IN VITRO EFFECTS OF PARTICULATE BONE SUBSTITUTE MATERIALS ON THE RESORPTION ACTIVITY OF HUMAN OSTEOCLASTS

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Abstract

Much research has been done on bone cells, but only a few studies deal with biomaterial-induced effects on human osteoclasts, which may take on an important role in the successful regeneration of bone. In order to highlight such effects, human peripheral blood mononuclear cells (PBMCs) were extracted from venous blood, differentiated to osteoclasts and then cultured in the presence of five particulate hydroxyapatite (HA)/β-tricalcium phosphate (TCP) biomaterials, on bovine bone slices and glass cover slips. The biomaterials, AlgOSS 50/50 (50 % HA/50 % TCP), AlgOSS 20/80 (20 % HA/80 % TCP), Algipore (98 % HA), Cerasorb (100 % TCP) and Bio-Oss (100 % HA) were chosen to assess their influence on cell morphology and numbers. Light microscopic evaluation was performed during ongoing cell culture. After 21 d of cultivation, the biomaterial-induced effects on osteoclastic resorption of the bone slices were evaluated by scanning electron microscopy (SEM). Osteoclast-like cells were identified by TRAP staining. All five biomaterials showed larger area fractions of resorbed bone than the control (5.6 ± 6.8 %), as measured on SEM images. The purely hydroxyapatite-based Algipore (9.8 ± 9.7 %) and Bio-Oss (7.9 ± 8.8 %) showed significantly elevated area fraction rates (p ≤ 0.05) of bone resorption. Light Microscope evaluation revealed a significant, but inhibiting effect of Cerasorb (p = 0.05). These data indicated that introducing of small biomaterial hydroxyapatite particles may have improved the performance of bone substitute materials.

Keywords: Bone substitute materials, biomaterials, bone resorption, phagocytosis, human osteoclasts, in vitro, hydroxyapatite, tricalcium phosphate.

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Introduction

In general, procedures for the regeneration of bone are aimed at stimulating and facilitating the growth of bone into defect sites and they should maintain the volume of regenerated bone. Autologous bone grafting is proven to be the most effective procedure for the regeneration of bone defects (Amini et al., 2012). Since it has the highest osteoconductive, osteoinductive and osteogenetic potential, it represents the gold standard for bone grafting. However, donor site morbidity, limited amount of autologous bone, and graft resorption promoted interest in the use of bone substitute materials as an alternative to autologous bone, especially for the regeneration of smaller defects (Laurie et al., 1984; Riachi et al., 2014; Schaaf et al., 2010). These bone substitute materials are categorized as being allogenous (bone harvested from same species), xenogenous (bone harvested from different species) or alloplastic (synthetic materials). They mainly offer osteoconductive properties and differ in their resorption kinetics (Kao and Scott, 2007; Taylor et al., 2002).

To optimize clinical results, biomaterials featuring slower resorption kinetics can lead to higher volume stability, as complete biomaterial resorption does not seem to be a prerequesition for regeneration success (Jensen et al., 2012; Schilling AF, 2006). To make up for the lack of osteoinduction by these materials, a combination of bone substitutes with autologous bone or bone marrow, has been found to be beneficial (Jensen et al., 2006; Russmueller et al., 2015).

Bone resorption is the physiological role of osteoclasts (Kylmaoja et al., 2015; Teitelbaum, 2000). Despite this unique ability to resorb the organic and inorganic matrix of bone, osteoclasts share several characteristics with foreign body giant cells (ten
Harkel et al., 2015; Wang et al., 1997b). Both cell types are part of the mononuclear phagocyte system and are derived from hematopoietic stem cells, whereas osteoblasts originate from mesenchymal stem cells (Miyamoto and Suda, 2003). Osteoclast differentiation is induced by macrophage colony-stimulating factor (M-CSF) and the ligand of the receptor activator of nuclear factor kappa B (RANKL) (Vaananen and Laitala-Leinonen, 2008), a member of the tumor necrosis factor family (Yasuda et al., 1998). M-CSF also stimulates progenitor cells to express RANKL. RANKL-activated osteoclast precursor cells fuse to form differentiated multinuclear osteoclasts (Schilling et al., 2004).

The interaction between osteoclasts and bone substitute materials seems to be one central part of successful bone regeneration, and understanding of the process at the cellular level may be necessary to achieve valid modeling of biomaterial effects in vivo (Detsch and Boccaccini, 2015; van Blitterswijk et al., 1985).

Osteoclastic bone resorption is coupled functionally with bone formation. Due to this intrinsic regulation, biomaterial-induced osteoclast function can influence bone formation (Kylmaoja et al., 2015).

The microscopic structure and the particle size of bone substitute materials seem to have more of an impact on cell differentiation, and consequently on bone resorption and formation, than do chemical composition and solubility (Sabokbar et al., 1998; Yamada et al., 1997).

Currently, there are no standardized protocols for comparative testing of biomaterials and their effects on osteoclast function. Standardized testing of biomaterial degradation patterns is carried out at extreme pH levels (pH 3). However, promising degradation properties retrieved from such tests may emerge as less favorable when analyzed under in vivo conditions (Keller et al., 2012; Zhang et al., 2012). It therefore seems evident that in vivo degradation depends on more factors than merely on solubility. The influence of enzymes, proteins and cellular activity, especially osteoclasts, is crucial to biomaterial degradation (Yamada et al., 1997). In an attempt to address these issues, several cell-based resorption assays are reported. They give useful information on how to develop and optimize bone substitute prior to clinical application (Friederichs et al., 2015; Winkler et al., 2010; Zhang et al., 2012).

Sabokbar et al. (1998; 2003) and Wang et al. (1997b) describe the effects of small-sized particles on osteoclast function. Their findings suggested that particulate bone substitute materials enhance the activity of human osteoclasts cultivated from precursor cells in vitro. In order to assess such effects on bone resorption behavior and cell morphology, the PBMCs were not cultured directly on the respective biomaterials but on bovine bone slices or glass cover slips along with small-sized biomaterial particles.

### Materials and Methods

#### Cell culture

All volunteers gave their written consent to participate in this study, and all procedures were conducted in accordance with the Declaration of Helsinki. After approval by the Institutional Review Board of the Ethics Committee of the Medical University of Vienna and Vienna General Hospital,EK 1092/2011, five healthy volunteers (three males, two females) were selected and venous whole blood (20 mL) was drawn from each individual by venipuncture and stored in EDTA tubes (GBO, Kremsmuenster, Austria) for further processing.

