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The effect of current used bone substitution materials and platelet-rich plasma on periosteal cells by ectopic site implantation: An in-vivo pilot study

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ABSTRACT

The aim of this study was to investigate de novo bone formation following ectopic site implantation of bone substitutes covered by periosteum, with and without the application of autologous platelet-rich plasma (PRP). Twenty-four weeks after subcutaneous implantation of various bone substitutes (bovine hydroxyapatite (bHAP), phycogenic hydroxyapatite (pHAP), and bioglass (BG)) in 35 mini-pigs, bone regeneration rates were compared microradiographically and histologically. Without PRP, bHAP showed a mean de novo bone formation of 32.41% ± 29.99, in contrast to the other substitute materials where no mineralization could be detected. In combination with PRP, in the bHAP (63.61% ± 12.98; $p \pm 0.03$) and pHAP (34.37 ± 29.38; $p = 0.015$) group, significantly higher de novo bone formation was ascertained than without PRP. No ossification could be detected in the BG group. In conclusion, bHAP and pHAP bone substitutes in combination with PRP showed a significant positive effect on periosteal cells by de novo bone formation after ectopic, subcutaneous, low-vascular site implantation.

1. Introduction

In the last decade new technologies have emerged for tissue engineering with new sources of scaffolds and bone substitutes for application in reconstructive surgery. Due to continuing improvement, bone substitute materials have become promising augmentation alternatives. Certain drawbacks, such as limited biocompatibility and the loss of osteoinductive potential due to various procedural techniques (to avoid disease transmission and immunogenic reactions), restricted their clinical application. To overcome these drawbacks and to further promote bone regeneration, osteoinductive growth factors (Kasten et al., 2008; Jungbluth et al., 2010; Li et al., 2010) and cells (Gassling et al., 2010; Hakimi et al., 2010) of various origin were added, mimicking autologous bone graft as the gold standard. The search for an ideal alternative among a broad variety of bone substitute materials with wide variability in material-specific physicochemical and geometric properties affecting bone metabolism, in combination with an ideal composition of osteoinductive growth factors, has remained a challenge and a topic of further research.

In the literature, various intrinsic osteogenic potentials (the ability to form bone in an ectopic site without the addition of osteogenic factors affecting cellular differentiation or proliferation) of various biomaterials have been described in experimental in-vitro and in-vivo approaches (LeGeros, 2008; Becker et al., 2010; Roberts et al., 2011). The objective of this present study was to investigate the impact of three routinely used surgical biomaterial substitutes of various origins and physicochemical configurations (bovine hydroxyapatite (bHAP), phycogenic hydroxyapatite (pHAP), and bioglass (BG)) on periosteal cells in-vivo.

Periosteal cells show a marked enhancement in tissue regeneration by the initially high proliferation and differentiation rate of periosteal mesenchymal stem cells, differentiated osteogenic progenitor cells, and osteoblasts, providing an essential prerequisite for bone graft incorporation (Hutmacher and Sittinger, 2003).

Simple harvesting, a reliable source, its osteogenic potential and promising results in response to growth factors (TGF-beta1

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(Olivos-Meza et al., 2010), PRP (Gassling et al., 2010), and BMP-2 (Runyan et al., 2010)) have brought periosteal cells into focus in bone tissue engineering during the last few years.

The second objective of this study was to evaluate the impact of autologous platelet-derived growth factors on periosteal cells and various bone substitutes on de novo bone formation. Platelet-rich plasma (PRP), releasing high concentrations of growth factors involved in bone healing, has shown conflicting results regarding it's osteopromoting potency in the literature, whether alone or in combination with bone substitutes and target cells (Alsousou et al., 2009). However, PRP has demonstrated its positive effect on bone turnover and the biological behaviour of periosteal cells in vitro as well as in vivo (Mizuno et al., 2008; Plachokova et al., 2008; Torres et al., 2009; Gassling et al., 2010). The simple procedural technique and the absence of risk factors (transmission of diseases or autoimmune response) make PRP a reliable source of autologous growth factors in tissue engineering. In order to exclude endogenous osteogenetic protein absorption (i.e., BMPs) and to compare these data with the human metabolism, we chose an ectopic, subcutaneous, low-vascular implantation site in the mini-pig (Ripamonti, 1999; Ripamonti and Tasker, 2000; Habibovic et al., 2006a,b; Habibovic and de Groot, 2007). To the best of the authors' knowledge, there is no study in the existing literature regarding these distinct aspects.

2. Material and methods

2.1. Bone graft materials

In this study, 3 current routinely used biomaterials of different origin, composition and physicochemical properties were evaluated.

