In general, it has been revealed that interaction of bone substitute material with the host immune system is dependent upon their physico-chemical properties. In the case of xenografts, different purification methods are applied to process the precursor tissue. One purification method that differs the most is the applied temperature. Materials treated with low and high temperatures are available. In this context, the question remains as to the influence of the different temperature treatments on the physical and chemical material properties and, thus, on the tissue reactions during the healing processes. It has been hypothesized that materials that induce mononuclear cells induce physiological healing processes, while a pathological reaction is accompanied with the induction of multinucleated giant cells (MNGCs). In this mini-review, the focus is on the comparison of preclinical research into tissue reactions to sintered and non-sintered bovine-derived xenograft. Interpretation of this data showed that an induction of higher numbers of MNGCs by sintered xenograft also induced a higher implant bed vasculization. Finally, the higher number of MNGCs and increased vasculization presumably resulted in a higher expression of anti-inflammatory molecules that may support the process of bone remodeling.

Key words: bone substitute, xenograft, multinucleated giant cells, implant bed vasculization, inflammation

Introduction

Bone tissue is a hard tissue and a type of dense connective tissue which has the ability to grow and heal itself in the case of minor defects. However, more pronounced bone defects and bone augmentation sites require a scaffold as a platform for bone regeneration. Bone substitution means the implantation of substitute materials into bone defects with the aim of allowing defect regeneration, ideally up to the condition of restitution ad integrum, i.e., the complete bone defect healing. A large variety of bone substitute materials are nowadays available on the market. Bone grafts can generally be classified based on their origin. Bone substitute materials can originate from autografts, allografts, xenografts and synthetic grafting materials. An autogenic graft is harvested from the patient itself, i.e., most often from the iliac crest bone. However, its harvesting is often accompanied with the effects of a surgical intervention, such as pain or infections at the donor side (1, 2). Furthermore, an allograft is derived from the individuals of same species, i.e., most often living human donors. Xenografts are derived from non-human species, i.e., mostly animal sources such as bovines. In contrast, synthetic grafting materials are manufactured mostly based on calcium phosphates such as hydroxyapatite (HA) or beta-tricalcium phos-
sphate (β-TCP) as these compounds are parts of the natural mineral component of bone tissue (3).

In general, an optimal bone graft should be easy to handle and should become incorporated, revascularized and integrated (4). Additionally, it should be biocompatible, non-immunogenic, physiologically stable and in simple words, it should be acceptable by patient and without the risk of disease transmission (4).

Interestingly, it has already been revealed that both "natural" bone substitute materials such as bovine-based xenografts and synthetic grafting materials induce an immune response within the implantation bed of the recipient, called a "foreign body reaction to biomaterials" (5, 6). In this cascade, macrophages and their fused relative cell type, the so-called multinucleated giant cell (MNGC), have manifoldly shown to be involved (5). It has been revealed that both these cell types are regulatory elements of the tissue reaction cascade as they express pro- and anti-inflammatory molecules that guide the cascade and, thus, the bone healing process (Figure 1.) (5, 7). In this context, it has been shown in more detail that the severity and the inflammatory alignment of the material-associated tissue reaction cascade is mainly influenced by different physical and chemical properties of bone substitute materials, such as their chemical composition, the granule size or the granule porosity, amongst others (8-10). Interestingly, these physicochemical properties of a bone substitute have also shown to have importance for the clinic as these factors have influence on the bony regeneration process (5, 11).

Figure 1. Schematic illustration of the correlation between cellular and inflammatory processes caused by bone materials, the process of implant bed vascularisation and the process of bone tissue regeneration

In case of both allo- and xenografts, the donor tissue has to be purified from immunologically effectual components such as cells or different proteins prior to their application as a bone graft material. Xenografts based on bovine donor tissue or bovine hydroxyapatite (BHA) are widely used and researched bone substitute materials due to their similar physiochemical properties compared to human bone, their osteoconductivity potential and availability (12). Two of the most popular and commonly used bovine-derived xenografts are Bio-Oss™ (Geistlich Bio-materials, Wolhusen, Switzerland) and cerabone® (botiss biomaterials, Berlin, Germany). Although it has been shown that both these bovine-derived bone substitutes provide acceptable regenerative potential, there are still essential differences in their purification processes (13, 14). The most prominent variation in these processes is the treatment of the precursor bone tissue at different temperatures. While Bio-Oss™ undergoes a low heat treatment with temperatures around 300 °C, cerabone® becomes treated at temperatures of up to 1250 °C (so-called
The inflammatory tissue reactions to both xenogeneic materials have comparatively been analyzed using the subcutaneous implantation model and established histomorphometrical methods (7-10, 13, 14, 16, 21-26). Different numbers of multinucleated giant cells (MNGCs), which showed partial expression of the lytic enzyme tartrate-resistant acid phosphatase (TRAP), have been found besides a large number of mononucleated cells such as macrophages (26). The comparative measurements showed initially that larger numbers of (TRAP-positive) MNGCs were found in the case of Bio-Oss™, which was related to the smaller material particles triggering the tissue reaction even at early study time points, while their numbers significantly decreased at later time points. In contrast, comparatively high numbers of MNGCs were found within the implantation beds of cerabone® starting after 10 days post implantation. However, the MNGC numbers did not decrease with time and remained at a comparable level up to 60 days post implantation. Interestingly, implant bed vascularization also differed: while a fast and continuously high implantation bed vascularization was measured for Bio-Oss™, vascularization was initially low and increased over time to a high level in case of cerabone®.

