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Detection of major histocompatibility complex molecules in processed allogeneic bone blocks for use in alveolar ridge reconstruction

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Objectives. Because processed allogenic bone blocks contain remnants of cells and other organic material, the present study examined the putative presence of major histocompatibility complex (MHC) molecules in protein extracts derived from processed allogeneic bone blocks.

Study Design. Protein content and the immunogenic potential of 3 different processed allografts (Osteograft, DIZG, Berlin, Germany; Caput femoris, DIZG, Berlin, Germany; Human Spongiosa, Charité Tissue Bank, Berlin, Germany) were assessed by protein extraction and analysis of the presence of MHC class 1 and 2 molecules prior to grafting. MHC concentration was measured by using enzyme-linked immunosorbent assay.

Results. Protein content in the allograft materials varied between 0.87 and 1.61 μg protein/mg. In the allograft Human Spongiosa, no MHC was detected, whereas in the allogeneic bone blocks Osteograft and Caput femoris MHC 1 (0.04–0.037 ng/mg graft material) and in Osteograft MHC class 2 molecules were detectable.

Conclusions. The results of the present study suggest that despite thorough processing, a potential antigenicity of allografts is not eliminated. MHC molecules in allografts may sensitize the immune system. (Oral Surg Oral Med Oral Pathol Oral Radiol 2018;■■:■■–■■)

In oral and maxillofacial surgery and orthopedics, allogeneic or xenogeneic bone grafting is frequently used for the treatment of bone defects because it circumvents the disadvantages of autogenous bone harvesting, which is associated with donor site morbidity.[1,2] Allografts and xenografts are available as blocks or struts to grant dimensional stability or as particulate grafts to fill osseous defects. In oral surgery, allogeneic block grafting with dental implant placement has become a widely used treatment strategy and appears promising in short-term clinical studies because the survival rates of the inserted implants are reported to be more than 90%.[3-5] The most common causes of early-stage allograft block failure described in the literature include membrane exposure and loosening of fixation screws usually accompanied by dehiscence and/or infection.[6] The current literature lacks information about failure mechanisms; however, clinical observations cannot exclude immunologic inflammation as a potential reason for graft failure. In allogeneic transplantation, the host's immune system recognizes major histocompatibility complex class I (MHC 1) and class II (MHC 2) on donor cells and elicits an immune response, which may lead to graft rejection.[7,8] MHC 1 surface molecules are expressed in all nucleated cells, whereas cells with an antigen-presenting function, such as macrophages, B lymphocytes, and activated T lymphocytes, express MHC 2 surface molecules.[9] In this context, several animal-based studies demonstrated a compromised incorporation of mismatched bone allografts when compared with syngeneic grafts in rats[10,11] and T-cell activation by donor MHC proteins in allogeneic murine bone in vitro.[12] Processing of the bone is aimed at reducing the risk of infection by removing bone marrow, cells, and proteins to minimize or prevent an immunologic response by the host.[2,8,13]

To examine whether commercially available bone allografts contain donor cells or protein residues after

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Statement of Clinical Relevance

Major histocompatibility complex class I and II antigens are detectable in allograft bone blocks despite thorough processing, and potential antigenicity is not eliminated. Major histocompatibility complex proteins may sensitize the immune system, and the resulting adverse effects remain to be investigated.

Table I. Data of examined allografts and xenografts

Sample	*Supplier*	*Architecture*	*Origin*	*Ref./Lot.*
Osteograft	Argon (DIZG)	Cancellous	Allogeneic	GT2765 / 42110000061059 GT 2601/ CHG0911150D
Caput femoris	DIZG	Corticocancellous	Allogeneic	41714000241002 / 070214BO 41714002501001 / 100714AD
Human Spongiosa	Charité	Cancellous	Allogeneic	160001062-8 / CH-B 20160216 160001050-4 / CH-B 20160216
Bio-Oss	Geistlich	Cancellous	Xenogeneic	30602.2 / 130395
Tutobone	RTI	Cancellous	Xenogeneic	68301 / 12832403 000000012847078
Native Bone	–	Cortical	Human	–

