

COMPARATIVE TESTING OF COLLAGEN BASED IMPLANTS: INTEGRITY AFTER EXPOSURE TO REMODELING ENZYMES

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INTRODUCTION

A variety of collagen based implants are currently available for the reinforcement of repaired soft tissue injuries. Once implanted, the collagen in these implants undergoes a process of organized removal or digestion, mediated by a variety of remodeling proteolytic enzymes belonging to the metalloproteinase family. The digestion phase is followed by restoration with newly synthesized host collagen.¹ Both implant derived and host derived factors influence this remodeling cascade, as tissue processing and sterilization methods greatly affect the total amount of collagen from both the implant and host that will ultimately be present in a repaired soft tissue defect.² Processes that structurally alter the collagen architecture of the implant may conceivably result in accelerated removal of donor collagen, potentially resulting in scar tissue formation, sub-optimal repairs, and possible failure of the implant function. Consequently, crosslinking of collagen-based implants is becoming a preferred choice for preparation given its ability to slow removal of donor collagen after implantation.

Collagen based implants differ according to the origin of raw materials and undergo different processing (e.g. crosslinking) and sterilization methods to render them safe and effective for clinical use. These methods include the use of chemicals, dry heat and gamma irradiation. Synovis Orthopedic and Woundcare, Inc. uses a proprietary chemical technology to crosslink and sterilize its highly organized, Type 1 collagen implant, the OrthADAPT® Bioimplant, which is derived from decellularized equine pericardium.³

Tests were performed in an enzymatic environment to compare the collagen stability of the OrthADAPT® Bioimplant to several commercially available collagen based implants. Specifically, the stability of different collagen-based implants was measured through two *in vitro* assays. Each implant was exposed to two enzymes, pronase and collagenase. These enzymes participate in the collagen turnover process during both normal remodeling and pathological states. The susceptibility of each tissue scaffold to digestion by these enzymes was measured as a means to predict durability of each scaffold to biological healing factors after implantation.

MATERIALS & METHODS

The tissue scaffolds listed in Table 1 were tested in the enzyme digestion assay using both pronase and collagenase. The tissue origin, crosslinking agents and sterilization methods of all the implants are detailed and these factors may be key contributors to the stability of processed tissues. Unprocessed equine pericardium was used as a control for the OrthADAPT® Bioimplant. The average mass of each test sample used in the enzyme digestion assay is listed

in Table 1. The average mass at time 0 was normalized according to the thickness of each implant.

Table 1. Mass Comparison of Materials Tested

Product Name	Source	Crosslinking Agent	Sterilization	Average Mass per cm² (mg)
OrthADAPT [®] Bioimplant	Equine Pericardium	EDC+	EDC+	15.95
GraftJacket [®]	Human Dermis	None	None	43.41
Pelvicol [™]	Procine Dermis	HMDIC	Gamma	44.91
TissueMend [®]	Bovine Dermis	None	EtO	27.86
CuffPatch [®]	Porcine SIS	EDC	Gamma	17.48

Enzyme solutions were prepared in a HEPES and NaCl buffer containing calcium chloride at the following concentrations: Collagenase (Sigma #C0773), 100 units/ml and Pronase (Sigma #P5147), 4.9 units/ml.

A minimum of five 1 cm x 1 cm pieces of each test tissue were cut under aseptic conditions. All samples were prepared according to the manufacturer's Instructions for Use prior to air drying overnight at room temperature. All samples were weighed before enzyme exposure. Test samples were incubated with 3 ml enzyme solution per cm² for 48 hours at 37°C for collagenase digestion, or for 24 hours at 50°C for pronase digestion. At the end of the indicated incubation periods, samples were removed from the incubator, blotted dry and left to air dry for no less than 24 hours at room temperature. Final mass was measured and the percentage of weight retained was calculated as a simple ratio.

RESULTS

The percentage of undigested tissue after enzyme exposure is shown in Figure 1 along with the respective standard error (as percent of retained tissue). TissueMend[®] samples were not tested in the pronase assay.

Although most other test samples had a larger mass per cm² than the OrthADAPT[®] Bioimplant samples, they were nearly or completely digested. Figure 1 shows the percentage of retained tissue for each test sample after the *in vitro* assays were performed. The OrthADAPT[®] Bioimplant demonstrated the greatest enzyme resistance among all of the tissue samples tested.