Moreover, it has been shown that the MNGCs in the implant bed of Bio-Oss™ seem to be foreign body giant cells (FBGCS), as also found in case of a synthetic hydroxyapatite-based bone substitute, which indicates that the different treatments based on different physical and chemical methods lead to a conversion of the former bone matrix in the direction of a foreign material (7). In this context, it is possible that the MNGCs found in the implant beds of cerabone® are also FBGCS. However, it has been shown that this cell type is not restricted to express only pro-inflammatory molecules but also anti-inflammatory mediators such as the vascular endothelial cell growth factor (VEGF) or the mannose receptor (MR, CD206), which leads to a related increased implant bed vascularization (22). Thus, it is presumable that a higher induction of MNGCs also might also cause a better bone regeneration, as implant bed vascularization is a key component for (bone) tissue regeneration (27, 28). Interestingly, the first results of a new study also confirm this theory, as it could be shown that a higher severity of a material-related inflammatory process, including MNGCs, supports directly and indirectly the bony regenerative process (unpublished data by Barbeck et al.).

Different preclinical implantation studies have been conducted to evaluate the material-related bone growth by means of Bio-Oss™ and cerabone® (Table 1) (29-39). In the case of cerabone®, only a few preclinical in vivo studies quantitatively analyzing bone regeneration have been conducted (Table 1) (29, 30). Interestingly, these studies report very diverse results. The studies give the range of newly built bone using cerabone® at different time points to be; 0 and 40% for between 21-28 days, 14-78% between 42-84 days and finally 21-30% for up to 168 days (Table 2) (29, 31, 39). In contrast, a variety of in vivo studies have been carried out to analyze the bone regeneration capacities of Bio-Oss™ (Table 1) (32-38, 39). A comparable variety of histomorphometrical results have been presented as in case of Bio-Oss™ (Table 1). Altogether, percent values of newly built bone tissue are between 8 and 34% for a time frame between 14-30 days, 4-57% for a time frame between 42-84 days and finally 39-47% for the time frame between 112-168 days have been found (Table 2) (32-38, 39). Altogether, the comparison of these preclinical data shows comparable bone healing capacities for both bone substitute materials (Table 2). However, even in case of cerabone®, more studies are necessary to evaluate the healing properties of this xenograft treated at high temperatures.
Table 1. Overview of preclinical in vivo studies analyzing the bone healing capacities of both xenogeneic bone substitutes

<table>
<thead>
<tr>
<th>Implantation model</th>
<th>Time point(s)</th>
<th>Bone growth</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerabone</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Calvarian critical size defect model, rat</td>
<td>28 and 56 days</td>
<td>28 days (42.10%)</td>
<td>Shakir et al. (31)</td>
</tr>
<tr>
<td>Calvarian critical size defect model, rabbit</td>
<td>60 days</td>
<td>55%</td>
<td>Huber et al. (29)</td>
</tr>
<tr>
<td>Periapical implantation model, cat</td>
<td>84 and 168 days</td>
<td>30.2% 5.7% at the grafted</td>
<td>Artzi et al. (30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>membrane-protected sites</td>
<td></td>
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<tr>
<td>Bio-Oss, Cerabone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calvarian critical size defect model, rabbit</td>
<td>21 and 42 days</td>
<td>cerabone® 60.6% new bone growth for</td>
<td>Institute of Bone Scienc, Seoul,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BioOss® 52.1% new bone growth for</td>
<td>Korea</td>
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<tr>
<td>Bio-Oss</td>
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<tr>
<td>Calvarian critical size defect model, rabbit</td>
<td>14 and 28 days</td>
<td>14 days (8.6 3.1%)</td>
<td>Park et al. (c) (34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28 days (15.7 5.4%)</td>
<td></td>
</tr>
<tr>
<td>Calvarian critical size defect model, rabbit</td>
<td>28 days</td>
<td>11.7 2.4%</td>
<td>Rokn, Khodadoostan (35)</td>
</tr>
<tr>
<td>Calvarian critical size defect model, rabbit</td>
<td>28 and 56 days</td>
<td>28 days (12.9 5.8%)</td>
<td>Park et al. (b) (33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>56 days (14 7.2%)</td>
<td></td>
</tr>
<tr>
<td>Calvarian critical size defect, rat</td>
<td>30 and 60 days</td>
<td>30 days (54.05% 5.78)</td>
<td>Oliviera et al. (36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 days (63.58% 5.78)</td>
<td></td>
</tr>
<tr>
<td>Calvarian critical size defect, rat</td>
<td>42 and 84 days</td>
<td>42 days (6.4 4.3%)</td>
<td>Park et al. (a) (32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>84 days (8.2 3.9%)</td>
<td></td>
</tr>
<tr>
<td>Calvarian critical size defect model, sheep</td>
<td>84 and 168 days</td>
<td>84 days (21 ± 1.2 %)</td>
<td>Scarano et al. (38)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>168 days (39 ± 3.3 %)</td>
<td></td>
</tr>
<tr>
<td>Calvarian critical size defect, rat</td>
<td>112 days</td>
<td>47.4 7.1%</td>
<td>Mah et al. (39)</td>
</tr>
<tr>
<td>Calvarian critical size defect model, rabbit</td>
<td>8 weeks</td>
<td>57.76 ± 7.75%</td>
<td>Takauti et al. (37)</td>
</tr>
</tbody>
</table>

