Razina pročišćenosti alogenoga koštanog bloka

Variant Purification of an Allogeneic Bone Block

Introduction

Numerous allogeneic and xenogeneic bone substitutes of different kinds have been introduced, since these classes of materials could exhibit a favorable regenerative potential based on their “natural” composition and bone tissue structure comparable to autografts (1, 2). Before their clinical application, the donor tissue must be purified and freed from all immunologically effective components such as the different cell types and matrix proteins (3). To accomplish this, a variety of purification techniques with different physical and/or chemical methods have been developed. In this context, most of the material companies have introduced their own purification methods, which follow relevant guidelines, i.e., standards such as the ISO 10993 (4, 5). However, before using general histochemical staining methods to detect the inorganic matrix and cellular or organic matrix components, we had previously found wide variations among different commercially available bone blocks, including two allogeneic and three xenogeneic bone blocks (4). Furthermore, we found some discrepancies between the manufacturer’s information and the composition of the bone...
Pročišćenost alogenoga koštanog bloka

Lorenz i sur.

Svrha ovog teksta bila je analizirati (ultra-)strukture komercijalno raspoloživih alogenih koštanih blokova Maxgraft®. Obavili smo njihovu strukturnu analizu kako bismo računali (ultra-)struktuру kalcificiranoga koštanog matriksa i procijenili prisutnost ostalih komponenata poput kolagena i organskih/staničnih ostataka.

Materijali i metode

Tri uzorka komercijalno raspoloživih alogenih koštanih blokova Maxgraft® histološki su pripremljeni i pregledani razdijeljena rađeni njihova sastava, kao što je već najavila naša grupa (4). Zbog određivanja kvalitete pročišćivanja posebno smo se usredotočili na otkrivanje mogućih organskih komponenata. Dodatno smo usporedili podatke proizvođača i rezultata istraživanja.

Maxgraft®

Maxgraft® (Botiss biomaterials GmbH, Berlin, Njemačka) alogen je mineralizirani zamjenski koštan blok dobiven od kosti glave živih humane dobara iz njemačkih, austrijskih i švicarskih bolnica (6). Koštan blokovi dobiveni su od certificirane neprofitne organizacije Cells + Tissuebank Austria, ustanove koju nadzire austrijsko Ministarstvo zdravstva (6, 7). Pročišćavanje koštanoga tkiva usklađeno je s odgovarajućim europskim smjerovima i austrijskim propisima o sigurnosti tkiva (6, 7). Za ovaj postupak pročišćivanja tvrdi se da su ga validirale neovisne institucije i austrijska Ministarstva zdravstva (7).

Postupak pročišćivanja, tzv. C+TBA postupak, opisan je potanko na stranica proizvođača (8). Ukratko, tvrdi se da je to iznimno siguran i kvalitetan postupak te da su s pritom poštovali najviši standardi kvalitete koji se koriste u inaktiviranju virusa i bakterija (7, 8).

Postupak pročišćivanja uključuje različite fizikalne i kemije procese (8). Tvrdi se da se primjenjuje tehnika na temelju ultrazvuka za uklanjanje krvi, stanica i staničnih komponenti, a koja bi uklonila najviše masnog tkiva kao fizikalnu metodu. Dodatno se postupci kemijskog i oksidativnog čišćenja dietilnim eteronom i etanolom različitog trajanja upotrebljavaju za inaktivaciju, kako patogeni poput virusa i bakterija, tako i nekolagenih proteina (8).

Sljedeći postupak oksidativnog pročišćivanja trebao bi ukloniti topline proteine i enzime potencijalnih antigena (8). Na kraju se primjenjuje gama-zračenje za liofilizaciju i sterilizaciju sa svrhom očuvanja prirodne strukture tkiva (8). Nisu dano informacije o sastavu koštanoga bloka, tako da dalo ni (ultra)struktura koštanoga matriksa ni druge komponente, poput kolagenog specifičnog za koštan tkivo.

Histološka priprema koštanog bloka

Tri uzorka dekalciificirane su u tris paferinanoj 10-postotnoj EDTA-i (Carl Roth, Karlsruhe, Njemačka), zatim su dehiderirani u nizu alkohola uzlaznih koncentracija te nakon ksilola.uklupljeni u parafin (kako je prethodno opisano) (4, 9–11). blocks as analyzed subsequently. Altogether, the results obtained in this study revealed that three out of five bone blocks contained organic/cellular remnants.

