X-VIVO™ Serum-Free Media Use in the Storage of Viable Osteochondral Allografts

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Introduction

Damaged articular cartilage within weight-bearing sites is associated with pain, loss of mobility, and reduced quality of life for many people. Fresh, non-frozen viable osteochondral allografts (OCAs) are the current gold standard for the restoration of a wide spectrum of chondral and osteochondral pathologies [1]. The overall number of these procedures performed in the United States nearly tripled from 2005 to 2011 [2]. Viability and structural integrity are dependent on the allograft preparation process, i.e. recovery, processing, and storage. Such changes have been shown to directly affect the biological function and thereby the clinical outcome of osteochondral allograft procedures [3]. Regardless of processing methods, almost all osteochondral allografts are typically implanted within 60 days after recovery because viability of chondrocytes has been shown to decrease over time [4].

Unfortunately, extending the potential storage time for viable osteochondral allografts is challenging. The use of standard fresh, non-frozen osteochondral allografts presents certain limitations. Currently, fresh osteochondral allografts have a very limited shelf life between release and expiration due to the fact that these grafts include viable chondrocytes which must be kept alive during storage. Current graft expiration times are generally fewer than 60 days, including the quarantine period of approximately two weeks during which the grafts are tested for bacterial, fungal, and viral pathogens. Since surgery cannot occur until a suitable match is found, this short shelf life contributes to the logistical complexity involved with using fresh allografts. In light of these challenges, several storage media have been developed to address the maintenance of graft integrity, viability, and durability over an extended amount of time. The advantages and disadvantages of these solutions will be discussed here and are reviewed in Table 1.

Lactated Ringer's Solution

The traditional storage system for fresh osteochondral allografts is Lactated Ringer's solution which is an electrolyte-replacing media commonly used for the replenishment of water and nutrients into the bloodstream [5]. For well over half a century, Lactated Ringer's has been used clinically as an electrolyte-replacing media. Its contents have remained relatively unchanged since the early 1980's and are characterized by a mixture of sodium chloride, sodium lactate, potassium chloride, and calcium chloride in water [6]. Lactated Ringer's has been used as a storage system for fresh osteochondral allografts, but has been shown to maintain biochemical and biomechanical integrity of allografts for only a short amount of time, resulting in a limited shelf-life for OCAs [7].

Fetal Bovine Serum (FBS)

FBS, a blood-derived cell culture supplement, is a frequent component of OCA storage media. Although it has successfully been used in cell culture for over fifty years, its use in tissue storage has been called into question [8]. A main concern regarding FBS, other than known ethical issues associated with procurement methods and certain religious beliefs that oppose the use of bovine-derived products, is its inability to provide a fully defined media component due to its inherent makeup. FBS is a serum derived from bovine fetuses and the standard method of collection leads to significant lot-to-lot variation, related to differences from one animal to another [9]. Consequentially, the undefined nature and variable composition of serum as part of serum-containing media has prompted the movement toward serum-free media options that can provide a more consistent and defined medium for use in cell culture applications and tissue storage.

X-VIVO™ Media

The advantage of non-serum-based media for osteochondral allograft storage is that it contains defined human components, avoiding the potential ethical and cultural issues and lot to lot variability associated with animal derived components. The BioWhittaker® X-VIVO™ media system (Lonza Wakersville, Inc.) is a complete, serum-free basal medium supplemented with clinical grade human albumin, pasteurized human transferrin, and recombinant human insulin. The clinical quality of these components combined with defined quantities give X-VIVO™ the same growth factor advantages of serum-containing media without the safety or variability concerns. Additionally, defined serum-free X-VIVO™ media are manufactured under current good manufacturing practices (cGMPs) and are listed with the FDA in a product Master File (21CFR864.2220) to monitor and ensure robust quality control procedures. X-VIVO™ has been used extensively in the culture and maintenance of stem cell and progenitor cell populations, as well as the storage and maintenance medium of fresh tissues for transplantation, including vein [10-12], skin [13], tendon [14], and osteochondral allografts [15-17]. And, perhaps most important to the orthopedic surgeon, it appears to effectively maintain allograft integrity and viability in post-operative stages after hypothermic storage [18].

X-VIVO™ Characterization

One of the most apparent biochemical changes affecting cartilaginous tissues during hypothermic storage is the gradual loss of glycosaminoglycans (GAGs) resulting in weakened biomechanical tissue characteristics over time. Below is a comparison of biomechanical and biochemical changes in refrigerated tissues stored in either Lactated Ringers solution or serum-free X-VIVO™.

