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Effect of Storage Temperature on Allograft Bone

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Keywords
Allogeneic bone graft · Temperature and duration storage bone bank · Biomechanical and biological properties · Processing and sterilization procedures · European directives

Summary
Background: The recommendations for storage temperature of allogeneic bone are varying between –20 °C and –70 °C and down to –80 °C. The necessary temperature of storage is not exactly defined by scientific data, and the effect of different storage temperatures onto the biomechanical and the biological behavior is discussed controversially. **Methods:** The historical development of storage temperature of bone banks is described. A survey on literature concerning the biomechanical and biological properties of allograft bone depending on the procurement and storage temperature is given as well as on national and international regulations on storage conditions of bone banks (European Council, American Association of Tissue Banks (AATB), European Association of Tissue Banks (EATB)). **Results:** Short-term storage up to 6 months is recommended with –20 °C and –40 °C for a longer period (AATB), and EATB recommends storage at –40 °C and even –80 °C while the regulations of the German German Medical Association (Bundesärztekammer) from 2001 recommend storage at –70 °C. Duration of storage at –20 °C can be maintained at least for 2 years. The potential risk of proteolysis with higher storage temperatures remains, but a definite impairment of bone ingrowth due to a storage at –20 °C was not shown in clinical use, and no adverse biomechanical effects of storage at –20 °C could be proven. **Conclusion:** Biomechanical studies showed no clinically relevant impairment of biomechanical properties of cancellous bone due to different storage temperatures. Sterilization procedures bear the advantage of inactivating enzymatic activity though reducing the risk of proteolysis. In those cases a storage temperature of –20 °C can be recommended for at least a period of 2 years, and the risk of undesired effects seems to be low for native unprocessed bone.

Schlüsselwörter
Allogenes Knochentransplantat · Lagerungstemperatur und Lagerungsdauer Knochenbank · Biomechanische und biologische Eigenschaften · Sterilisation und Aufbereitung · Europäische Richtlinien

Zusammenfassung
Hintergrund: Die Empfehlungen für die Lagerungstemperatur von allogenen Knochentransplantaten schwanken zwischen –20 °C und –70 °C und bis zu –80 °C. Detaillierte wissenschaftliche Daten zur exakten Definierung einer Lagerungstemperatur liegen nicht vor, und der Effekt verschiedener Lagerungstemperaturen auf die biomechanischen und biologischen Eigenschaften wird kontrovers diskutiert. **Methoden:** Die historische Entwicklung der Lagerungstemperatur wird beschriebenund ein Überblick über die Literatur bezüglich biomechanischer und biologischer Eigenschaften von allogenen Knochen in Abhängigkeit von Verarbeitung und Lagerungstemperatur sowie die nationalen und internationalen Regulierungen zu Lagerungsbedingungen der Knochenbank (European Council, American Association of Tissue Banks (AATB), European Association of Tissue Banks (EATB)) gegeben. **Ergebnisse:** Es wird keine einheitliche Lagerungstemperatur empfohlen. Die kurzzeitige Lagerung bis zu 6 Monaten kann bei –20 °C und längere Lagerung bei –40 °C erfolgen (AATB); die EATB empfiehlt eine Lagerung bei –40 °C und bis zu –80 °C, während die deutsche Bundesärztekammer (2001) eine Lagerung bei –70 °C vorschlägt. Die Lagerungsdauer kann bei –20 °C mindestens 2 Jahre betragen. Proteolyse ist ein potenzielles Problem bei höheren Lagerungstemperaturen, aber eine Verschlechterung der ossären Integration von Knochentransplantaten bei Lagerungstemperatur von –20 °C konnte nicht bewiesen werden, und nachteilige Effekte der Lagerung bei –20 °C auf biomechanische Eigenschaften wurden nicht festgestellt. **Schlussfolgerung:** Die Lagerungstemperatur von –20 °C beeinflusst die biomechanische Qualität von spongiösen allogenen Knochentransplantaten im Vergleich zu einer Lagerung bei tieferen Temperaturen nicht negativ. Da Sterilisationsverfahren Enzyme inaktivieren, wird Proteolyse reduziert oder vermieden. Für derartig behandelte Transplantate kann eine Lagerungstemperatur bei –20 °C empfohlen werden. Für native allogene Knochentransplantate scheint das Risiko von unerwünschten Auswirkungen gering zu sein.