Mononuclear cells (PBMCs) were extracted from the peripheral blood of the five donors and these cells were differentiated to osteoclasts. Differentiation was carried out based on the original protocol of Flanagan and Massey (2003). Modifications were applied, according to Sabokbar and Athanasou (2003). The protocol was further modified after initial results were obtained, in order to improve repeatability and reproducibility (Table 1).

After dilution with phosphate buffered saline (PBS) (Sigma Life Science, St. Louis, MO, USA) in a 1:1 ratio, pipetting to 16 mL Ficoll-Paque PLUS (GE Life Science, Little Chalfont, UK) and centrifugation at 500 x g without brake set off for 20 min, the layer of PBMCs was collected and mixed 1:1 with 350 x g for 8 min.

<table>
<thead>
<tr>
<th>Procedure step</th>
<th>Original protocol (Flanagan and Massey, 2003)</th>
<th>Modified protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ficoll-Paque centrifugation</td>
<td>350 x g for 30 min. w/o brake set off</td>
<td>500 x g for 20 min. w/o brake set off</td>
</tr>
<tr>
<td>Centrifugation after collection of cell layer</td>
<td>in 10 mL PBS with 350 x g for 10 min</td>
<td>twice in 10 mL DMEM with 350 x g for 8 min</td>
</tr>
<tr>
<td>Target cell count per well</td>
<td>2 x 10⁶</td>
<td>5 x 10⁶</td>
</tr>
<tr>
<td>Washing after incubation</td>
<td>washing with PBS+ antibiotics, then transfer to fresh well plates</td>
<td>no washing before transfer to new well plates</td>
</tr>
<tr>
<td>Additives to culture medium</td>
<td>30 ng/mL RANKL 25 ng/mL M-CSF</td>
<td>30 ng/mL RANKL 25 ng/mL M-CSF 100 nM dexamethasone 10 nM vitamin D₃</td>
</tr>
</tbody>
</table>
suspension was washed twice with 10 mL DMEM (Sigma Life Science) and the cell pellet resuspended with 3 mL of medium. For counting, by light microscopy, cells were stained with Trypan Blue (Sigma Life Science).

5 × 10^5 cells were placed in each well on bovine bone slices (diameter 6 mm, thickness 0.65 mm, IDS Immunodiagnostic Systems, Tyne & Wear, UK) and on glass cover slips (diameter 10 mm, Marienfeld-Superior, Lauda-Königshofen, Germany) and incubated at 37 °C, 5% CO₂ and 95% humidity for 3 h.

The bone slices and glass cover slips were then transferred into well plates containing DMEM medium, supplemented with 10% fetal calf serum (FCS) (Gibco® by Life Technologies, Carlsbad, NM, USA), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco® by Life Technologies), 25 ng/mL M-CSF (R&D Systems, Minneapolis, MN, USA), 30 ng/mL RANKL (PeproTech, Inc., Rocky Hill, NJ, USA), 10 nM vitamin D₃ (Sigma Life Science) and 100 nM dexamethasone (Sigma Life Science).

After the cells attached onto the bone slices or the glass cover slips, they were transferred into new well plates. To allow sedimentation, the particulate biomaterials were added to the culture along with the supplemented DMEM medium. The respective bone substitutes were added with a target concentration of 100 μg/mL. To determine this target amount of biomaterial, a concentration series was run prior to cell culture experiments and measured by scanning electron microscopy (SEM). A concentration of 100 μg/mL gave an even biomaterial sedimentation, covering approximately half of the surface of the respective cover slips or bone slices.

The cells were cultivated for 21 d (at 37°C, 5% CO₂ and 95% humidity) and half the culture medium was changed every third day. Culture medium was removed by slow pipetting only from the outer edges of the wells, so as not to remove adherent cells or sedimented biomaterials at the bottom and center of the well, and new medium slowly added to the culture. Before this procedure, images of the glass cover slip containing wells were taken by light microscopy for evaluation of cell maturation.

After 21 d, the plates were fixed (2.5% glutaraldehyde (Sigma Life Science), potassium-phosphate buffer 0.1 M (pH 7.4, Sigma Life Science)) and stored at 4°C for at least 2 h and prepared for SEM. Five experimental groups and one control group were examined per donor. Groups BM1 to BM5 contained the five respective biomaterials; the control contained no bone substitute material.

### Bone substitute materials

Biomaterials that were chemically based on high crystallinity forms of the two calcium orthophosphates, hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) or a combination of both were used (Table 2 and 3). Such compound materials, called biphasic bone substitute materials, are available in order to control the velocity of biodegradation by adapting the β-TCP to HA ratio (Chow, 2009; Schopper et al., 2005; Sheikh et al., 2015).

In general, HA (Ca₉(OPO₄)₆(OH)₂) offers little chemical solubility (at 25°C, −log (KS) = 116.8) on a cellular basis with a slow biodegradation rate in vivo (Bauer, 2007; Chow, 2009). Solubility of HA changes when it is calcium deficient (at 25°C, −log (KS) = 85) or ion substituted (F, at 25°C, −log (KS) = 120) (Dorozhkin, 2011). If such calcium phosphates feature high crystallinity, they show considerably lower solubility than so-called amorphous calcium phosphates (at 25°C, −log (KS) = 25) (Dorozhkin, 2011).

#### Table 2. Overview of analyzed bone substitute materials (Accorsi-Mendonca et al., 2008; Dorozhkin, 2011; Ewers, 2005; Schopper et al., 2003; Schopper et al., 2005; Spassova, 2007; Tadic and Eppe, 2004). The phase composition ratio was estimated from X-ray diffraction powder patterns (CuKα radiation) using the Rietveld method. ([a] No F⁻ substitution in this phase.)

