ALLOSOURCE

THE BIOLOGICAL PROPERTIES OF ALLOMEND® ACELLULAR DERMAL MATRIX: GROWTH FACTOR STUDY

Ryan Delaney, MS, Reginald Stilwell, BS, CTBS
AlloSource®, Centennial, CO

The Biological Properties of AlloMend® Acellular Dermal Matrix: Growth Factor Study

Ryan Delaney, MS, Reginald Stilwell, BS, CTBS

AlloSource®, Centennial, CO

ABSTRACT

Acellular dermal matrices can successfully be used to replace or repair integumental soft tissues compromised by disease, injury or surgical procedures. A consideration when utilizing this biomaterial *in vivo* is whether it retains native proteins and growth factors after the decellularization process, which aid in structuring and signaling cellular activity. This is critical in a variety of applications—for example when the graft is anchored to soft tissue during plastic surgery procedures and hernia repairs, or when the graft is anchored to bone in rotator cuff surgeries or other sports medicine procedures.

In this study by AlloSource researchers, an enzyme-linked immunosorbent assay (ELISA) was used to test AlloMend® Acellular Dermal Matrix (ADM) samples for the presence of four primary growth proteins.

Introduction

AlloMend ADM (**Fig. 1**) (AlloSource®, Centennial, CO) is produced through a proprietary process of cleaning and rinsing donated human dermal tissue. The process does not require the use of detergents or enzymes, thereby mitigating the possibility of harmful residuals in the tissue.

The tissue undergoes a terminal e-beam sterilization process, resulting in a 10^{-6} Sterility Assurance Level (SAL), meeting the same stringent sterility levels required by the U.S. Food and Drug Administration for implantable biomedical devices.

Because of its terminal sterilization, AlloMend ADM can be stored at room temperature for up to two years. Unlike some acellular dermal matrices, AlloMend ADM is pre-hydrated and ready for immediate use without requiring a lengthy rehydration period. In addition, due to its handling characteristics, the tissue can be easily placed in a variety of anatomical areas, including soft tissue and tendon-to-bone environments.

The AlloMend ADM proprietary process results in a three-dimensional, collagen-rich, biocompatible, non-cytotoxic matrix that retains its biomechanical properties. The resulting properties help ensure the graft will be readily accepted by the recipient through subsequent revascularization and cell repopulation. The mild decellularization process removes cellular debris (including DNA and RNA) and certain proteins and antigens, while retaining native growth factors normally present in tissues.

The purpose of this study was to qualitatively investigate the presence of growth factors within AlloMend ADM. The research utilized ELISA testing for the presence of:

- Fibroblast Growth Factor (bFGF)
- Platelet Derived Growth Factor (PDGFbb)
- Transforming Growth Factor (TGFb)
- Bone Morphogenic Protein 2 (BMP2)

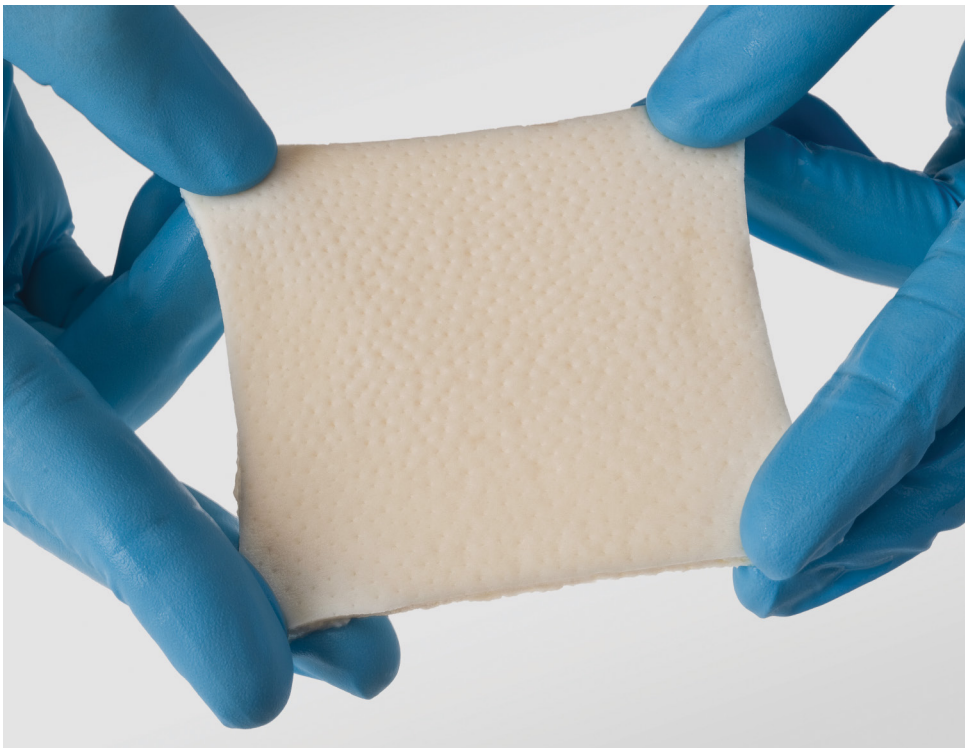

Figure 1. AlloMend ADM tissue.

