Variability in the Processing of Fresh Osteochondral Allografts

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Abstract

The indications for fresh osteochondral allograft continue to increase. As a result, variations in graft processing and preservation methods have emerged. An understanding of these techniques is important when evaluating the optimal protocol for processing fresh osteochondral allografts prior to surgical implantation. The aim of this study is to review the literature and understand various tissue processing protocols of four leading tissue banks in the United States. Donor procurement, serological and microbiological testing, and storage procedures were compared among companies of interest. Similarities between the major tissue banks include donor screening, aseptic processing, and testing for microorganisms. Variability exists between these companies with relation to choice of storage media, antibiotic usage, storage temperature, and graft expiration dates. Potential exists for increased chondrocyte viability and lengthened time-to-expiration of the graft through a protocol of delicate tissue handling, proper choice of storage medium, adding hormones and growth factors like insulin growth factor-1 (IGF-1) to serum-free nutrient media, and storing these grafts closer to physiologic temperatures.

Keywords
- allograft
- processing
- storage
- chondrocyte
- viability

The use of fresh osteochondral allografts has steadily increased, with over 5 million grafts being utilized by surgeons in the United States from 2004 to 2014.[1] These grafts are commonly used for osteochondral defects to alleviate pain, restore anatomy, preserve the native joint, and avoid arthroplasty, especially in young patients.[2–4] Outcomes of these procedures are generally favorable, as multiple studies have reported >80% graft survival at 10 years.[5–7] The success of an allograft transplantation is directly correlated to the viable chondrocyte density of the graft (defined by Cook et al as the quantity of live chondrocytes per area of cartilage), and it is well established that maintaining an increased chondrocyte viability via tissue har`vesting, processing, and storage protocols correlates with a longer shelf-life and improved efficacy of the graft postimplantation.[3,8–14] Cook et al found that successful allografts have a viable chondrocyte density >70% at time of implantation.[14] Thus, the extraction, processing, and preservation of the tissue, all play critical roles in the success or failure of the allograft transplantation.[4] Since 1998, the U.S. Food and Drug Administration (U.S. FDA)

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and the American Association of Tissue Banks (AATB) have established federal guidelines for standardizing the processing and preserving of fresh tissue.[15] Tissue banks, while abiding by these guidelines, implement unique methods surrounding graft preparation.[4,16–22]

Commonly utilized tissue banks include JRF Ortho (Englewood, CO), LifeNet Health (Virginia Beach, VA), MTF Biologics (Edison, NJ), and RTI Surgical (Alachua, FL).[23] These banks have developed distinct techniques of tissue processing, introducing variability in fresh osteochondral allografts options. Allograft tissue is already limited in availability and constrained geographically, and this competitive market drives companies to continue developing advancements in preservation techniques.[17,24,25] The purpose of this review is to investigate the similarities and differences between each company's processing techniques and evaluate any underlying basic science to help further understand such techniques.

Methods

Four tissue banks (JRF Ortho, LifeNet Health, MTF Biologics, and RTI Surgical) were individually contacted to gather information regarding standardized company protocols for tissue acquisition, processing, and graft preservation. Additionally, package inserts from the fresh osteochondral allografts were referenced to determine the specific company techniques. Donor screening, aseptic processing of tissue from extraction to storage, tissue screening for microorganisms, temperature of graft storage, and antibiotic usage within storage media were identified. Expiration dates were determined by the independently reported duration of cartilage viability for each tissue bank. These methods were compared between each company's protocols and explored in reference to literature (►**Table 1**).

Screening

Each osteochondral allograft undergoes screening as required by the U.S. FDA of the Department of Health and Human Services for any human tissue that may be used in a transplant.[26] The screened diseases and conditions include human immunodeficiency virus, hepatitis B, hepatitis C, and others. Furthermore, all companies review medical records of the donors for pertinent risk factors of communicable diseases.