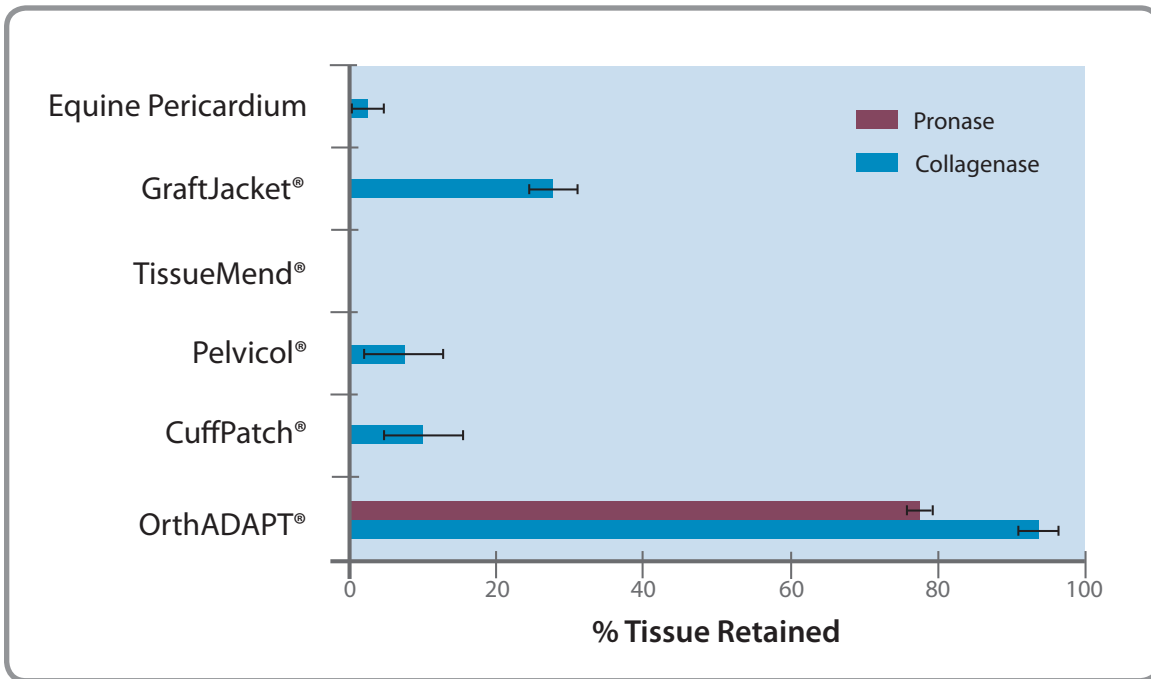


Figure 1. Susceptibility of Enzymatic Degradation

DISCUSSION

The results of this study demonstrate that the OrthADAPT® Bioimplant is significantly more resistant to enzymatic degradation than all other products tested. Collagen based implants that were unable to endure this *in vitro* test included those that were crosslinked by chemical means and those labeled as “permanent grafts”, indicating poor stability of these tissues in an *in vitro* remodeling milieu and highlighting the effects of destructive sterilization methods. Even in the presence of a non-specific protease, such as pronase, the OrthADAPT® Bioimplant was highly resistant to digestion, indicating the exceptional stabilization by the crosslinking technology and the tissue sparing properties of the sterilization technology.

High levels of proteases and collagenases are known to be present in both damaged tendons as well as in their respective joint spaces.^{4,5} Furthermore, local inhibition of the activity of collagenase is shown to improve bone-tendon healing of soft tissue grafts⁶. These data point to the presence of a highly catabolic state in damaged, injured and subsequently repaired joint spaces. Tissue implant used for repairs in such an environment may be exposed to these remodeling enzymes. Presumably, tissue scaffolds that are readily digested may not have a clinical advantage. It stands to reason that after implantation into the body, the OrthADAPT® Bioimplant may provide consistent performance over time which should allow for more guided cellular integration and true remodeling.

CONCLUSIONS

The ideal characteristics for a soft tissue scaffold have been identified as being able to withstand the expected anatomic forces at the suture-graft interface, be non-reactive, remain present until the repaired tendon is healed and be easy to store, prepare, and use.⁷ In addition, the strength of many commercially available collagen based scaffolds have been investigated prior to implantation.⁸ This study suggests the retention of strength over time of the OrthADAPT® Bioimplant due to their ability to resist proteolytic destruction. The OrthADAPT® Bioimplant is an advanced soft tissue scaffold crosslinked to prevent premature enzymatic degradation and sterilized by a proprietary method that preserves the inherent tissue properties without the use of harsh chemicals or gamma irradiation.

REFERENCES

1. Nagano J, Shink K, Maeda A, et al. The remodelling process of allogeneic and autogenous patellar tendon grafts in rats: a radiochemical study. *Arch Orthop Trauma Surg.* 1996;115(1):10-16.
 2. Toritsuka Y, Shino K, Horibe S, et al. Effect of freeze-drying or gamma-irradiation on remodeling of tendon allograft in a rat model. *J Orthop Res.* 1997; 15(2):294-300.
 3. Passes USP Sterility Testing. Data on File: Synovis Orthopedic and Woundcare, Inc.
 4. Lo IK, Marchuk LL, Hollinshead R, et al. Matrix metalloproteinase and tissue inhibitor of matrix metalloproteinase mRNA levels are specifically altered in torn rotator cuff tendons. *Am J Sports Med.* 2004; 32(5):1223-9.
 5. Yoshihara Y, Hamada K, Nakajima T, et al. Biochemical markers in the synovial fluid of glenohumeral joints from patients with rotator cuff tear. *J Orthop Res.* 2001; 19(4):573-9.
 6. Demirag B, Sarisozen B, Ozer O, et al. Enhancement of tendon-bone healing of anterior cruciate ligament grafts by blocking matrix metalloproteinases. *J Bone Joint Sur Am.* 2005; 87(11):2401-10.
 7. Coons DA, Barber FA. Tendon graft substitutes-rotator cuff patches. *Sports Med Arthrosc Rev.* 2006; 14(3):185-190.
 8. Barber FA, Herbert MA, et al. Tendon augmentation grafts: biomechanical failure loads and failure patterns. *Arthroscopy.* 2006; 22: 534-538.
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