Growth Factors

The presence of growth factors in an implanted ADM can stimulate healing and revascularization, as well as inhibit scarring[1,2] in a variety of procedures, including tendon-to-bone repair and soft tissue repair.

Generally, tendon-to-bone repairs (e.g., rotator cuff surgeries) may have a high rate of failure due to insufficient tissue incorporation. However, the presence of growth factors within the graft can help guide the activity of recipient cells, leading to better incorporation and recovery. In soft tissue procedures, the use of a graft containing growth factors helps promote scar-free healing and proper graft incorporation without allogeneic rejection.

In this study, AlloSource researchers tested for the presence of four specific growth factors widely known to contribute to a healing response following tissue injury:

- **Fibroblast Growth Factor (bFGF)**. bFGF is essential for coordinated cell activity. It acts as a chemotactic and proliferative marker for fibroblasts and endothelial cells, and a guide for the process of angiogenesis and the development of new membrane layers.[1] Applying bFGF to a full-thickness skin graft is reported to improve clinical outcomes by decreasing the incidence of tissue necrosis.[3] The application of bFGF has also been shown to be effective in mediating scar-free healing[4] and spurring new vessel growth in the healing of surgical wounds,[4,5] both of which are essential for soft tissue repair. In tendon repair surgeries, bFGF augmentation has been found to lead to better tendon-to-bone incorporation and strength.[6,7] This is especially apparent when acellular dermal matrices are used for the repair.[8]
- **Platelet Derived Growth Factor (PDGFbb)**. PDGFbb helps coordinate a number of steps in the healing process that are especially important for soft tissue repair. It attracts cells to the area of the injury, guides the production of collagen and elastin, and induces cell differentiation. It also directs the signaling cascade to begin the process of reverse remodeling, coordinating the reduction in scarring.[9] PDGFbb was the first growth factor to be approved by the FDA for direct application and has proved to be effective in wound healing.[10,11] In tendon-to-bone procedures, PDGFbb improves the mechanical strength[12] and the histologically-evident healing of the repair.[13]

- **Transforming Growth Factor (TGFb)**. TGFb is an important signaling factor in all stages of the healing process, guiding the proliferation and extracellular protein production of fibroblasts. It also helps prevent over-proliferation and limits the inflammatory response of keratinocytes.[14,15] Through the control of the responses of these cells, TGFb mediates scar-free wound healing.[16] Conversely, TGFb-deficient mice have been shown to have impaired healing processes.[17] TGFb works with other growth factors to coordinate tendon-to-bone incorporation and its presence can reduce the incidence of failure in these procedures.[18,19] Studies have also shown that TGFb acts as an important guide for the long term acceptance of allografts.[20]
- **Bone Morphogenic Protein 2 (BMP2)**. BMP2 is a potent osteogenic protein that directs the development and healing of bone and has been proven to affect tendon-type cells, directing differentiation and matrix production.[21] This signaling enhances tendon-to-bone healing[22] by inducing the production of new cartilage at the insertion site.[23] BMP2 is also a potent chemotactic factor for stem cells and serves to drive adipogenesis of such cells.[24] This would prove useful for an allograft used in soft tissue reconstructive work such as breast reconstruction.

Methods and Process

TISSUE HOMOGENIZATION

The study utilized AlloMend® ADM tissues from four donors. The grafts were cut into 1.0 mm square cubes and homogenized in Raybiotech Lysis buffer. Thermo Scientific Halt Protease Inhibitor cocktail was added. The process utilized a VWR VDI 25 Homogenizer and the homogenate was kept on ice for 30 minutes and sonicated for two minutes. The homogenates were centrifuged at 12,000 G for 10 minutes and the supernatant was collected and stored at -80˚ C until use.