processing, which may impair graft incorporation, we recently performed a histologic and biochemical study on different freeze-dried block allografts destined for clinical application.[14] Each of the evaluated processed allogeneic bone blocks demonstrated organic material consisting of cells that varied in type and number, cell debris, and fibrous tissue within the donor bone graft. The bone blocks contained osteocytes, chondrocytes, and adipocytes. In addition, variable concentrations of DNA were isolated.[14] Results published by other authors confirmed these findings demonstrating cell and cell remnants in processed and fresh-frozen allografts prior to grafting.[15,16]

Cell residues, fat, and proteins in processed allografts may evoke an immune response after grafting because they still can exhibit MHC molecules on their surfaces that can later become a target to the host immune response.[17,18]

Because donor cells are detectable in processed allogenic bone blocks, we hypothesize that cell and/or protein remnants in allogeneic and xenogeneic bone blocks destined for alveolar ridge reconstruction might be able to interact with the host's immune system. The objective of the present study was, therefore, to test if proteins can be extracted from purified allogeneic and xenogeneic bone blocks and, if so, whether these protein fractions contain immunologically relevant molecules, such as MHC 1 and MHC 2.

MATERIALS AND METHODS

Allografts and xenografts

Processed allograft bone block products from three different suppliers were examined. From each tested allogenic graft product, 2 blocks with different batch/lot number were analyzed as specified in Table I. In detail, allogeneic graft materials included Osteograft (DIZG, Berlin, Germany), Caput femoris (DIZG, Berlin, Germany) and Human Spongiosa CHB (Charité Tissue Bank, Berlin, Germany). As negative control, 2 different xenogeneic bone block products were used (Bio-Oss, Geistlich AG, Switzerland; and Tutobone, RTI Surgical, Neunkirchen am Brand, Germany) because according to the manufacturer, the enzyme-linked immunosorbent assay (ELISA) does not show cross-reaction between different species. To verify the results from the protein and ELISA-based MHC analyses, fresh harvested bone biopsies were used as a positive control. The bone samples were taken during augmentation surgery with avascular cortical bone grafts from the fibula. The harvesting of bone samples was approved by the Ethics Committee of the Albert-Ludwigs-University, Freiburg, Germany (vote No. 603/15).

Protein extraction and ELISA

The extraction of proteins from bone grafts was performed by using a method previously described by Sroga et al. and Dong et al.[19,20] First, bone graft blocks were frozen at −80°C, subsequently pulverized in a sterile prechilled bone mill (Medicon, Tuttlingen, Germany), divided into 4 samples of 150 mg bone powder per test material group and immediately mixed with 2 mL extraction buffer containing 0.05 M ethylenediaminetetraacetic acid (EDTA; Sigma-Aldrich, Taufkirchen, Germany), 4M guanidine hydrochloride (Carl Roth, Karlsruhe, Germany), 0.03 M Tris hydrochloride (Carl Roth, Karlsruhe, Germany), 15% glycerol (Sigma-Aldrich), and cOmplete Mini Protease Inhibitor Cocktail (Roche Diagnostics, Mannheim, Germany). Bone biopsy specimens were immersed in liquid nitrogen and stored at −80°C until processing, in analogy to the bone graft blocks. The demineralization and protein extraction process was then performed simultaneously using dialysis at 4°C in a Slide-A-Lyzer MINI dialysis device (Life Technologies GmbH, Darmstadt, Germany) with 3.5 K molecular weight cutoff against 3 changes of the phosphate buffered saline (Sigma-Aldrich), pH 7.4 (4°C overnight incubation). After mini-dialysis, the four samples per material group were pooled and transferred to Amicon Ultra-4 centrifugal filter units (10 K molecular weight cutoff; Merck Chemicals, Darmstadt, Germany) to concentrate the proteins in the protein extracts by centrifugation at 4000 g for 20 minutes at 4°C. Protein concentration was determined by using the Pierce BCA Protein Assay Kit (Life Technologies, Darmstadt,

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