(I) Xenogenic bovine anorganic bone hydroxyapatite (bHAP), Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland.
(II) Phycogenic hydroxyapatite of red algae (pHAP) (Corallina officinalis, Frios® Algipore®; Dentsply Friadent, Mannheim, Germany), which is prepared by hydrothermal conversion of the calcium carbonate of algae, preserving the porosity (particle size 0.3 mm–2 mm, pore range 5–100 μm).
(III) Absorbable amorphous bioglass (BG) Biogran® (Orthovita, Implant Innovations, Palm Beach Gardens, FL, USA), supplied in granules with an approximate diameter of 300–355 μm.

Autogenous bone (AB) served as the reference material (Schlegel et al., 2003a,b).

2.2. Test groups

Seven test groups were created and examined 24 weeks after implantation. Each group comprised 5 mini-pigs each. The following material combinations were randomly selected:

Group A: Bio-Oss®/Periosteum
Group B: Algipore®/Periosteum
Group C: Biogran®/Periosteum
Group D: Bio-Oss®/Periosteum/PRP (1.5 ml per defect)
Group E: Algipore®/Periosteum/PRP (1.5 ml per defect)
Group F: Biogran®/Periosteum/PRP (1.5 ml per defect)
Group G: Autogenous bone/Periosteum

2.3. PRP preparation

Before the procedure, 250 ml of blood was drawn from the jugular vein of each animal to produce PRP, using a 2-tube technique (Curasan AG, Kleinostheim, Germany). This technique is suitable and easy to perform in clinical use, and the platelet concentration in the final product PRP is 4.1 times higher compared to that of untreated whole blood ($118.0 \pm 12.0 \times 10e3$ platelets/μl), resulting in a total of $483.8 \pm 97.2 \times 10e3$ platelets/μl (Wiltfang et al., 2004). Leucocytes were increased from $4.3 \pm 1.9 \times 10e3$ in untreated whole blood to $24.8 \pm 8.9 \times 10e3$/μl in PRP. Growth factor concentrations of 79.7 ng/ml for TGF-B1, 314.1 ng/ml for PDGF-AB, and 69.5 ng/ml for IGF-1, measured prior to the in-vivo application of this study, can be achieved by this technique (Wiltfang et al., 2004). For Groups E, F, and G, 1.5 ml of PRP was available for each augmentation site.

2.4. Selection of the study animal

The bone regeneration rates in mini-pigs are closely correlated with those in humans (pigs, 1.2 mm–1.5 mm per day; humans, 1.0 mm–1.5 mm per day). Its suitability for evaluation of bone substitute materials before clinical use in maxillofacial surgery was shown by Schlegel et al. (2009). The study was approved by the local animal committee of the government of Midfrankonia, Ansbach, Germany (approval no. 31-05/00).

2.5. Surgical procedure

All surgical procedures were performed under general anaesthesia. Perioperative antibiotics were administered 1 h preoperatively and for 2 days postoperatively (Streptomycin, 0.5 g/day, Gruenenthal, Stolberg, Germany). Following an incision in the skin of the forehead region, the periosteum was prepared and a piece 4×4 cm in length was harvested and stored in phosphate buffered saline (PBS). Bone was harvested in the frontal region using a 1 cm trephine drill (Roland Schmid, Fuerth, Germany) and particulated in a bone mill (Quentin Dental Products, Leimen, Germany) to an approximate size of 1 mm. The skin was finally sutured in 2 layers (Vicryl® 3.0; Vicryl® 1.0; Ethicon GmBH and Co KG, Norderstedt, Germany). PRP was produced according to the manufacturer's recommendations by using a 2-tube technique (Curasan AG, Kleinostheim, Germany). Next, an incision was made to the paravertebrala back bone region; and the specimens from Groups A to G, covered by the periosteum of the anterior skull and sutured with Vicryl® 1.0 (Ethicon GmBH and Co KG, Norderstedt, Germany), were implanted in subcutaneous adipose tissue, marked by a lead ball (Fig. 1). The inner cambial layer of the periosteum containing the osteoprogenitor cell was attached to the bone substitute. In Groups D–F, a further 1.5 ml of prepared PRP was added. In addition, the full-thickness flap was repositioned and sutured in 2 layers (Vicryl® 3.0; Vicryl® 1.0; Ethicon GmBH and Co KG, Norderstedt, Germany). The animals were sacrificed to allow recovery of the material after a period of 24 weeks. The animals were sedated by an intramuscular injection of azaperone (1 mg/kg) and midazolam (1 mg/kg). Then they were euthanized by an intravascular injection of 20% pentobarbital solution into an ear vein until cardiac arrest.

2.6. Removal and preparation of the specimens

The specimens were removed and immediately frozen at minus 80 °C. Immersion fixation was carried out using 1.4% paraformaldehyde at 4 °C to render the organic matrix insoluble. Then the specimens were dehydrated in an ascending alcohol series at room temperature in a dehydration unit (Shandon Citadel 1000, Shandon GmBH, Frankfurt, Germany). Xylol was used as an intermediate fixation. We used Technovit 9100® (Heraeus Kulzer, Kulzer Division, Wertheim, Germany) for embedding, according to Donath and Breuner (1982).