Testing for Disease/Aseptic Processing

Aseptic techniques of each company meet the federal guidelines. Companies perform aseptic handling of their grafts beginning at the time of procurement from the donor. Prior to removing the tissue, the donor is screened through a series of serological tests. The tissue is tested for bacteria, viruses, and fungi through cultures. Each company swabs the allograft directly with the exception of MTF Biologics which does not swab directly because of marginal culture sensitivity (78–92%).[3,27] Each company's methods maintain live cells in cartilage but possible adverse effects of these treatments need to be further investigated. Although recent literature regarding infection following fresh osteochondral allograft implantation is limited, prior discussion has demonstrated the potential for infection.[28] Intrinsic risk of procedures involving arthrotomy must be accounted for, and it is commonly known that much of this risk of infection can be attributed to intrinsic patient factors. Advancements in disease testing and aseptic processing methods have likely caused historical infection rates to decline, and further research is needed to report current infection rates and properly define an optimal aseptic method of processing that does not decrease the viability of the tissue while limiting the risk of disease transmission.

Storage Media

Historically, lactated Ringer's solution has been the solution of choice for short-term graft preservation.[29] This isotonic solution is composed of lactate and electrolytes $CaCl_2$, KCl, and NaCl, but it lacks nutrients that help sustain tissue cells.[30] Studies have shown that nutrient-containing storage media is more effective in maintaining cells than lactated Ringer's solution alone.[29,31] One example of this is Dulbecco's modified eagle's media (DMEM), a widely-used, water-based, synthetic cell culture medium. This solution consists of a concentrated blend of selected vitamins, amino acids, and other desired additions to supply nutrients for tissue metabolism.[32] Specifically regarding chondrocytes, DMEM has been shown to yield improved cell viability compared with lactated Ringer's solution alone.[29] Teng et al demonstrated that at 2-week storage time, lactated Ringer's solution maintained only 20.4% viability, while DMEM maintained a 54.8% viability.[29] To optimize viability, serum-free medium has been developed by combining DMEM with additional amino acids and antibiotics, as in LifeNet Health's X-VIVO storage media and MTF Biologic's Missouri Osteochondral Allograft Preservation System (MOPS) protocol.

Although the companies in this study reported serum-free media, another option supported in literature and used for storage media is fetal bovine serum. Pennock et al found that fetal bovine serum outperformed a serum-free medium in maintaining viable chondrocyte density (82.1% compared with 27.3%) while maintaining cell density and metabolic activity.[33] The metabolic production of proteoglycan and the density of the cartilage was superior in fetal bovine serum compared with serum-free media.[33] However, use of fetal bovine serum may raise ethical questions regarding morality of acquisition and integrity of composition.[34–36] Additionally, the risk of infectious disease transmission through fetal bovine serum in storage media is not yet fully understood.[33,37]

Building off these principles, companies have developed undisclosed, proprietary nutrient medias to facilitate maintenance of fresh osteochondral allograft chondrocyte viability. JRF Ortho uses a proprietary nutrient media to store its tissues after removal from the donor, asserting that its nutrient media maintains adequate cell viability until

Table 1 Details of fresh osteochondral allograft processing

Protocol variables	JRF Ortho[20]	LifeNet Health[19]	MTF Biologics[21]	RTI Surgical[22]
Expiration date	28 Days	45 Days	60 Days	45 Days
Donor information obtained	• Medical history • Social history • "Physician assessment of the donor"	• Donor medical record • Autopsy report (if performed) • "Risk assessment"	• Medical history • Social history • Autopsy report (if performed)	• Family/next-of-kin interview • Medical/hospital records • Donor physical assessment • Radiology/pathology reports • Death certificate • Autopsy report (if performed)
Donor screening	Yes	Yes • "Each tissue is swabbed at recovery using a 100% swabbing method"	Yes • Does not swab tissue directly for testing	Yes • "Eight cultures are taken during processing and packaging episodes to detect microbial contamination" • Graft tissue itself is swabbed for culture
Aseptic processing	"Aseptic environment" processing	"Test methods are validated and adapted from the U. S. Pharmacopeial Convention (USP) <71> volume 36."[53]	• "Not terminally sterilized" • "Passes USP <71> Sterility tests" • MOPS[SM] technology	• "Aseptic processing in a certified International Organization for Standardization (ISO) Class 5 clean environment to ensure a high-quality processing setting"
Storage media	"Unique nutrient media"	• X-VIVO media which may contain human albumin, recombinant human insulin, and pasteurized human transferrin • X-VIVO is a serum-free, xeno-free medium	• "Serum-free media" which may contain, DMEM, glucose, dexamethasone, ascorbate 2-phosphate, L-Proline, sodium pyruvate, transferrin, and selenous acid	• "Nutrient media"
Antibiotics used	"Antibiotic media"	• Gentamicin • Vancomycin	• Amphotericin B • Penicillin • Streptomycin sulfate	• "Proprietary antibiotic media"
Temperature (°C)	1–10	1–10	25	1–10