The aim of this short communication was to analyze the (ultra-)structure of commercially available Maxgraft® allogeneic bone block. Specifically, we performed a structural analysis of the bone block to analyze the (ultra-)structure of the calcified bone matrix and to investigate the presence of other components such as collagen and organic/cellular remnants.

Materials and Methods

Three samples of a commercially available Maxgraft® allogeneic bone block were histologically prepared and examined to evaluate their composition as previously published by our group (4). We particularly focused on the detection of possible organic components to establish their purification quality. Additionally, the manufacturer’s information and the study results were compared.

Maxgraft®

Maxgraft® (Botiss biomaterials GmbH, Berlin, Germany) is an allogeneic cancellous bone substitute block derived from the bone of femoral heads of living human donors from German, Austrian and Swiss hospitals (6). The bone blocks are processed by the Cells + Tissue bank Austria, a certified and audited non-profit organization that is regulated by the Austrian Ministry of Health (6, 7). The purification of the bone tissue is in accordance with the respective European Directives and the Austrian Tissue Safety Act (6, 7). This purification process is stated to be “validated by independent institutes” and by the Austrian Health Ministry (7).

The purification process, the “C+TBA process”, is described in more detail on the manufacturer’s homepage (8). Briefly, it is stated to be a highly secure quality process that is in compliance with the highest quality standards that are employed when inactivating viruses and bacteria (7, 8). This purification process includes different physical and chemical purification steps (8). It is stated that an ultrasonic-based removal of blood, cells and tissue components is applied, which should predominantly remove adipose tissue, as a physical method. Additionally, chemical and oxidative cleaning steps by means of diethyl ether and ethanol at different durations were employed to inactivate both pathogens, such as viruses and bacteria, and also non-collagen proteins (8). Furthermore, the oxidative purification step should eliminate soluble proteins by denaturation and potential antigens (8). Finally, lyophilization and sterilization via gamma irradiation were applied to preserve the natural tissue structure (8). No information was given about the composition of the final bone block, so that neither the (ultra-)structure of the bone matrix nor other components, such as bone tissue-specific collagen, are described.

Histological preparation of the bone blocks

Three material samples were initially decalcified in Tris-buffered 10% EDTA (Carl Roth, Karlsruhe, Germany), dehydrated in a series of increasing alcohol concentrations followed by xylol application and embedded in paraffin as
Histološka analiza

Histološka analiza obavljena je na košanim blokovima kao što je opisano (4, 9 – 11). Ukratko, histološki prerezi košanih komponenata su za svojstva materijala poput strukture koštanoga matriksa i drugih komponenata, uključujući i kolagen ili stanice/stanične ostatke. Ovaj dio neovisno su obavila dva autora (JL i SG) svjetlosnim mikroskopom (Nikon Eclipse 80i, Tokio, Japan). Za dokumentiranje histoloških slika korištena je digitalna kamera DS-Fi1 i digitalni slide controller DS-L2 (oboje Nikon, Tokio, Japan).

Histološki rezovi od 3 do 5 mm dobiveni su na rotacijskom mikrotrom (Leica RM2255, Wetzlar, Njemačka).

Korištena su tri histokemijska bojenja, uključujući hematoxilin i eosin (HE), Masson-Goldnerov trikrom i Sirius red te Giemsa. Dodatni presjek obojen je za histokemijsku metodu otkrivanja tartarat-rezistentne fosfataze (TRAP) da se identificiraju osteoklasti. Za kontrolu kvalitete TRAP bojenja korišten je presjek zdrave kosti.