<table>
<thead>
<tr>
<th>Biomaterial</th>
<th>Phase composition</th>
<th>Ratio (w/w)</th>
<th>Origin</th>
<th>Porosity &amp; pore size</th>
<th>Commercial grain size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BM1</strong></td>
<td>Calcium deficient ion substituted HA Ca₉Mg₅(OPO₄)₆(CO₃)₂(OH,F)₀</td>
<td>51.9%</td>
<td>phycogenic; red marine algae</td>
<td>67%</td>
<td>2 sizes; 0.1-2.0 mm</td>
</tr>
<tr>
<td></td>
<td>Mg-β-Tricalcium phosphate Ca₅Mg₂H₂(OPO₄)₆</td>
<td>46.7%</td>
<td></td>
<td>1-10 μm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcite CaCO₃</td>
<td>1.4%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BM2</strong></td>
<td>Calcium deficient ion substituted HA Ca₉Mg₅(OPO₄)₆(CO₃)₂(OH,F)₀</td>
<td>19.6%</td>
<td>phycogenic; red marine algae</td>
<td>78%</td>
<td>2 sizes; 0.1-2.0 mm</td>
</tr>
<tr>
<td></td>
<td>Mg-β-Tricalcium phosphate Ca₅Mg₂H₂(OPO₄)₆</td>
<td>80.2%</td>
<td></td>
<td>1-6 μm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcite CaCO₃</td>
<td>0.2%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BM3</strong></td>
<td>Calcium deficient ion substituted HA Ca₉Mg₅(OPO₄)₆(CO₃)₂(OH,F)₀</td>
<td>97.8%</td>
<td>phycogenic; red marine algae</td>
<td>78%</td>
<td>3 sizes; 0.3-2.0 mm</td>
</tr>
<tr>
<td></td>
<td>Mg-β-Tricalcium phosphate Ca₅Mg₂H₂(OPO₄)₆</td>
<td>1.9%</td>
<td></td>
<td>1-10 μm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcite CaCO₃</td>
<td>0.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Effects of particulate biomaterials on human osteoclasts

Table 3. Characterization of particulate biomaterials. The d10, d50 and d90 values were assessed by SEM analysis. The crystallite sizes and crystallinity were estimated from X-ray diffraction powder patterns (CuKα radiation) using the Rietveld method.

<table>
<thead>
<tr>
<th></th>
<th>BM1 AlgOSS 50/50</th>
<th>BM2 AlgOSS 20/80</th>
<th>BM3 Algipore</th>
<th>BM4 Cerasorb</th>
<th>BM5 Bio-Oss</th>
</tr>
</thead>
<tbody>
<tr>
<td>d10 [μm]</td>
<td>1.83</td>
<td>2.28</td>
<td>1.66</td>
<td>3.04</td>
<td>1.87</td>
</tr>
<tr>
<td>d50 [μm]</td>
<td>3.73</td>
<td>4.72</td>
<td>3.97</td>
<td>6.68</td>
<td>4.08</td>
</tr>
<tr>
<td>d90 [μm]</td>
<td>8.29</td>
<td>9.93</td>
<td>9.06</td>
<td>13.08</td>
<td>10.5</td>
</tr>
<tr>
<td>Crystallite size [nm]</td>
<td>44/99</td>
<td>49/38</td>
<td>60</td>
<td>420</td>
<td>19</td>
</tr>
<tr>
<td>Crystallinity [%]</td>
<td>70</td>
<td>81</td>
<td>77</td>
<td>100</td>
<td>83</td>
</tr>
</tbody>
</table>

In contrast, β-TCP is chemically more soluble (at 25 °C, \( -\log (K_s) = 28.9 \)) than HA and offers a higher biodegradation rate (Chow, 2009; Horch et al., 2006). Similar to HA, ion substitution leads to changes in solubility of β-TCP (Mg\(^{2+}\)), at 25 °C, \( -\log (K_s) \approx 33 \) (Li et al., 2009a). Despite the stoichiometric phase composition and β-TCP to HA ratio, bioreactivity can be influenced by crystallinity and crystallite size (Gallinetti et al., 2014; LeGeros et al., 2003).

BM1 (AlgOSS 50/50, AlgOss Biotechnologies, Austria), BM2 (AlgOSS 20/80, AlgOss Biotechnologies, Austria) and BM3 (Algipore, Dentsply Implants, USA) derive from two species of lime-incrusted naturally-grown red marine algae: Corallina officinalis and Amphiroa ephedra. These algae contain a highly porous three-dimensional hard tissue skeleton, consisting mainly of calcium carbonate bound as calcite (CaCO\(_3\)). This algal calcite is converted either into a monophasic calcium deficient ion substituted phycogenic HA (with traces of calcite an Mg-β-TCP), or into a biphasic biomaterial containing HA and Mg-β-TCP (with traces of calcite) by hydrothermal chemical transformation in alkaline aqueous phosphate solution (Table 2). By varying the addition of magnesium and tuning parameters of the hydrothermal process, the concentration ratio of HA and β-TCP can be controlled obtaining biphasic composites with a β-TCP content up to 95 % by weight (Spassova, 2007).

BM4 (Cerasorb, Curasem, Germany) is a fully synthetic substitute from pure phase β-TCP. It can be obtained by calcining of calcium-deficient hydroxyapatite at temperatures over 800 °C and reflects the rhombohedral low temperature form of tricalcium phosphate (Destainville et al., 2003).

BM5 (Bio-Oss, Geistlich Pharma, Switzerland) is a bone substitute originating from bovine material and consists of pure phase HA. The bovine material undergoes a low heat treatment (300 °C) and a chemical extraction process, by which organic components are removed but the natural structure of bone is maintained (Baldini et al., 2011).

Because the commercially available grain sizes of the biomaterials did not match the requirements of our experiment, we ground specimens of each biomaterial (2.0 g) in a micronizing mill under aqueous conditions, washed them with ethanol and dried them at 110 °C. The samples were then sieved (mesh 40 μm) and sterilized by gamma radiation (25.5 kGy/min, exposure period of 26 h).

Phase abundances (weight percentages), crystallinity and crystallite sizes of the particulate biomaterials were estimated from X-ray diffraction powder patterns on a PANalytical X’Pert PRO powder diffractometer (Tables 2 and 3). Measurement conditions were CuKα radiation (\( \lambda = 0.154060 \) nm, \( \lambda = 0.154441 \) nm), primary beam X-ray mirror (Bragg-Brentano, HD), X’Celerator detector, scan length 2.546°, recording time per scan length 25 s, scan range 20 = 5-70°, total recording time approximately 11 min, rotation of samples during the measurement at 4 s/rotation. The measured X-ray diffraction patterns were analyzed using the Rietveld Method as implemented into the TOPAS program (version 4.1, Bruker AXS, Karlsruhe 2008). Chebyshev polynomials were used for the modeling of the background.