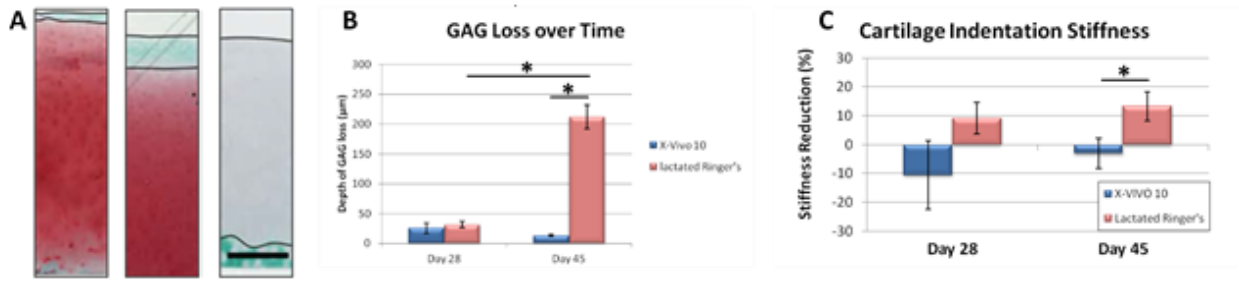

Figure 1: Biochemical and Biomechanical Changes in Hypothermally Stored Fresh Porcine Non-Frozen Osteochondral Allografts. A) Exemplary observation of glycosaminoglycan (GAG) loss from articular cartilage over time; Safranin O staining on formalin-fixed tissues, GAGs appear in the red while collagen appears light green; scale bar=0.5mm, n=6, B) GAG loss depth of porcine osteochondral segments stored in Lactated Ringer's or serum-free X-VIVO™ for 28 or 45 days at 4 °C (*$p < 0.05$), C) Relative reduction in cartilage stiffness of porcine osteochondral segments compared to fresh segments at day 0 following storage in Lactated Ringer's solution or serum-free X-VIVO™ for 28 or 45 days at 4 °C (*$p < 0.05$, n=6). Data on file at LifeNet Health.

The biochemical and biomechanical characteristics of osteochondral allografts stored in Lactated Ringer's solution or serum-free X-VIVO™ were evaluated to determine whether these media supported allografts during extended periods of hypothermic storage. Osteochondral plugs were isolated from porcine condyles and stored for up to 45 days at 4˚C in Lactated Ringer's solution or serum-free X-VIVO™. The degree of GAG loss over time was determined on Safranin O stained histologic sections at day 0, 28 and 45. The tissues' biomechanical characteristics were evaluated by indentation testing and reported as change to fresh tissue. Osteochondral allografts showed a dramatic loss of GAGs by 45 days when stored in Lactated Ringer's solution. In contrast, allografts stored in serum-free X-VIVO™ did not show a significant loss of GAGs (Figure 1A+B), indicating retention of key structural elements. Serum-free X-VIVO™ also supported the maintenance of biomechanical characteristics over 45 days (Figure 1C). Allografts stored in Lactated Ringer's solution showed a significantly reduced level of indentation stiffness by day 45. Also, Csonge et al. showed that storage of osteochondral allografts in serum-free medium might be possible for up to 60 days [19]. Additionally, recent studies have indicated that serum-free media, including X- VIVO™, may also be used for the long-term storage of fresh, vitrified osteochondral allografts and allografts at physiologic temperatures [17, 18, 20, 21].

Summary

Effective storage of fresh osteochondral allografts has proven to be challenging, particularly over an extended period of time. The integrity, viability, and durability of the allografts is dependent on the maintenance of biochemical and biomechanical properties of the tissue, which are directly affected by the processing and storage of the allograft. The chief purpose of solutions used in processing and storage of allografts is to maintain the natural structure and bio-processes of the human tissue for transplantation, resulting in better post-operative outcomes. X-VIVO™ provides a serum-free, nutrient-rich medium that not only delivers a more consistent medium but also maintains viability of osteochondral allografts over an extended period of time. These characteristics combine to provide a safer, more consistent and reliable solution than historically used storage media.

Table 1: Comparison of common storage media options for OCAs

	X-VIVO™	Lactated Ringer's Solution	Fetal Bovine Serum (FBS)
Biological Source	Human-based, serum-free medium	Serum-free solution	Bovine-based serum
Media Components	Clinical grade human albumin, pasteurized human transferrin, and recombinant human insulin	Mixture of sodium chloride, sodium lactate, potassium chloride, and calcium chloride in water	Fetal bovine blood with growth factors
Package	Clear, translucent liquid	Clear, translucent liquid	Colored liquid
Primary Use	Storage media for human cell and tissue culture	Fluid and electrolyte replenishment	Storage media for different cell culture applications
Produced under cGMPs	Yes	Yes	Yes
FDA Recognition	Yes, listed in a product Master File (21CFR864.2220)	Yes, approved new drug application (018681)	No
Advantages	Human tissue compatible, maintains allograft integrity and viability for extended period of time, no safety or variability concerns	Sterile solution, consistent content, traditional use of solution in cell cultures with no safety concerns	Contains growth factors and low content of antibodies, maintains tissue integrity and viability during prolonged storage
Limitations	More expensive than other commonly used storage media	Maintains allograft integrity and viability for a limited amount of time	Inherent batch variation, safety concerns of xenographic components, ethical issues

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