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Slika 1. Histološke slike alogenih koštanih blokova Maxgraft® s fokusom na (ultra-) strukturu i sastav; (A) poprečni presjek koštanoga bloka pokazuje trabekularnu strukturu anorganskoga koštanog matriksa (crna zvjezdica); fragmenti koštanoga matriksa (crne strijelice) mogu se uobičajeno uočiti u međuprostorima trabekularnih fragmenata (Masson-Goldnerovo bojenje, total scan, 100 x povećanje); (B) koštani matriks (zvjezdica) ima lamelarni podraspored; u većini lakuna osteocita nađene su stanice i stanični ostatci (crvene kratke strijelice), a samo je manji dio lakuna bio prazan (zelene kratke stanice); stanice/stanični ostatci i ekstracelularni matriks također su bili u Haversovim kanalima (plave strijelice) (Giemsovo bojenje, 400 x povećanje, mjerilo = 10 µm); (C) na vanjskim površinama koštanoga matriksa (zvjezdica) identificirane su stanice ili stanični ostatci (strijelice), a dodatno su otkriveno jednojezgrene (cijanske strijelice), višejezgrene (crvene strijelice) (Giemsovo bojenje, 400 x povećanje, mjerilo = 10 µm); (D) i (E) unutar trabekularnih prostora uočavaju se stanične i stanično tkivne ostatke (plave strijelice u D) i vezivno-tkivni ostatci sa stanicama ili staničnim ostacima (cijanske strijelice u E) i izvanstanični matriks (žute zvjezdice u E) (koštani matriks = zvjezdica) (D: Masson-Goldnerovo bojenje, 200 x povećanje; E = Giemsovo bojenje, 400 x povećanje, mjerilo = 10 µm).

Figure 1 Histological images of the Maxgraft® allogeneic bone block with a focus on its (ultra-) structure and composition. (A) A cross-section of the bone block illustrates the trabecular structure of the inorganic bone matrix (black asterisks). Fragments of bone matrix (black arrows) can be regularly observed within the interspaces of the trabeculae fragments (Masson Goldner-staining, "total scan", 100 x magnification). (B) The bone matrix (asterisk) exhibits a lamellar sub-arrangement. In most of the osteocyte lacunae cells or cell remnants (red arrowheads) were found with only a few empty lacunae (green arrowheads). Cells/cell remnants and extracellular matrix are also apparent in the Haversian channels (blue arrow) (Giemsa-staining, 400x magnification, scale bar = 10 µm). (C) At the outer surfaces of the bone matrix (asterisks), cells or cellular remnants are identified (arrows). In addition to mononuclear cells (cyan arrows), multinucleated cells (red arrow) are detected (Giemsa-staining, 400x magnification, scale bar = 10 µm). (D) and (E) Within the trabecular interspaces, fatty-like tissue structures (black arrowheads in D) and connective tissue-like strands include both cells or cellular remnants (cyan arrows in E) and extracellular matrix (yellow asterisk in E) (bone matrix = asterisk) (D: Masson-Goldner-staining, 200x magnification; E = Giemsa-staining, 400x magnification, scale bars = 10 µm).
Ovom kontekstu je potreban komercijalni allogenecni bočni blok analiziran standardnim histološkim metodama, s fokusom na (ultra-)struktur i otkrivanje staničnih komponenata matriksa. Usporedo smo opis sastava materijala proizvođača s našim histološkim rezultatima. Zanimljivo, u tri od pet koštanih blokova stanice otkrili smo stanice ili stanične ostatke, što upućuje na to da se nedostajeja učinkovitosti pročišćavanja ovih materijala. Različitim metodama pročišćavanja analizirani koštani blokovi raspoređeni su u četiri skupine. Ova klasifikacija ima raspon od potpunog uspješnog pročišćavanja koštana matriksa s gubitkom komponenata matriksa, do materijala koji sadržavaju i koštani matriks s izvornom komponentom matriksa. Kolagenom strukturom pročišćava kvalitetna i stanični ostacima. U ovom su istraživanju allogenecni koštani blokovi Maxgraft analizirani istom histološkom metodom. Rezultati pokazuju da imaju trabekularnu strukturu s komponenata matriksa. Pronađeni su stanični ostaci unutar lakuna osteocita i na vanjskim površinama trabekula, tj. bivši osteoblasti i osteoklasti zajedno s ostatcima masnog i vezivnog tkiva kao kolagenih struktura te stanica i staničnih ostataka vezivnog tkiva. Usporedbom navedenih podataka proizvođača i onih dobivenih u našem istraživanju, ustanovili smo da primijenjena metoda pročišćavanja omogućuje očuvanje trabekularne strukture kvalitetnog tkm. Uklopljeni su naizmjeničnim ispiranjem dietil-eter ethanolom (8). Očito je nemoguće ukloniti sve intra- i ekstratrabekularne stanične ostatke iz donorskoga tkiva, što može biti, među ostalim, posljedica nedovoljnog prodora otapala unutar tvrde mineralizirane struktura. Ovi rezultati, kao npr. prisustvo stanica i staničnih ostatka u Maxgraftovim koštanih blokova, mogla bi biti važna za kliničku primjenu ovih zamjene za kost, jer su istraživanja sa procjenom transplantacije ksenogenog tkiva pokazala da razni ostaci mogu potaknuti nefiziološku proupalnu tkivnu reakciju (13 – 16). Sve ukupno ovi rezultati pokazuju da se Maxgraftovi koštani blokovi trebali uvestiti u četvrtu skupinu koja uključuje koštane blokove s najvišim udjelom ostataka, kao i Puros Allograft Spongiosa i OsteoBiol. Sp koštani blokovi prema ranijem objavljenom istraživanju naše grupe (4). Konačno, ovom istraživanju ponovno pokazuje da korištenje različitih metoda pročišćavanja za koštane zamijenske materijale na temelju njihova sastava može rezultirati pojavom ostataka različitih staničnih i staničnih ostataka. Ovo su ipak samo histološki nalazi opisanih tehnika i potrebna su klinička istraživanja za dokaživanje utjecaja staničnih ostataka na klinički rezultat nakon primjene ovih specifičnih materijala. Nema podataka koji je stupanj pročišćavanja potreban za sigurnu kliničku primjenu ovih koštanih zamjena. Također je važno spomenuti klinički izvještaj Prussa i suradnika koji su izvijestili o uspješnoj kliničkoj primjeni različitih allogenecnih tkivnih transplantata steriliziranih parom octene kiseline i etanolom, no bez podataka o mogućoj tkivnoj reakciji (17). Treba ipak istaknuti da ovdje opisane histološke metode mogu prikazati samo postojanje staničnih ostataka. Unutar ograničenja tih metoda, na pi-
degree of purification that is necessary for safe clinical application of such bone substitutes. In this context, it is important to note that a clinical report has been published by Pruss and colleagues that reports on successful application of different kinds of allogeneic tissue transplants sterilized, also, by a mixture of peracetic acid and ethanol but without presenting any data concerning the tissue reaction (17).