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Introduction

MacEwen performed the first interhuman transplantation in a case of osteomyelitis in 1878, and Lexer reported the use of bone allografts in 1908. The necessity for storage of bone has been proclaimed early in the last century by Carrell in 1912, and Inclan, Cloward and Bush made the beginning of modern bone banking due to technical improvements in the 1940s. In March 1930 the first brand distributing deep frozen vegetable was founded in USA and the storage temperature of 0 °F which matches –17.8 °C was implemented. The recommendation of storage at minus 18 °C was continued by the European Guidelines for storage of food (89/108/EWG) [1] and the corresponding national regulations. Different storage temperatures for frozen allogeneic bone between –10 and –196 °C have been discussed [2–5]. The eutectic point of biological tissue is considered relevant for definition of storage temperature which is –21 °C in physiologic salt solution [6]. At the eutectic point of bone at –28 °C maximum ice crystal formation is achieved, and empirically a storage temperature below the eutectic point at –39 to –40 °C was recommended [7]. The absence of free water should inhibit microbial and enzymatic activity. An influence of the temperature and the velocity of freezing onto the biological behavior of the allogeneic bone and the formation of ice crystals were discussed [8–10].

Good clinical results after implantation of allogeneic unprocessed bone were found for storage temperature between –10 and –20 °C [2, 11]. A storage temperature as low as –80 °C for a bone bank has been set empirically [2, 5, 12, 13] and was continued by the German Medical Association (Bundesärztekammer) in 2001 [14]. The costs for this storage procedure are higher compared with a normal fridge. The recommendations of the American Association of Tissue Banks (AATB) [15] and the European Association of Tissue Banks (EATB) [16] and recent interpretations of the German Drug Law (Arzneimittelgesetz) [17, 18] have increased the storage temperature consecutively. As the bone grafts stored within a bone bank are sterile, the question arises whether a storage temperature similar to food could be adequate for bone as well. It remains uncertain whether a relevant affection of biomechanical and biological properties would result from a storage at higher temperature. Proteolytic activity could be expected in fresh frozen allograft bone but processing procedures eliminate enzymatic activity within the bone graft [3, 19]. Though enzymes activity in general appeared to be reduced by freezing, it has to be considered that collagenase gets inactivated below –80 °C [4]. The relevance of a residual enzymatic activity onto the quality of the transplant seems unclear, but chemical alterations due to oxidation are known to reduce vitamin content of food and influence its quality and taste. A shorter period of storage could eventually minimize the influence of the temperature onto the biomechanical and biological behavior. Storage up to 6 months at –20 °C is being recommended by the AATB [15]. It remains in discussion which is the necessary storage temperature and possible length of storage for allograft bone. For these reasons we had previously studied the influence of storage of cancellous bone at –20 °C and –80 °C up to 2 years onto the biomechanical behavior [20] and did not found any difference between the two storage temperatures.

