LifeNet Health Fresh Storage Media

ORIGINAL RESEARCH ARTICLE

Introduction

For over 30 years, osteochondral allografts (OCAs) have been successfully used to treat articular cartilage defects by improving function and alleviating pain.[1,2] Although osteochondral grafts have demonstrated clinical efficacy, their use is limited by availability due to logistical issues involving procurement, processing, and storage.[1,3] Following procurement, osteochondral grafts must be stored for a minimum of 14 days to allow time for results from bacterial, fungal, and viral tests to be completed. However, prolonged storage time has been shown to decrease chondrocyte viability, and thus negatively impacting the maintenance of the biomechanical and biochemical properties of the osteochondral allografts.[1,3] The reduction of these properties can impact the long-term success of osteochondral transplantation.[1,4]

Based on these limitations, various storage solutions have been developed in an attempt to maintain graft viability, integrity, and durability over an extended period of time. Currently, LifeNet Health stores osteochondral allografts in X-VIVO™ 10 (Lonza Group Ltd.), a chemically defined, serum and phenol-red free medium. Recently, the LifeNet Health Research & Development team identified an alternative media solution based on its similar properties to X-VIVO 10.

Osteochondral Allograft Storage Solutions

X-VIVO

The BioWhittaker® X-VIVO media system (Lonza Wakersville, Inc.), is a complete, serum-free media system designed for the culture of hematopoietic cells and stem cell populations. This media system, which includes X-VIVO 10 and X-VIVO 15, is chemically defined and is supplemented with clinical grade human albumin, pasteurized human transferrin and recombinant human insulin. The use of human derived, defined components avoids potential ethical or cultural issues while reducing lot to lot variability seen with animal derived components. X-VIVO has been used for the storage and maintenance medium of fresh tissues for transplantation, including hypothermic storage of osteochondral allografts.[5,6]

Dulbecco's Modified Eagle Media (DMEM)

One of the most common solutions for osteochondral allograft storage is "minimal" cell culture media, such as Dulbecco's Modified Eagle's Medium (DMEM).[4] This widely used basal medium contains high glucose and L-glutamine, but is phenol-red and albumin free. DMEM is readily available from multiple manufactures due to its nonproprietary formulation. DMEM has been successfully used in the storage of musculoskeletal allografts, including osteochondral tissue.[7,10]

Characterization

The biochemical and biomechanical characteristics of osteochondral allografts stored in X-VIVO 10, X-VIVO 15, hybrigro SF, or DMEM were evaluated to determine whether these media supported allografts during extended periods of hypothermic storage. Hybrigro is a complete, animal serum-free, defined medium formulated for cell culture and served as an additional comparison in this study. Osteochondral plugs were isolated from porcine condyles and stored for up to 45 days at >0-10°C in X-VIVO 10, X-VIVO 15, hybrigro SF, or DMEM. The degree of glycosaminoglycan (GAG) loss over time was determined by Safranin O stained histologic sections at day 0, 28 and 45. GAG concentrations were also evaluated due to the role of GAGs in osteochondral tissue shock-absorbing properties.[11,12] Changes in GAG content can provide additional evidence to potentially explain biomechanical changes after tissue

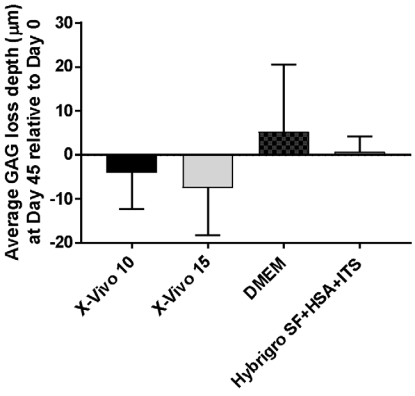

Figure 1: Average reduction in cartilage stiffness of porcine osteochondral segments following storage in X-VIVO 10, X-VIVO 15, DMEM, and hybrigro SF at 0-10 °C on day 28 and 45 relative to day 0.