Structure data for the crystalline phases were taken from the Inorganic Crystal Structure Database (version 2016-2, FIZ Karlsruhe-Leopoldshafen): hydroxyapatite Ca\(_{10}\)(PO\(_4\))\(_6\)OH\(_2\), whittlockite Ca\(_3\)Mg\(_2\)H\(_2\)(PO\(_4\))\(_4\), β-tricalcium phosphate β-Ca\(_3\)(PO\(_4\))\(_2\), calcite CaCO\(_3\), and corundum α-Al\(_2\)O\(_3\). The latter phase (α-Al\(_2\)O\(_3\)) was required for the determination of crystallinity by adding a certain amount of the standard reference material (NIST 676a) to the original samples. The samples were measured under the conditions mentioned above, and their XRD patterns analyzed by using the TOPAS program. The amorphous phase abundance in a given sample can be calculated from the ratio of percentage corundum as determined to percentage corundum as known. This ratio must be >1 for samples containing amorphous phase, and crystallinity (%) is 100 % – % amorphous phase. Crystallite sizes were estimated using the TOPAS program from the X-ray reflection widths (Scherrer equation, spherical crystallites, Fig. 4).

For assessment of particle size distribution (Table 3), the five particulate bone substitute materials were applied to glass cover slips and analyzed by SEM (JSM 6310, JEOL Ltd., Tokyo Japan). The mean Feret’s Diameter of the particles was defined using a semi-automated image analysis system (NIS- Elements AR 2.3.0, Nikon Corporation).
Light microscopy
Before every change of culture medium, light microscope images of the respective glass cover slips were taken (IMT-2 microscope, Olympus, Tokyo, Japan) and stored on a local computer. In order to assess the quality of phenotypic cell morphology during ongoing cell culture, a score system was developed based on cell number and multinuclearity of cells per field of view (Barnaba et al., 2012; Kurihara et al., 1990) (Table 4). Scores ranged from Score 0 (PBMCs) to Score 3 (osteoclast-like morphology) (Fig. 1). Assessments were carried out by three individuals, working independently, and their results were averaged for statistical evaluation.

TRAP-staining
Tartrate-resistant acid phosphatase (TRAP) is an enzyme released by osteoclasts during bone resorption (Vaananen and Laitala-Leinonen, 2008). TRAP-staining provides standard cytochemical detection and quantification of osteoclasts (Kirstein et al., 2006).

<table>
<thead>
<tr>
<th>Cell number per field of view</th>
<th>Multinucleated cells</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 60</td>
<td>≥ 3</td>
<td>3</td>
</tr>
<tr>
<td>30-60</td>
<td>1-2</td>
<td>2</td>
</tr>
<tr>
<td>10-20</td>
<td>0-1</td>
<td>1</td>
</tr>
<tr>
<td>≤ 10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

After 21 d of cultivation, the glass cover slips were treated with fixative solution (citrate, acetone, formaldehyde 37 %, Sigma Life Science) for 30 s and rinsed with double distilled water. A commercial acid phosphatase leucocyte kit (Sigma Life Science) was used for the TRAP-staining. Cells were treated with the staining solution (Fast Garnet GBC, sodium nitrite, H₂O, Naphtol AS-BI phosphoric acid, acetate and tartrate solution) and incubated at 37 °C for 1 h. After rinsing, counterstaining with hematoxylin (Gill No 3) was carried out.

Two cover slips were analyzed per donor and group. One was treated with tartrate solution,
while the second served as negative control without specific staining. In order to quantify osteoclast-like cells, TRAP-positive cells were counted by light microscopy (BX60 microscope, Olympus, Tokyo, Japan). Reddish to brownish staining, featuring at least 3 nuclei, were classified as TRAP-positive (Kirstein et al., 2006). For evaluation, TRAP-positive cells were summarized per group and donor.

**SEM and histomorphometric analysis**

4 bone slices and 2 glass cover slips were examined per donor and bone substitute, by SEM. In the control group without bone substitute, 2 bone slices were examined per donor. The bone slices containing cells and their respective biomaterial particles were dehydrated in a graded ethanol series, and critical point dried (Polaron CPD7501, Quorum Technologies, East Sussex, UK). For detection of the resorption lacunae, a confluent cell layer was removed prior to imaging by ‘dabbing’ with an adhesive strip. The samples were fixed on specimen mounts, coated with gold (Polaron SC7620 sputter coater, Quorum Technologies) and analyzed by SEM (JSM 6310 scanning electron microscope, JEOL, Tokyo, Japan) using an accelerating voltage of 15 kV.

To quantify osteoclastic resorption activity, the SEM images underwent histomorphometric analysis (ImageJ, National Institutes of Health, Bethesda, ML, USA). A “region of interest” (ROI) was standardized for every slice. The ROI included the maximum area of the bone slices excluding the very margin of the slices. The bottom arc was skipped because of the presence of automated annotations by the SEM. The photographs were inverted, brightness adapted, and the background was removed to diminish the consistent gray level. A threshold for gray levels was determined and area fractions of osteoclastic resorption pits were assessed (2d,e).

**Statistics**

Collected data were converted into descriptive statistics and their statistical significance assessed using a generalized linear model (GLM). As Poisson distribution is considered to be the best method for modeling areas, this distribution was used in the GLM to model the area fraction of resorbed bone on the respective bone slices. The cell-maturing score, TRAP-positive cells, and area fractions of resorbed bone were estimated by the biomaterial (BM) as dummy factors, the control group served as a baseline (intercept). Each effect was tested using Wald’s Test, and a p-value of < 0.05 was defined as being significant. A post-hoc power analysis was performed in support of a sample size of five independent experiments (blood donors). To evaluate the inter-rater reliability (IRR) of the scoring system in light microscopy, a Fleiss’ Kappa for more than two raters was calculated. All calculations were performed using the statistical programming environment “R” (version 2.15.1, Vienna, Austria).

**Results**

**Light microscopy**

To determine the influence of the particulate biomaterials on the phenotypic differentiation of PBMCs towards osteoclast-like morphology on the days that the culture medium was changed, cells cultured on glass cover slips were evaluated by light microscopy. On day 1 of culture, in all groups, only PBMCs (score 0) were found. Despite insignificant differences in maturing on day 4, all biomaterial groups, including the control, were at a similar stage of osteoclast-like morphology (score 1.79 ± 0.06) on day 7. After day 7, cell morphology differed greatly between the tested groups, but remained fairly stable from day 11 to day 21 in all groups. BM1 (AlgOSS 50/50) reached the highest overall levels of osteoclast-like morphology and cell number (score 2.45 ± 0.91), contrary to BM4 (Cerasorb), which showed a statistically significant inhibition (p = 0.004) throughout the observational period (score 1.27 ± 0.51). The other groups (BM2, BM3, BM5, and control) had comparable scores from day 11 onwards, with a slight increase towards the end. In addition, statistical evaluation featured a significant effect of the factor “day” (p = 0.049), indicating more pronounced osteoclast cell morphology and cell number with the progress of time (Fig. 5, Table 5).