Recommendations and Regulations on Bone Bank Storage Temperature

In the early time of bone banking during the 1940s and 1950s higher freezing temperatures as nowadays were applied by Bush, Böhler, Wilson and Bürkle de la Camp [7]. In 1947 Bush [21] recommended the storage of bone grafts within sealed containers at a temperature of –25 °C, and in 1951 Wilson [2] reported a freezing temperature between –10 and –20 °C. No general regulations and standards of tissue banking existed at that time. With increasing frequency of transplantation of human tissues, the AATB was established in 1976. The storage temperature of a bone bank was empirically set at –80 °C [12–14]. The demand for harmonization and implementation of standards for tissue banking in Europe became evident after the reunification of Europe and led to the foundation of the EATB in June 1992 while different storage temperatures were still used for bone banking [22]. Within the 1990s further political efforts were made in Europe to harmonize and regulate tissue banking and transplantation. In 2002 the European Council (EC) made a proposal to the Euopean Union Council and the parliament to set standards for tissue banking which resulted in the first supranational framework directive 2004/23/EC [23] regulating donation, procurement, testing, processing, preservation, storage, and distribution of human tissues and cells. Accordingly, the EATB and the European Association of Musculo-Skeletal Transplantation (EAMST) set 'Common Standards for Tissue and Cells Banking' [16]. The necessary storage temperature was only defined to be acceptable. There was no international agreement on a specific storage temperature. In Europe the national regulations are made according to the European Directive 2004/23/EC [23]. The resulting guidelines are 2006/17/EC [24] and 2006/86/EC [25] for technical and biological aspects of tissue banking: during storage the allograft bone should retain its structure and preserve the biomechanical properties. and the limitation of storage up to 5 years is recommended but no exact temperature of storage is stated. The EC directives implemented the German Transplantation Law Tissue Enactment (Transplantationsgesetz(TPG)-Gewebeverordnung) and an update of the Arzneimittelgesetz March 26, 2008 [26].

Recent statements of the AATB recommend a storage temperature of –20 °C for up to 6 months of storage and –40 °C for longer periods of deep frozen preservation [15]. Deeper temperatures of –70 °C were recommended by the Bundesärztekammer 2001 [14] and freezing at even lower

temperature at –80 °C is still being performed [27–29]. According to the Arzneimittelgesetz a storage temperature between –35 and –45 °C was allowed for bone banks up to 2 years of storage [17]. EATB recommends a storage of –40 °C but also considers –80 °C [16]. The directive for storage of food in Europe (89/108/EC) recommends a storage temperature of –18 °C suppressing all microbiologic activity implementing the German directive [1]. Obeying to these conditions food is being stored longer than 12 months and during transport a temperature up to –15 °C can be tolerated temporarily. These recommendations remained unchanged in the recent update of the German regulations on deep frozen food.

Effect of Temperature on Biologic Behavior of the Bone Transplant

The storage temperature should ideally suppress proteolysis and lipolysis and a reduction of immunogenicity due to freezing was assumed [9]. But an increase of storage temperature was found to reduce immunogenicity of a graft but very low temperatures were suspected to increase immune response due to denaturation [8]. Storage at –10 to –20 °C evoked no adverse reaction within the host [2]. A long-term follow up of patients who had received bone allografts stored at –17 °C in the early 1950s had satisfactory results in 85%, and no difference was found between incorporation of autograft and allograft [11]. Bassett [30] presumed that storage above the eutectic point of –28 °C might impair the desired host osteogeneic response. The effects of soft tissues, cells, and enzymes of the allograft bone onto the host are not entirely understood. It should be discussed which microstructural changes of the tissue appear primarily due to temperature and secondarily due to enzymatic activity; their effects onto the biologic behavior of the allograft needs to be determined. The inactivation of enzymes does not occur completely at a temperature above –80 °C [5] but within processed bone no enzymatic activity leading to degradation should be present [19]. This should be an advantage of thermodisinfection, chemical processing, or irradiation of the allograft bone. Some potential osteoinductive proteins should be present in native allograft, but their activity level and relevance could not be measured [30, 31]. Positive effects of processing procedures onto angiogenesis and incorporation of allograft bone in mice have also been shown for osteochondral grafts [32].

There is evidence that allograft bone might achieve similar clinical results compared with autograft bone indicating that cells attached to the autograft are not essential for the incorporation [2, 11]. No relevant promotion of ingrowth of the graft due to cellular or humoral components of its own can be expected [5, 9]. Fresh allografts were found to induce an immune response due to badly denaturated matrix while preservation with freezing reduced antigenicity [29, 32]. Besides collagen, bone matrix [33], bone itself, and bone marrow induced antigenic responses [9, 34]. The immunogenic capacity was found to be higher for grafts stored at –79 °C than for those stored at –20 °C [35–37], indicating that denaturation might be one reason for immunogenic reactions [4, 9]. The study of Burwell [37] was in favor for a storage at only –20 °C but early cell infiltrations of the implant and metabolic differences of the cells had to be considered generally [2, 9, 36, 37]. Bush [21] showed the safety of storage at –25 °C for an indefinite time for rabbit bone.