However, it has to be stated that histological methodology, which has been described in this paper, can only show the presence of cellular remnants. Within the limit of present methods, the question about the vitality of the remnants cannot be answered by this methodology. Therefore, a potential immunologic response within the human organism needs to be investigated by immunologic methodologies in a clinical setting, as the question arises if the observed cellular remnants have any influence on the integration process of such bone substitute materials and the healing process. To date, the final impact of insufficient purification on the clinical performance of bone substitute materials is still unresolved. However, the process of quality insurance of non-homogeneous bone graft materials makes interpretation of etiology of complications difficult and, also, calls into question the validity of interpretation of failure, technique or material.

Conclusion

In the present study, the structure of a commercially available allogeneic bone block was histologically analyzed, with the analysis particularly focused on its composition, including the appearance of cell or tissue remnants. The results demonstrate that this bone block exhibits a trabecular structure with lamellar sub-organization that harbors cellular remnants within the osteocyte lacunae and at the outer trabecular surfaces with remnants of the former inter-trabecular fatty and connective tissue, i.e., collagenous structures and connective tissue cells or cell remnants. In conclusion, clinical application will have to confirm the relevance of the results obtained in this study.

Acknowledgments

The authors would like to thank Ms. Verena Hoffmann for her excellent technical assistance. This research was funded solely by the authors’ own research funds.

Conflict of Interest

The authors declare no conflict of interest.
Abstract

Objective: This short communication reports on a histological analysis of the composition of the commercially available Maxgraft® allogeneic bone block. Materials and Methods: Based on previously published, easily applicable histological methods, blank samples of the Maxgraft® allogeneic bone block have been decalcified, dehydrated and embedded in paraffin before histological and histochemical staining. Afterwards, the slides were evaluated for their material characteristics, such as the bone matrix structure and other components, including collagen or cells/cell remnants. Results: The results show that this bone block exhibits a trabecular structure with lamellar sub-organization. Additionally, cellular remnants within the osteocyte lacunae and at the outer trabecular surfaces reside together with remnants of the former inter-trabecular fatty and connective tissue, i.e., collagenous structures and connective tissue cells or cell remnants. Conclusion: Consistent with a previous study on this topic, the data presented here demonstrate that some of the certified purification techniques might not allow for the production of allogeneic materials free of organic cell and tissue components.

Received: May 15, 2017
Accepted: June 3, 2017

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Key words
Transplantation, homologous; Bone Substitutes; Biocompatible Materials; Decalcification Technique; Desiccation

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