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Potential lack of "standardized" processing techniques for production of allogeneic and xenogeneic bone blocks for application in humans

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ABSTRACT

In the present study, the structure of two allogeneic and three xenogeneic bone blocks, which are used in dental and orthopedic surgery, were histologically analyzed. The ultimate goal was to assess whether the components postulated by the manufacturer can be identified after applying conventional histological and histochemical staining techniques. Three samples of each material, i.e. allogeneic material-1 and -2 as well as xenogeneic material-1, -2 and -3, were obtained commercially. After decalcification and standardized embedding processes, conventional histological staining was performed in order to detect inorganic matrix, cellular or organic matrix components. Allogeneic material-1 showed trabecular bone-like structures, which were free of cellular components as well as of organic matrix. The allogeneic material-2 showed trabecular bone structures, in which connective tissue and cellular remnants were embedded. Additionally, some connective tissue, which resembled fat-like tissue, was found within this material. The xenogeneic material-1 showed trabecular bone-like structures and contained organic components comparable to that demonstrated for the allogeneic material-2. The xenogeneic material-2 showed trabecular bone structures with single cells located in lacunae. The xenogeneic material-3 also showed trabecular structures. Neither cellular nor organic matrix components were found within this material. According to the data of the present study, the allogeneic material-1 and the xenogeneic material-3 were the only investigated materials for which the obtained histological data were in accordance with the manufacturer's advertised information. The remaining three materials showed discrepancies—although the manufacturers of all five bone substitute materials stated that their blocks were free of organic/cellular remnants. These data are of great clinical and material science interest. It seems that even patented processing techniques are not always able to deliver reproducible materials. Although the manufacturers of all five bone blocks stated that their blocks were free of organic/cellular remnants, our histological analysis revealed that three out of five bone blocks did contain such remnants. Such specimens might be able to induce an immune response within the recipient.

1. Introduction

Regenerating and enlarging local bone volume frequently requires procedures involving either bone transplants or bone substitute materials. Although still postulated as the gold standard [1], the use of autologous bone transplants is associated with several disadvantages including the need for a second surgical stage and the risk of donor site morbidity [2]. In the last decades, biomaterials research and the related industry have developed a large number of different bone substitute materials, which all aim to avoid the need to use autologous bone transplants, either from the mouth or the iliac crest region. Among the possible sources for the bone substitute materials, human bone from deceased or living donors (allografts), as well as bone from different species (xenografts) have been proposed as reliable alternative concepts to autografts, when considering the biological performance of the grafts in patients [3–5].

However, organic residues within these "naturally derived" bone substitutes might contain pathogenic agents or genetic material and should therefore be thoroughly removed during the manufacturing process [6]. On the other hand, these materials should contain components such as extracellular bone matrix and tissue-specific collagen, which can support the natural bone

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remodeling process and consequently enhance the formation of new bone [7]. In some cases, however, these purification processes could result in failure of these "naturally derived scaffolds" because stimulation and support of the proliferation and differentiation of osteoblastic cells as well as mesenchymal progenitor cells in the host tissue—and with this the process of osteoconduction—are diminished.

Although the application of the allografts and xenografts leads in many cases to satisfactory clinical results [8,9], there is still a lack of distribution of manufacturing information on the available products on the market. Especially with respect to the processing technique, as well as the purity and the content of organic components, the given information ranges from complete transparency to a minimum (quantity and quality) of data.

Following the concept of our group to systematically analyze the cell and tissue reaction to biomaterials of different classes, the present study aimed at analyzing the histological architecture and components of five commercially available different allogeneic and xenogeneic bone substitute material blocks, in order to evaluate the information given by the manufacturer. The main focus was on the presence of organic, i.e. cellular and matrix components, vs. inorganic components within the bone blocks.

2. Materials and methods

Five commercially available bone graft blocks, i.e. DIZG Human-Spongiosa, Tutobone®, Puros® Allograft Spongiosa, OsteoBiol® Sp and Bio-Oss®, were histologically prepared and analyzed according to standardized techniques, in order to evaluate their matrix structure and to detect possible organic components contained in the different biomaterials, i.e. control and assurance of their purification quality. Additionally, the literature and the manufacturer's data were evaluated with regard to manufacturing process, material characteristics and components.