After storage of human femoral heads at –80 °C for at least 6 months viable cells with osteoblastic characteristics were found and the cell growth was independent of the period of freezing [36] but other studies did not find viable cells after freezing [38, 39]. A negative consequence of the presence of viable cells can be graft rejection. Marrow-free bone grafts showed less reactivity than complete allogeneic bone which provoked cellular and humoral immune response [34] but collagenous matrix itself was found to be locally mitogenic [40]. The host reaction towards the implant should be minimized to enhance the incorporation process, the removal of fat and marrow cells from the implant are recommended, and adverse denaturation of the allograft bone due to processing procedures and storage should be avoided [9, 32, 33]. Biologic changes of the allograft during storage which do not increase host immune reaction should not be relevant for the incorporation of the graft. In the literature there is no proven impact of a higher storage temperature at –20 °C onto the suppression of proteolysis in fresh frozen allografts. Fresh frozen food is being stored at –18 °C for more than 12 months without unacceptable changes of its quality. Therefore, storage of fresh frozen bone allografts at –20 °C seems reasonable. After sterilization procedures no relevant impact onto the biological behavior should be expected. Enzymatic activity leading to structural changes of the graft should be restricted [5, 31]. In combination with processing procedures the alteration of the storage temperature to –20 °C can be recommended. In case of fresh frozen allograft bone an adverse effect of the higher storage temperature onto the biological behavior seems unlikely. In a previous study we could show that the biomechanical properties are not more affected by a temperature of –20 °C than at –80 °C [20]. In the beginnings of bone banking good clinical results were reported after transplantation of bone grafts stored at temperatures higher than –20 °C [2]. The storage at –20 °C for up to 2 years was supported by our study [20].

Effect of Temperature on Biomechanical Features of Allogeneic Bone

An influence of duration of storage and freezing temperature onto biomechanical properties of bone has been discussed controversially [10]. Linde [10] could not find changes of trabecular structure due to freezing at –20 °C for up to 100 days and found only minor effects on stiffness but slight

changes of viscoelastic properties. Elasticity of bone is affected by mineralization while plastic behavior is related to collagen [41, 42] and the strength of the bone graft depends on the interaction of mineral content and collagen [42]. Other studies found no influence of deep freezing at –20 °C onto the biomechanical properties neither of goat bone within 12 months of storage [43] nor of rat bone after 2 weeks [3]. No adverse effect onto biomechanical features was shown at lower temperatures of –70 and –196 °C [3, 42]. Another study on cadaver vertebrae stored at –18 °C up to 7 months revealed no biomechanical change due to freezing [44], but a recent study revealed deterioration of dynamic mechanical properties of bovine femoral bone due to deep freezing [45]. No change of the biomechanical properties of bone was found after up to 5 years of storage at –80 °C [19]. The duration of freezing did not affect the biomechanical properties of human osteochondral grafts as well [46]. Our previous study, which in contrast to the study of Salai et al. [19], studied two different temperatures, compared the effect of storage for up to 2 years at –80 °C to a higher temperature at –20 °C; no adverse effect of deep freezing onto biomechanical properties even after 2 years was noted [20]. From the biomechanical point of view the storage at –20 °C is not inferior to a lower temperature.

Conclusion

The effect of processing procedures onto the quality of the allograft bone has to be considered while biomechanical properties are not adversely affected by storage at –20 °C in comparison to lower storage temperatures. Sterilization procedures might reduce or eliminate the risk of proteolysis as enzymes might be inactivated. The storage temperature of –20 °C should be recommended for storage of sterilized bone allografts for a period of at least 2 years. No relevant adverse biological effects onto the clinical outcome should be expected for fresh frozen bone allograft stored similarly.

Disclosure Statement

The authors declare no conflict of interest.

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