Estimation of inter-rater reliability (IRR) for the semi-quantitative evaluation by light microscopy showed a Fleiss’ Kappa of 0.495 for three observers (H₁,(kappa = 0), H₁,(kappa > 0): p < 0.001), indicating moderate agreement.

**TRAP-staining**

For the cytochemical detection of osteoclastic differentiation, PBMCs were cultured on glass cover slips and TRAP stained after 21 d of culture. Despite clear indications by light microscopy of osteoclast-like cell morphology, only few cells reached standard positive TRAP staining criteria (cells with reddish to brownish staining with ≥ 3 nuclei). Surprisingly, most TRAP-stained cells had only 1-2 nuclei, while multinuclear cells (≥ 3 nuclei) did not show distinctive TRAP-staining in every case. There were no statistical effects to be found between the different biomaterials in the evaluation of TRAP-positive cells (Fig. 6, Table 6).

**SEM and histomorphometric analysis**

To determine the influence of the calcium phosphate based biomaterials on the key function of human osteoclasts, namely bone resorption, PBMCs together with the triturated biomaterials were cultured on bovine bone slices for 21 d. The area fractions of resorbed bone (resorption pits) on the bone slices were then assessed by SEM, to measure the rate of
Fig 2. SEM. Detailed view of an active osteoclast (dyed purple) after phagocytosis of biomaterial (BM4-Cerasorb) embedded in resorption pits, featuring polarized cell morphology and filopodia (a, image taken before taping of the bone slice). Starfish-shaped osteoclasts (b, dyed purple) surrounded by resorption pits (b, left half) and unscathed surface of a bone slice (b, right half). Bovine bone slice featuring distinct resorption pits assessed after cell removal (taping) (c). Histomorphometric assessment of bone resorption by scanning of a taped bone slice (d) and subsequent image processing (e).
bone resorption. The average values of resorbed bone ranged from $5.56 \pm 6.78\%$ (control group) to $9.82 \pm 9.71\%$ (BM3 Algipore). BM5 (Bio-Oss) featured $7.86 \pm 8.82\%$. BM1 (AlgOSS 50/50) led to $6.96 \pm 6.98\%$, BM2 (AlgOSS 20/80) $6.55 \pm 7.09\%$ and BM4 (Cerasorb) to $6.68 \pm 6.24\%$ of resorbed bone. Resorption pits appear as bright spots on the surface of the respective bone slices. (Images were taken after removal of cells and biomaterial particles.)

It must be noted that the two purely hydroxyapatite-based biomaterials gave the highest rates of osteoclastic bone resorption (Fig. 3c,e). This was shown by regression analysis, which confirmed BM3 (Algipore, $p < 0.001$) and BM5 (Bio-Oss, $p = 0.029$) as significant predictive factors of more osteoclastic bone resorption (Fig. 7, Table 7).

In support of a sample size of five independent experiments (blood donors), post-hoc power analysis estimated an actual power of 99.9\% with a significance level of 5\% (alpha), a base rate of 3.5\% ($e^{1.2498}$) bone resorption in the control group, and an effective bone resorption of 5\% ($e^{1.6}$) in the biomaterial groups (BM1 to BM5).
Fig 4. X-Ray diffraction analysis. Composition, crystallinity and crystallite sizes were estimated from X-ray diffraction powder patterns (Cu Kα radiation) using the Rietveld method as implemented in the TOPAS program (version 4.1, Bruker AXS 2008).
Fig 5. Descriptive analysis (run-sequence plot) of light microscopy. For statistical evaluation and results of light microscopy see Table 5.

Fig 6. Descriptive analysis (boxplot) of TRAP-staining. The horizontal lines of the plot represent the smallest observation, lower quartile (Q1), median (Q2), upper quartile (Q3), and largest observation. A small circle indicates outliers. For statistical evaluation and results of TRAP-staining see Table 6.

Table 5. Light microscopy (generalized linear mixed model with Poisson distribution). The score of phenotypic cell morphology and number was estimated by intercept (i.e. control), time and biomaterial as dummy variables.

| Factor              | Estimate | Std. Error | t value | p (>|t|) |
|---------------------|----------|------------|---------|---------|
| Intercept (= control) | 1.76940  | 0.24086    | 7.346   | < 0.001 |
| Day                 | 0.02586  | 0.01259    | 2.053   | 0.049   |
| BM1 (AlgOSS 50/50)  | 0.36111  | 0.25780    | 1.401   | 0.172   |
| BM2 (AlgOSS 20/80)  | −0.16667 | 0.25780    | −0.646  | 0.523   |
| BM3 (Algipore)      | 0.03704  | 0.25780    | 0.144   | 0.887   |
| BM4 (Cerasorb)      | −0.81481 | 0.25780    | −3.161  | 0.004   |
| BM5 (Bio-Oss)       | −0.21296 | 0.25780    | −0.826  | 0.416   |

Table 6. Evaluation of TRAP positive cells (generalized linear model with Poisson distribution). The cell count of TRAP positive cells was estimated by intercept (i.e. control) and biomaterial as dummy variables. (Since TRAP evaluation was done once at end of culture experiments, time was not applicable as a variable.)

| Factor              | Estimate | Std. Error | z value | p (>|z|) |
|---------------------|----------|------------|---------|---------|
| Intercept (= control) | 1.43508  | 0.21822    | 6.576   | < 0.001 |
| BM1 (AlgOSS 50/50)  | 0.04652  | 0.30508    | 0.152   | 0.879   |
| BM2 (AlgOSS 20/80)  | −0.04879 | 0.31244    | −0.156  | 0.876   |
| BM3 (Algipore)      | −0.47957 | 0.35291    | −1.359  | 0.174   |
| BM4 (Cerasorb)      | 0.04652  | 0.30508    | 0.152   | 0.879   |
| BM5 (Bio-Oss)       | −0.04879 | 0.31244    | −0.156  | 0.876   |

Discussion

This study focused on the in vitro bone resorption activity of human osteoclasts, under the influence of five different particulate bone substitute materials. For this purpose, peripheral blood mononuclear cells (PBMCs) were isolated and differentiated to osteoclasts by the addition of cytokines and cultured for 21 d. All the cultures were conducted according to a modified protocol of Flanagan and Massey (2003) for the differentiation of PBMCs. In order to assess effects on bone resorption, the PBMCs were not cultured directly on the respective biomaterials, but on bovine bone slices along with the small-sized biomaterial particles. To analyze time-dependent effects of the particulate bone substitute materials on the PBMCs, cell morphology and cell number were studied by light microscopy on glass cover slips during the cultivation. Cytochemical detection of osteoclast-like cells was performed by TRAP-staining at the end of the culture experiments. For the assessment of osteoclast mediated bone resorption, bone slices were studied by SEM, and the area fractions of resorbed bone determined by histomorphometry.