2.1. Bone grafting substitutes

2.1.1. DIZG Human-Spongiosa

The DIZG (Deutsches Zentrum für Zell- und Gewebeersatz, GmbH [DIZG]/German Institute for Cell and Tissue Replacement, Berlin, Germany) Human-Spongiosa is an allogeneic cancellous bone substitute block derived from demineralized bone matrix from deceased or living human donors (Table 1). Bone transplants from DIZG, a non-profit organization organized as a tissue bank, are available from various donor sites, in many sizes and in many forms, i.e. as blocks, granules/powder or as custom-built models. After application of different purification steps the analyzed bone substitute material is stated to contain only demineralized cancellous bone matrix without other cellular or organic contents (Table 1).

2.1.2. Tutobone®

The Tutobone® block (Tutogen Medical GmbH, Neunkirchen am Brand, Germany) is a xenogeneic bone substitute material, which originates from bovine donor animals (Table 1). After purification by the "Tutoplast® process", the Tutogen block is stated to contain cell-free trabecular bone matrix with a native extracellular collagen I matrix (Table 1).

2.1.3. Puros® Allograft

The Puros® Allograft block (Zimmer Dental GmbH, Freiburg, Germany) is an allogeneic bone substitute with a corticocancellous structure (Table 1). The Puros® Allograft block, which is purified by the "Tutoplast® process", is stated to contain corticocancellous bone matrix with a preserved collagen matrix (Table 1).

2.1.4. OsteoBiol® Sp

The OsteoBiol® Sp-Block (Tecnoss®, Giaveno, Italy) is a xenogeneic bone substitute containing heterologous cancellous bone blocks. The OsteoBiol® Sp-Block is purified by the "Tecnoss® process" and is stated to contain a calcified extracellular bone matrix combined with collagen components (Table 1).

2.1.5. Bio-Oss® Spongiosa

The xenogeneic Bio-Oss® block (Geistlich Biomaterials, Wolhusen, Switzerland), which contains only the mineral component of bovine bone, was used as control bone substitute material because of it is thoroughly documented in the literature [10]. Bio-Oss® is commercially available in the form of a block or in granular form with different particle sizes. The Bio-Oss® materials are stated to contain the demineralized bovine bone matrix without organic remnants such as cells or extracellular components such as collagen (Table 1).

2.2. Sample preparation

Three samples for each of the five biomaterials were randomly purchased as bone substitute blocks with varying dimensions from the manufacturers at two different time points, which were at least 6 months apart. This approach was chosen in the belief that ts was not sufficient to evaluate only one batch of each biomaterial.

For further microscopic inspection and analyses the material samples were histologically processed as previously described [11–13]. In summary, all samples were divided into two parts and initially decalcified for 4 days at 37 °C in Tris-buffered 10% EDTA (Carl Roth, Karlsruhe, Germany). Afterwards, the bone blocks were dehydrated in a series of increasing alcohol concentrations followed by xylol application and paraffin embedding. After that five sections 4 μm thick were cut from every block with a rotation microtome (Leica RM2255, Wetzlar, Germany), so that slides from two different parts of every bone block were prepared for the following histological staining methods as previously described. Briefly, the first, second and third sections were stained with hematoxylin and eosin (H&E), Masson0Goldner's trichrome and Giemsa, respectively [12–14]. The fourth section was used to identify osteoclasts by histochemical staining for tartrate-resistant acid phosphatase (TRAP), while a bone section was used as control for the quality of the staining procedure [11,15].

2.3. Histological analysis

Histological and histopathological evaluation was performed as previously described [11,13,16]. Briefly, the two section series of all five biomaterials were investigated microscopically with respect to material/matrix characteristics such as porosity and physicochemical structure by S.G. and M.B. using a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan). The analysis also focused on other parameters including the presence of organic components such as collagen or vital cells to describe the efficacy of processing techniques and manufacturing procedures. High-resolution microphotographs were taken using a Nikon DS-Fi1digital camera and a DS-L2 digital sight control unit (both from Nikon, Tokyo, Japan) that were connected to the above-mentioned microscope.

3. Results

The aim of the present study was a comparative analysis of the microscopic structure of five commercially available allogeneic and xenogeneic bone substitute materials with respect to the identification of the calcified bone matrix as well as other components such as collagen and possible cellular remnants.

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