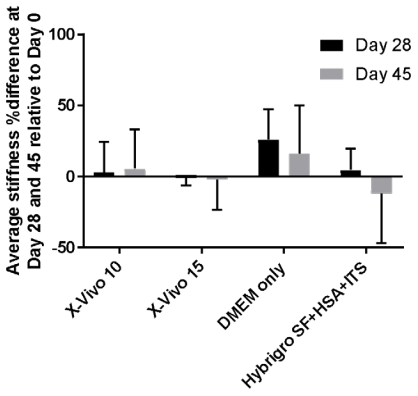

Figure 2: Average GAG loss depth of porcine osteochondral segments following storage in X-VIVO 10, X-VIVO 15, DMEM, and hybrigro SF at 0-10°C on day 45 relative to day 0.

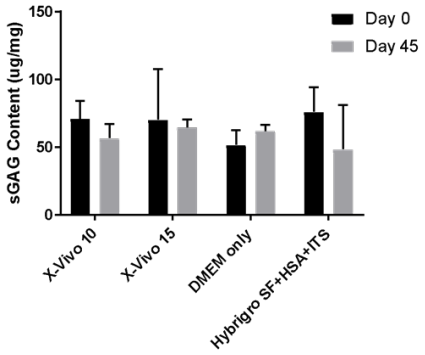

Figure 3: Cartilage sGAG content by quantified by Blyscan assay following storage in X-VIVO 10, X-VIVO 15, DMEM, and hybrigro SF at 0-10°C on day 0 and 45.

storage. Histologically, osteochondral grafts showed no significant loss of GAG distribution, irrespective of the media solution. Furthermore, there were no significant changes in the tissues' GAG content irrespective of the media for up to 45 days. The biomechanical properties of the tissue were evaluated via cartilage indentation stiffness following 0, 28, and 45 days in storage and reported as a change relative to fresh tissue. Osteochondral grafts stored in X-VIVO 10, X-VIVO 15, and DMEM retained their indentation stiffness for up to 45 days indicating retention of biomechanical graft stability. However, grafts stored in hybrigro SF showed a reduction in indentation stiffness, although not statistically significant.

Summary/Conclusion

Identifying an effective storage solution for osteochondral allografts has proven to be challenging. It is important that storage solutions retain the biomechanical and biochemical properties of osteochondral allografts. Several studies have suggested that minimal cell culture and serum-free media are effective solutions for osteochondral allograft storage. Previous pre-clinical bench top studies have reported that osteochondral grafts stored at 4°C in DMEM demonstrated greater chondrocyte viability, and maintained glycosaminoglycan (GAG) content and cartilage load bearing functions when compared to other storage methods.[10,13] Garrity et al. showed that canine OCA grafts stored in DMEM demonstrated optimal maintenance of chondrocyte viability, ECM biochemical composition GAG, and biomechanical properties. 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, addressing some of the storage limitations of fresh allografts.[5,6,8] Based on these studies and the data presented above, both DMEM and X-VIVO (10 and 15) are suitable media solutions for the hypothermic storage of fresh osteochondral allografts for up to 45 days.

LifeNet Health Fresh Storage Media

Cell culture media utilized by LifeNet Health

	X-VIVO™	DMEM
Biological Source	Human-based, serum-free medium	Basic serum-free medium
Media Components	Clinical grade human albumin, pasteurized human transferrin, and recombinant human insulin	High glucose, L-glutamine, HEPES
Package	Clear, translucent liquid	Clear, translucent liquid
Primary Use	Storage media for human cell and tissue culture	Storage media for cell and tissue culture
Produced under cGMPs	Yes	Yes
FDA Recognition	21CFR864.2220	21CFR876.5885
Advantages	Human tissue compatible, maintains allograft integrity and viability for extended period of time, no safety or variability concerns	Human tissue compatible, maintains allograft integrity and viability for extended period of time, no safety or variability concerns
Limitations	Proprietary formulation with single manufacturer	None identified

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