It was possible to differentiate PBMCs toward osteoclast-like morphology after the addition of RANKL, M-CSF, dexamethasone and vitamin D3 in all cases. SEM of cells cultured on glass cover slips
revealed multinuclear cells up to 300 μm in size. The evaluation of bone slices, in all groups, showed pronounced lacunar resorption and typical feeding traces of up to 400 μm in length. As the results of the cell culture experiments proved reproducible, this indicated that the modified Flanagan and Massey (2003) protocol was successful. Using PBMCs proved to be a useful method for generating human osteoclasts. Precursor cells were obtained rapidly. Venipuncture and differentiation, with the addition of RANKL and M-CSF, avoid the need for mesenchymal cell co-culture and consequently facilitate the evaluation of culture results (Friederichs et al., 2015; Sabokbar and Athanasou, 2003). Nevertheless, the requirement for adding externally manufactured cytokines complicates the assessment of culture medium supernatants by standard assays (Lange et al., 2009).

Differences between human and murine cells are well known, especially with regard to the effects of M-CSF and TGF-β. The use of human cells is preferable for in vitro studies involving the interaction of osteoclasts with biomaterials (Flanagan and Massey, 2003).

Unfortunately, the amount of PBMCs in peripheral blood is variable and depends on gender and age. Therefore, it is important to consider that the required volume of blood drawn can increase up to 40 mL per donor in order to reach the required total cell counts of about 5 x10⁶ or more. A drawback of in vitro assays involving human osteoclasts, is that elaborate and difficult procedures are needed to ensure reproducible results (Sabokbar and Athanasou, 2003).

Understanding the phenotypic characteristics of osteoclast-like cells, as viewed by light microscopy (Kurihara et al., 1990), makes it possible to analyze both multinucularity and cell number per field of view in the context of studying the effect of electromagnetic fields on human osteoclasts cultures (Barnaba et al., 2012). In addition, light microscopy based score systems are used in other areas, such as cartilage research or cytotoxicity assays (Changoor et al., 2011; Grogan et al., 2006). In order to determine the influence of the particulate biomaterials on the ability of PBMCs to develop osteoclast-like cell morphology during culturing, evaluations of cells cultured on glass cover slips, by light microscopy, were performed using a new scoring system. Analysis of PBMCs cultures revealed that all groups scored 0 on day 1 of culture, increased by day 4 and reached similar scores by day 7. After day 7, cell morphology progressed differently in the five treatment groups.

Schilling et al. (2004) are the first to report signs of osteoclastic differentiation from day 4, and the current work confirmed this. Similarly, Sabokbar and Athanasou (2003) describe the second week as being crucial to cultivation. BM1 (AlgOSS 50/50) increased until day 11 and featured the highest overall scores. This indicated that these cells differentiated most rapidly to osteoclast-like morphology. The control group and BM3 (Algipore) behaved similarly, but featured slower differentiation throughout and reached the highest levels by the end of culture period. BM2 (AlgOSS 20/80) and BM5 (Bio-Oss) showed comparable results, but less than the control group and BM3 (Algipore). To summarize, BM1, BM2, BM3 and BM5 showed distinct differentiation, but did not differ significantly from the control.

In contrast, cells from the BM4 (Cerasorb) group developed regularly over the first week of cultivation,

Table 7. Histomorphometric analysis of bone resorption (generalized linear model with Poisson distribution). The area fractions of bone resorption were estimated by intercept (i.e. control) and the bone materials as dummy variables. (Since this evaluation was done once at the end of culture experiments, time was not applicable as a variable.)

| Factor     | Estimate | Std. Error | z value | p (>|z|) |
|------------|----------|------------|---------|---------|
| Intercept  | 1.2498   | 0.2679     | 4.666   | < 0.001 |
| BM1 (AlgOSS 50/50) | 0.2293 | 0.1626     | 1.410   | 0.158464 |
| BM2 (AlgOSS 20/80) | 0.1688 | 0.1640     | 1.030   | 0.303174 |
| BM3 (Algipore) | 0.5734 | 0.1560     | 3.675   | < 0.001 |
| BM4 (Cerasorb) | 0.1880 | 0.1635     | 1.150   | 0.250345 |
| BM5 (Bio-Oss) | 0.3504 | 0.1600     | 2.189   | 0.028584 |

Fig 7. Descriptive analysis (boxplot) of SEM. The horizontal lines of the plot represent the smallest observation, lower quartile (Q1), median (Q2), upper quartile (Q3), and largest observation. A small circle indicates outliers. For statistical evaluation and results of SEM see Table 7.
but then failed to confirm the effect of BM4 (Cerasorb), as determined by the light microscopy score, with a comparable reduction of bone resorption as detected by SEM. Estimation of inter-rater reliability (IRR) for the semi-quantitative evaluation by light microscopy revealed moderate agreement (Fleiss’ Kappa of 0.495). This indicated that evaluation studies of bone resorption by SEM remained of higher validity than those conducted by light microscopy.