Table 2. Comparison of the preclinical in vivo data

<table>
<thead>
<tr>
<th></th>
<th>Bio-Oss</th>
<th>cerabone</th>
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<tbody>
<tr>
<td>14 – 30 days</td>
<td>8 – 34% (18.69%)</td>
<td>0 – 40% (20%)</td>
</tr>
<tr>
<td>42 – 84 days</td>
<td>4 – 57% (23.76%)</td>
<td>14 – 78% (46.56%)</td>
</tr>
<tr>
<td>112 – 168 days</td>
<td>39 – 47% (43.2%)</td>
<td>21 – 30% (25.9)</td>
</tr>
</tbody>
</table>
Conclusion

The sintering temperature of bone substitutes including bovine hydroxyapatite based materials has shown to be an important parameter that can affect the properties of HA. In this context, the sintering temperature has influence on phase stability, densification behavior, crystallinity and porosity of HA. The data outlined in the present mini-review show that the heat treatment at different temperatures influence the tissue response to the bone matrix based bone substitute materials. Although it has been shown that both Bio-Oss™, which is purified at temperatures of 300 °C, and cerabone® with a treat-ment at 1250 °C, allow for comparable outcomes of bone healing, the number of the MNGCs and the related implant bed vascularization seem to be influenced by the material differences, induced by the different temperature treatments. Thus, it is also conceivable that variations in the expression of pro- and anti-in-flammatory molecules by both macrophages and MNGCs are induced by these material differences. Thus, the question arises as to how the temperature treatment affects material properties to be more favorable for optimal bone tissue regeneration.

Conflicts of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

References

EFEKAT TERMIČKOG TRETMANA KSENOGENIH KOŠTANIH ZAMENIKA NA TKIVNI ODGOVOR – MINI PREGLED

Mike Barbeck¹, Željka Perić-Kačarević², Faraz Kavehei³, Patrick Rider⁴, Stevo Najman⁵, Sanja Stojanović⁶, Denis Rimashevskiy⁷, Sabine Wenisch⁷, Reinhard Schnettler⁸

¹Odeljenje za oralnu i maksilofacijalnu hirurgiju, Univerzitetska bolnica Hamburg-Ependorf, Hamburg, Nemačka
²Departman za anatomsku histologiju i embriologiju, Fakultet za dentalnu medicinu i zdravstvo, Univerzitet u Osijeku, Osijek, Hrvatska
³Departman za hemijsko inženjerstvo, Imperial College London, UK
⁴Istraživanje i razvoj, Kompanija botis biomaterijali GmbH, Berlin, Nemačka
⁵Univerzitet u Nišu, Medicinski fakultet, Katedra za čelijsko i tkivno inženjerstvo; Institut za biologiju i humanu genetiku, Niš, Srbija
⁶Departman za traumatologiju i ortopediju, Ruski univerzitet narodnog prijateljstva, Moskva, Rusija
⁷Klinika za male životinje, Institut za veterinarnu anatomiju, histologiju i embriologiju, Justus Liebig Univerzitet u Gisenu, Gisen, Nemačka
⁸Ostali autorovi

Kontakt: Mike Barbeck
Univerzitetska bolnica Hamburg-Ependorf, Hamburg, Germany
E-mail: mike.barbeck@icloud.com


Ključne reči: koštani zamenik, ksenograf, multinuklearne gigantske ćelije, vaskularizacija ležišta implantata, inflamacija

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