clinical potential of this work.

Conclusions

All three biomaterial scaffold groups tested in this intra-oral mini-pig model enabled Ti dental implants to guide new vertical bone growth. The biphasic calcium phosphate SBC+BMP-2 scaffold exhibited the best bone formation followed by the HA-coated collagen, Healos+BMP-2. All of the scaffold+BMP-2 groups showed greater bone formation than the scaffolds without BMP-2. Additionally, bone formation outcomes appear to show a direct association with overlying soft tissue healing.

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References


### Supporting Information

**Additional Supporting Information** may be found in the online version of this article:

**Table S1:** Descriptive statistics for the measured histomorphometric and microCT outcomes.