For the cytochemical proof of osteoclast presence and quantification, TRAP-staining of the cells on glass cover slips was conducted at the end of culture and after final light microscopic assessment of cell differentiation. This cytochemical staining is the only defining characteristic that permits a cell to be classified as an osteoclast (Sabokbar and Athanasou, 2003). Only very few cells were recognized as TRAP-positive (cells with reddish to brownish staining with ≥ 3 nuclei). Mixed model analysis could not describe the statistical data either. As a consequence, TRAP-staining did not lead to any conclusive results. TRAP-positive staining similarly varies from reddish, purple, yellow and brown color (Hoshino et al. 2010; Jorgensen et al., 2002; Li et al., 2009b; Narducci et al., 2010; Newa et al., 2011; Paloneva et al., 2003; Schilling et al., 2004; Winkler et al., 2010). This low TRAP count was in clear contrast to light microscopy results (many multinucleated cells) and the bone resorption assay that featured distinct lacunar resorption throughout the slices for 21 days with ≥ 3 nuclei). Mixed model analysis could not describe the statistical data either. As a consequence, TRAP-staining did not lead to any conclusive results. TRAP-positive staining similarly varies from reddish, purple, yellow and brown color (Hoshino et al. 2010; Jorgensen et al., 2002; Li et al., 2009b; Narducci et al., 2010; Newa et al., 2011; Paloneva et al., 2003; Schilling et al., 2004; Winkler et al., 2010). This low TRAP count was in clear contrast to light microscopy results (many multinucleated cells) and the bone resorption assay that featured distinct lacunar resorption throughout all groups. Alternatively, the measurement of TRAP secreted by the supernatants is possible, because Kirstein et al. (2006) report a correlation between TRAP secretion and bone resorption. Staining of vitronectin receptors (VNR) or calcitonin receptors (CTR) with monoclonal antibodies is an alternative to TRAP-staining, but seems to show reduced dependability (Flanagan and Massey, 2003; Miyamoto and Suda, 2003; Sabokbar and Athanasou, 2003; Schilling et al., 2004).

In order to assess the influence of the calcium phosphate-based biomaterials on bone resorption of human osteoclasts, PBMCs together with the tritutated biomaterials, were cultured on bovine bone slices for 21 d and resorption pits were analyzed by determining their area fraction. Osteoclasts are solely dedicated to the resorption of bone and hard tissue (Kylmaaja et al., 2015). In contrast to this, foreign body giant cells (FBGCs) are not able to resorb bone (ten Harkel et al., 2015). Despite many cellular similarities between these two cell types, FBGCs seem to be able to dissolve the superficial mineral fraction of bone but they are not capable of resorbing the organic matrix of bone and are consequently incompetent for osteoclastic bone resorption (ten Harkel et al., 2015). Resorption pits and osteoclastic feeding traces connected to cells featuring osteoclast-like morphology were detected by SEM. In all cases, these cells were cultured on bovine bone slices. Due to the lack of any other cell type capable of bone resorption, we were convinced that we were able to culture functional (bone resorbing) human osteoclasts. The observation of osteoclast-derived bone resorption by SEM corresponded to published information (Kleinhaus et al., 2015; Sabokbar et al., 2003; Sabokbar et al., 1998).

The smallest area fractions of bone resorption were observed in the control group. They increased from BM2 (AlgOSS 20/80), BM4 (Cerasorb), BM1 (AlgOSS 50/50), and BM5 (Bio-Oss) to BM3 (Algipore). BM3 and BM5 showed the highest absolute area fractions of bone resorption and Poisson regression confirmed that these results were significant. Consequently, the two substitute materials composed of pure HA resulted in the highest impact on resorption activity.

The stoichiometry of the tested biomaterials also differed significantly, ranging from pure phase β-TCP (BM4) or HA (BM5), combined calcium deficient ion substituted HA, ion substituted β-TCP and traces of calcite (BM1, BM2 and BM3). In general, HA (Ca$_{10}$ (PO$_4$)$_2$ (OH)$_2$) offers little chemical solubility (at 25 °C, –log (Ks) = 116.8) and is resorbed on a cellular basis with a slow in vivo biodegradation rate (Bauer, 2007; Chow, 2009). Solubility of HA changes when it is calcium deficient (at 25 °C, –log (Ks) = 85) or ion substituted (F, at 25 °C, –log (Ks) = 120) (Dorozhkin, 2011). β-TCP is chemically more soluble (at 25 °C, –log (Ks) = 28.9) than HA and offers a higher biodegradation rate (Chow, 2009; Horch et al., 2006). Similar to HA, ion substitution leads to changes in solubility of β-TCP (Mg$_4^+$, at 25 °C, –log (Ks) = 33) (Li et al., 2009a). These differences in biodegradation seem to influence volume stability of regenerated bone significantly in favor of HA (Jensen et al., 2012; Russmueller et al., 2015).

Despite stoichiometric phase composition, solubility, and β-TCP to HA ratio, it is important to note that bioreactivity can be strongly influenced by crystallinity, crystallite size and particle size (Gallinetti et al., 2014; LeGeros et al., 2003). Assessment of crystallinity showed that all tested biomaterials were highly crystalline, presenting values ranging from 70 % (BM1) to 100 % (BM4). As this study was inspired by observations of the influence of small-sized particles on osteoclast function (Hallab and Jacobs, 2009; Sabokbar et al., 2003; Sabokbar et al., 2006).
1998), all biomaterials were ground down from their commercially available grain size and featured d₅₀-values ranging from 3.73 µm to 6.68 µm before being applied to the cultures. Such small-sized particles can be phagocytized and may directly influence cellular activity (Noordin and Masri, 2012; Sabokbar et al., 2003; Wang et al., 1997a; Wang et al., 1997b).

Osteoclastic bone resorption was mostly activated by small-sized HA particles.

Whether and how osteoclasts are activated by biomaterial chemical properties, their specific surface features and similarity to bone structure still needs to be elucidated in detail: Lange et al. analyze the immunological effects of HA and TCP, and reveal a significantly lower induction of GM-CSF and RANKL in the presence of TCP particles (Lange et al., 2009). Lange's research group also shows that larger TCP particles, which cannot be phagocytosed can cause a higher inflammatory response when compared to smaller particles. Therefore, they ask whether the absence or inefficiency of phagocytosis due to TCP particle size, shape, or surface, can induce cytotoxic effects (Lange et al., 2011).

A limitation of the current study was that the use of osteoclast differentiation media (GM-CSF and RANKL) for differentiation of FBGCs inhibited detailed insight into immunological effects. As a consequence, the results may not completely correspond to the ability of the biomaterials to stimulate osteoclast differentiation. We were not able to rule out that giant cells were forced into an osteoclastic lineage by addition of GM-CSF and RANKL to the cultures. To evaluate this in detail, an experimental setting without the external addition of these cytokines is needed (Lange et al., 2009).

Detsch et al. show the solubility of HA and TCP in buffered sodium chloride solution, revealing that TCP has a significantly higher degradation than HA. According to this, a higher concentration of calcium in the surrounding of TCP, inhibits osteoclasts (Detsch et al., 2008). In contrast to this, Taylor et al. (2002) report higher calcium concentrations near HA surfaces inhibiting osteoclast differentiation. Similar effects are described by Shiwaku et al. (2015).