Abbreviations: CLIA, Clinical Laboratory Improvement Amendments; DMEM, Dulbecco's modified eagle's media.
Notes: Regarding Donor Screening, all companies meet the requirements of the American Association of Tissue Banks (AATB) and the Federal Drug Administration (FDA). Testing by all companies is conducted by a CLIA certified facility.
Source: LifeNet Health.[19]

transplantation.[20] RTI also specifies the use of an undisclosed "nutrient media" to promote cell viability in their product. Further, companies have included additives to improve storage lifespan. LifeNet Health's serum-free X-VIVO 10 claims to be hematopoietic in nature. MTF also includes a combination of DMEM, glucose, dexamethasone, and other additives within their media to promote chondrocyte viability.[38]

Antibiotics

Though antibiotic inclusion into storage media is necessary to prevent infection following host receipt, the specific types of antibiotics used by companies vary. LifeNet Health adds vancomycin and gentamicin, while MTF Biologics includes amphotericin B, penicillin, and streptomycin sulfate.[19] Both JRF Ortho and RTI Surgical use proprietary, undisclosed "media with antibiotics."

While certain antibiotics, such as penicillin and streptomycin, are commonly used in culture media across various cell lines, the effects of other agents that are used for in vitro chondrocyte storage and in vivo surgical site infection prophylaxis have yet to be determined.[39] For example, mixed opinions exist on the effect of vancomycin on chondrocytes. Röhner et al note that vancomycin is significantly toxic to chondrocytes, paralleling conclusion derived by Shaw et al and Antoci et al.[39–41] In contrast, Dogan et al concluded that vancomycin and other key choices for treating *Staphylococcus aureus* do not demonstrate toxicity to chondrocytes in both cellular dimensions and molecular levels, arguing that previous investigations were flawed in measuring chondrotoxicity indirectly.[42] Röhner et al argued, however, that vancomycin levels were below clinically applicable levels in the study by Dogan et al, therefore impairing the detection of existing chondrotoxicity.[39] While studies have shown that higher concentrations of certain antibiotics are increasingly cytotoxic to certain chondrocyte cell lines in vitro, methods for preserving a noninfectious graft are required, and further evaluation of effects of antibiotics on chondrocyte viability is warranted to optimize osteochondral allograft storage.[43,44]

Growth Factors and Hormones

Opinions regarding the inclusion of growth factors and hormones varies in literature, and different combinations were described within individual company protocol. While JRF Ortho and RTI Surgical both maintain privacy of storage media contents. Although free of artificial growth factors, LifeNet Health's storage media is noted to contain recombinant human insulin.[47] Though opinions vary, the inclusion of insulin has been supported by some studies demonstrating that the addition of recombinant insulin or insulin growth factor-1 (IGF-1) may improve chondrocyte viability.[29,48,49] Further study regarding efficacy and safety of adding growth factors to storage media is needed to create the best practice model for osteochondral allograft preservation and storage.

Storage Temperature

In accordance with standard tissue bank practice, all companies studied, with the exception of MTF, maintain grafts in temperatures between 1 and 10°C.[47,50] Many banks store grafts in a refrigerated environment due to concerns regarding infection if stored at higher temperatures.[4] However, according to a study by Pallante et al, preservation of fresh osteochondral allografts at 4°C leads to a significant reduction in the viability of the cartilage by 28 days.[8] Furthermore, it was found that osteochondral allografts stored at 37°C had improved chondrocyte viability after 28 days when compared with 4°C, with the largest improvement in viability seen on the articular surface of the cartilage.[8] It is important to note that variations in storage temperature did not significantly affect cartilage thickness, collagen content, or glycosaminoglycan content. Storing tissue at 37°C maintains chondrocyte ability to carry out glycosaminoglycan metabolism, maintain extracellular matrix, and sustain homeostasis, improving viability.[4,8,50]