ELISA

The ELISA investigative technique utilizes the activity of immunosorbent antibodies to measure the specific protein content of a homogenate. The known concentration of a protein is used as a standard curve to measure concentrations in a sample. The tissue homogenate produced earlier was analyzed using ELISA sandwich kits (RayBiotech, Atlanta, GA) to detect BMP2, bFGF and PDGFbb as illustrated in **Figure 2**. An additional ELISA kit (Enzo Biochem, Farmington, NY) was used to detect TGFb. Researchers utilized the kit manufacturers' recommended procedures as well as the provided reagents. All washes were performed with a BioTek 405 Select TS. Researchers gathered colorometric readings immediately at 450 nm with a BioTek Synergy H1 Hybrid Reader with curve normalization at 560 nm. Finally, standard reader software was used to perform the curve fitting.

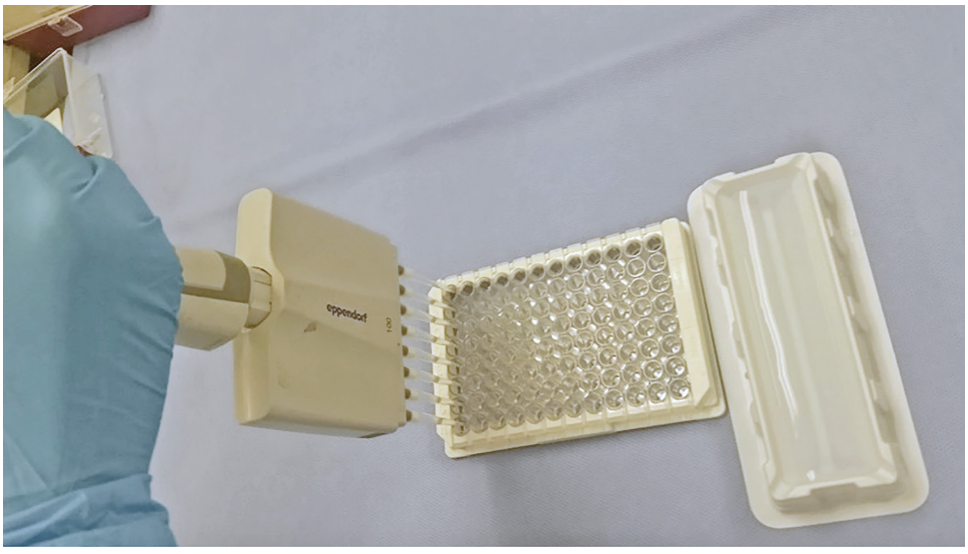

Figure 2. Pipetting process during ELISA analysis of AlloMend ADM.

Results

The ELISA testing revealed the presence of BMP2, PDGFbb and bFGF in all four of the grafts. TGFb was found in three of the four grafts.

Implications

The presence of growth factors in AlloMend® ADM indicates the tissue offers an advantage over synthetic grafts, which lack inherent growth and signaling capacity to aid healing. More and more researchers are recognizing the value of growth factors in a healing environment and these proteins are even becoming commercially available for direct application in wound healing.[10]

As described above, the role each of these proteins plays in a range of procedures is summarized in **Table 1**.

	Tendon Reconstruction Procedures	Soft Tissue Reconstruction	Total Surgical Site Benefits
bFGF	Prevents necrosis Assists in angiogenesis Increases attachment strength	Assists in angiogenesis basal membrane formation Prevents necrosis	Prevents necrosis Promotes scar-free healing Promotes surgical wound closure
PDGFbb	Reduces inflammation Serves as a chemotactic guide to graft for host cells Improves tendon strength	Reduces inflammation Serves as a chemotactic guide to graft for host cells	Promotes scar-free healing
TGFb	Assists in the production of new matrix in graft Promotes allograft acceptance Increases tendon-to-bone incorporation	Assists in the production of new matrix in graft Promotes allograft acceptance	Prevents inflammation Coordinates the wound healing process.
BMP2	Serves as a chemotactic guide to graft for host cells Improve tendon-to-bone healing Signals tenocytes to form new tissue	Serves as a chemotactic guide to graft for host cells Induces adipogenesis in soft tissue spaces	Promotes stem cell differentiation

Table 1. Significance of growth factors in allograft procedures.

Conclusion

The presence of the tested growth factors in AlloMend ADM makes this an ideal acellular graft tissue for numerous types of reconstructive surgeries, including soft tissue repair (e.g., breast reconstruction procedures) and sports medicine (e.g., rotator cuff repairs or other tendon-to-bone attachments). Collectively, these growth factors coordinate cellular incorporation, proper bone attachment and proper differentiation of cells. At the same time, they prevent untoward inflammation and necrosis at the implantation site.