Given that in the current study cell differentiation was conducted on bovine bone slices and particulate biomaterial particles were added once at the beginning of culture, the granules were able to sediment onto the bone slices and were not affected by changing the culture medium. Published work mostly reveals work done by direct cultivation of cells on plates or discs made out of biomaterial with dentin chips serving as control group. The possible comparison of our findings with those of others is limited, but a favorable role of HA can also be deduced (Keller et al., 2012; Schilling et al., 2004; Zhang et al., 2012).

Keller et al. cultivated human osteoclasts on 10 different biomaterials and report that HA-based biomaterials (Tutogen bovin and Tutogen human) increase nuclei count and resorption, but they find no increase formation of mature osteoclasts (Keller et al., 2012). Detsch et al. cultured osteoclast-like-cells deriving from a human monocytic leukemia cell line on HA and TCP ceramics. HA increases the formation of giant cells and significantly more and larger lacunae are detected. The count of TRAP-positive multinuclear cells is similar between the HA and TCP group (Detsch et al., 2008). Taylor et al. reports the detection of fewer neonatal rabbit osteoclasts, less typical morphology, less frequent and smaller lacunae, and less resorption when cells are cultured on pure Bio-Oss plates after 4 d. Here, bovine cortical bone serves as a control (Taylor et al., 2002). Similarly, Perotti et al. (2009) reports slower bone resorption of osteoclasts on Bio-Oss plates than on bovine bone. As these results are based on different study setups (processing of biomaterial, cultivation length and cell line used), in future, a standard assay for the evaluation of cell based resorption effects should be established to facilitate the comparison of these various reports (Kleinhaus et al., 2015). For example, Schilling et al. (2004) recommend the simultaneous use of dentin as a control, so as to allow comparison of biomaterials. The so-called resorbability coefficient (RRC) describes the ratio between resorption of biomaterial and resorption of dentin within the very same culture (Keller et al., 2012; Schilling et al., 2004). Co-culture systems are developed to gather information concerning bidirectional communication of osteoclasts and osteoblasts (Atkins et al., 2005; Narducci et al., 2010). However, there is currently no model for the simultaneous analysis of osteoconductive and osteoinductive features of bone substitutes (Keller et al., 2012). As a consequence, a standardized and easy to conduct in vitro assay, such as the one presented here, may be a valuable supplement.

ten Harkel et al. (2015) claim that resorbable bone graft substitutes, such as calcium phosphates, should allow for remodeling by bone resorbing osteoclasts that is important for normal bone homeostasis, bone coupling, and osteogenesis. Culturing of PBMCs on bone slices and studying osteoclastic bone resorption may be valuable. At sites of bone augmentation and regeneration, osteoconductive ingrowth commonly starts at the border between endogenous bone and the biomaterial, resulting in a so-called bioactive area (Pearsall et al., 1992; van Blitterswijk et al., 1985). Any promotion or impairment of the osteoclast-osteoblast coupling may influence augmentation success (Kylmaajo et al., 2015). The current study was designed based on observations of the role of this bioactive area between endogenous bone and biomaterial, and was inspired by observations of the influence of small-sized particles on osteoclast function (Sabokbar et al., 2003; Sabokbar et al., 1998; Wang et al., 1997b). Consequently, it was shown that an addition of small-sized hydroxyapatite particles could improve the performance of conventional biomaterials. The next step would be to assess
whether these in vitro results correlate with the behavior of the studied bone substitute materials in vivo (Lange et al., 2009; Zhang et al., 2012).

Conclusions

The influence of different particulate bone substitute materials on human osteoclasts that were cultured from peripheral blood mononuclear cells in vitro was demonstrated. It was possible to show that small-sized particles of the purely hydroxyapatite-based biomaterials Algisop and Bio-Oss significantly enhance osteoclast-mediated bone resorption. As a limitation, assessment of differences in cell morphology, as observed by light microscopy, may have been influenced by the inaccuracy of this semi-quantitative method. Nevertheless, our results indicated that further study of the cellular effects of hydroxyapatite-based biomaterials could be revealing.

Acknowledgements

The authors want to thank AlgOss Biotechnologies for providing the biomaterials AlgOss 50/50, AlgOss 20/80 and Algisop. AlgOss Biotechnologies supported the author Else Spassova in the form of a salary, but did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. All other authors declare no conflict of interest.

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Discussion with reviewers

Stefan Tangl: How did you determine sample size?

Authors: The conduction of three independent experiments (samples) as triplets (three repetitions within one independent experiment) is common in cell culture experiments. We exceeded this number by two, thus, a total of five independent experiments (five donors). In addition, each independent experiment was conducted as quadruple in our investigation.

The generation of human osteoclasts from peripheral blood mononuclear cells (PBMCs) remains an ongoing field of research with few novel data published within the last three years. Thus, the number of publications to make an a priori sample-size calculation is still very limited in 2017.

Since data to make an a priori sample size calculation was not available, we based our experimental set-up as described above. We conducted a post-hoc power analysis in support of our sample size (number of independent experiments).

With a significance level of 5 % (alpha), a base rate of 3.5±% (= e±17) bone area resorption in the control group, and an effective bone area resorption of 5±% (= e±17) in the tested groups (biomaterial 1 to 5), for the sample size of 5 an actual power of 99.9± % was estimated.


Authors: Our study was inspired by observations of Sabokbar et al. (1998) on the promoting influence of small diameter particles on osteoclast function (Such effects may lead to aseptic loosening of joint implants in orthopedic surgery). This led us to believe that all the particulate bone substitute materials will enhance the activity of human osteoclasts cultivated from precursor cells in vitro. In order to assess such effects on bone resorption behavior and cell morphology, the PBMCs were not cultured directly on the respective biomaterials but on bovine bone slices or glass cover slips along with small diameter biomaterial particles.

As expected, all five biomaterials led to higher rates of bone resorption than the control (without biomaterial). The average values of resorbed bone ranged from 5.56 ± 6.78 % (control group) to 9.82 ± 9.71 % BM3 (Aligpore), BM5 (Bio-Oss) featured similar high rates of bone resorption (7.86 ± 8.82 %) as BM3 in SEM evaluation. BM1 (AlgOSS 50/50) led to 6.96 ± 6.98 %, BM2 (AlgOSS 20/80) to 6.35 ± 7.09 % and BM4 (Cerasorb) to 6.68 ± 6.24 % of resorbed bone.

Editor’s note: The Scientific Editor in charge of this paper was Chris Evans