The MOPS process utilized by MTF stores grafts at 25°C.[3,13–15,18,38,50,51] Following initial testing in a preclinical canine model, Stoker et al asserted that storage of osteochondral allografts using a proprietary technique at 25°C preserved the grafts at higher levels while not increasing the potential risk for microbial contamination when compared with grafts stored at 4°C using the same proprietary technique.[3] Furthermore, additional study revealed that storage at 25°C resulted in lower inflammatory and degradative responses to rewarming for transplantation when compared with grafts stored at lower temperatures.[51] While further investigation is required, the impact of temperature appears to be a major factor in viable chondrocyte density. To improve chondrocyte viability and graft success, additional research on optimal temperature is indicated and may guide future federal recommendations.[4,15,50,51]

Expiration

The most distinct difference between the four leading companies is the expiration date for each graft. The common standard for allograft viable chondrocyte density is >70% at 28 days of storage.[14,15,29,30,33] Below 70% viability, it is thought that physical integrity of the graft is unable to be sustained, and the graft is therefore expired.[14,15,17] LifeNet Health and RTI Surgical report a maximum of 45 days prior to tissue expiration. In contrast, JRF Ortho reports a maximum of 28 days. Stoker et al determined that the proprietary MOPS technology of MTF Biologics maintains chondrocyte quality and viability at least 56 days.[3] Though each tissue bank follows AATB guidelines, proprietary approaches introduce heterogeneity to tissue preparation. A recent large clinical series (194 patients) found that success of grafting of large defects correlated to the tissue preservation technique, with a significant improvement in success with the MTF MOPS^{M} grafts compared with grafts from other tissue banks accredited by the AATB.[52] The variation in expiration dates may be due to differences in the storage media and storage

temperatures used by each company, but further research is indicated to determine causation.

Potential for increased osteochondral allograft viability exists in the field of orthopaedics. Currently, it is reported that between 20 and 29% of grafts are discarded due to expiration.[23] If the length of time that cartilage tissue viability can be maintained during storage can be extended, the number of allografts available and the amount of time for tissue processing and surgery can increase. Moreover, access to quality grafts may increase for surgeons who currently may not have easy access to fresh osteochondral allografts.[24,25] Determining the optimal approach of storage media composition, temperature, antibiotic use, and aseptic processing may lead to a generalized protocol that improves allograft longevity.

Limitations

Limitations exist of this investigation and others that examine the processing and preservation of grafts. It is important to note that reported expiration dates are primarily from internal research of each tissue bank rather than independent studies that are published in peer-reviewed literature. Stoker et al found major decrement in the viability of grafts from some AATB accredited tissue banks compared with what the expiration date of the graft would have suggested.[13] Additionally, there is industry-wide heterogeneity in graft preservation techniques which are often proprietary with little publicly available information. Research is needed to confirm the reported correlation between storage method and clinical outcomes. Furthermore, investigation is warranted to study the effects of the various aseptic processing methodology on fresh osteochondral allografts, particularly in determining the impact that these treatments have on the viability of chondrocytes, the transmission rate of infectious diseases, and the long-term success rate of the graft itself. Patients may benefit from evidence-based guidelines that ensure maximal graft preservation with minimal risk.

Conclusion

While companies share similarities regarding donor screening, aseptic processing, and testing for microorganisms, variation exists in choice of storage media, storage temperature, and antibiotics used, ultimately yielding different expiration dates of fresh osteochondral allografts. Evidence suggests that the most effective method of extending chondrocyte viability and the longevity of these allografts may be through maintaining cartilage integrity through delicate tissue handling, adding hormones and growth factors like IGF-1 to serum-free nutrient media, and storing these grafts closer to physiologic temperatures. Standardized, evidence-based processing, and storage methods may ultimately improve access to, and viability of, fresh osteochondral allografts.

Conflict of Interest
None declared.

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