References

1. Ching YH, et al. The use of growth factors and other humoral agents to accelerate and enhance burn wound healing. Eplasty. 2011;11.
2. Wang H, et al. Acceleration of skin graft healing by growth factors. Burns. 1996;22(1):10-14.
3. Matsumine H. Treatment of skin avulsion injuries with basic fibroblast growth factor. Plastic and Reconstructive Surgery Global Open. 2015;3(4).
4. Nissen NN, et al. Basic fibroblast growth factor mediates angiogenic activity in early surgical wounds. Surgery. 1996;119(4):457-465.
5. Akita S, Akino K, Hirano A. Basic fibroblast growth factor in scarless wound healing. Advances in Wound Care. 2013;2(2):44-49.
6. Zhao S, et al. Biological augmentation of rotator cuff repair using bFGF-loaded electrospun poly(lactide-co-glycolide) fibrous membranes. Int J Nanomedicine. 2014;9:2373-85.
7. Ide J, et al. The effect of a local application of fibroblast growth factor-2 on tendon-to-bone remodeling in rats with acute injury and repair of the supraspinatus tendon. J Shoulder Elbow Surg. 2009;18(3):391-8.
8. Ide J, et al. The effects of fibroblast growth factor-2 on rotator cuff reconstruction with acellular dermal matrix grafts. Arthroscopy. 2009;25(6):608-16.
9. Heldin CH, Westermark B. Mechanism of action and *in vivo* role of platelet-derived growth factor. Physiological Reviews. 1999;79(4):1283-1316.
10. Meyer-Ingold W. Wound therapy: growth factors as agents to promote healing. Trends in Biotechnology. 1993;11(9):387-392.
11. Pierce GF, et al. Tissue repair processes in healing chronic pressure ulcers treated with recombinant platelet-derived growth factor BB. The American Journal of Pathology. 1994;145(6):1399.
12. Hee CK, et al. Augmentation of a rotator cuff suture repair using rhPDGF-BB and a type I bovine collagen matrix in an ovine model. Am J Sports Med. 2011;39(8):1630-9.
13. Uggen C, et al. The effect of recombinant human platelet-derived growth factor BB-coated sutures on rotator cuff healing in a sheep model. Arthroscopy. 2010;26(11):1456-62.
14. Pakyari M, et al. Critical role of transforming growth factor beta in different phases of wound healing. Advances in Wound Care. 2013;2(5):215-224.
15. Coffey R, et al. Selective inhibition of growth-related gene expression in murine keratinocytes by transforming growth factor beta. Molecular and Cellular Biology. 1988;8(8):3088-3093.
16. Penn JW, Grobbelaar AO, Rolfe KJ. The role of the TGF-beta family in wound healing, burns and scarring: a review. Int J Burns Trauma. 2012;2(1):18-28.
17. Brown RL, et al. Wound healing in the transforming growth factor beta 1– deficient mouse. Wound Repair and Regeneration. 1995;3(1):25-36.
18. Bedi A, et al. Cytokines in rotator cuff degeneration and repair. J Shoulder Elbow Surg. 2012;21(2):218-27.
19. Manning CN, et al. Sustained delivery of transforming growth factor beta three enhances tendon-to-bone healing in a rat model. J Orthop Res. 2011;29(7):1099-105.
20. Daley SR, et al. A key role for TGF-beta signaling to T cells in the long-term acceptance of allografts. The Journal of Immunology. 2007;179(6):3648-3654.
21. Pauly S, et al. BMP-2 and BMP-7 affect human rotator cuff tendon cells *in vitro*. J Shoulder Elbow Surg. 2012;21(4):464-73.
22. Chen B, et al. Enhancement of tendon-to-bone healing after anterior cruciate ligament reconstruction using bone marrow-derived mesenchymal stem cells genetically modified with bFGF/BMP2. Sci Rep. 2016;6:25940.
23. Chen CH, et al. Enhancement of rotator cuff tendon-bone healing with injectable periosteum progenitor cells-BMP-2 hydrogel *in vivo*. Knee Surg Sports Traumatol Arthrosc. 2011;19(9):1597-607.
24. Sottile V, Seuwen K. Bone morphogenetic protein-2 stimulates adipogenic differentiation of mesenchymal precursor cells in synergy with BRL 49653 (rosiglitazone). FEBS Lett. 2000;475(3):201-4.

6278 S Troy Cir
Centennial, CO 80111

MAIN 720. 873. 0213
TOLL FREE 800. 557. 3587
FAX 720. 873. 0212

allosource.org

M8S0115.001 – 11/2016