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Viscoelastic Behavior of Tendons During Repair: Does Biodegradable Nanofibrous Membrane Augmentation Work?

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ABSTRACT

Restoring normal tendon function after injury requires reestablishing the tendon fibers and the gliding mechanism between the tendon and its surrounding structures. An electrospun PLGA/collagen/drug nanofibrous membrane was used for augmentation of tendon repair in the study. From the result, host type I collagen is affected by the donor type I collagen, i.e., delay proliferation. The elastic module increased over time, though no tendons in three groups reached the elasticity of the control tendon by the 12th week. The viscous modules reached their maximum value in the 4th week in all tendons; these values were higher than that of the control tendon over the whole study period. No tendon in any group reached the yielding load of the control tendon at the 12th week. In the current study, however, the nanofibrous membranes seem not to improve the tendon healing process when compared with the tendons in the primary suture group.

Keywords: *Tendon repair, Biodegradable nanofibrous membrane, Viscoelasticity*

BACKGROUND

Restoring natural tendon function after injury requires reestablishing the fibers and soft tissue between the tendon and its surrounding structures[1]. Initial repair involves the scar tissue formation, which provides continuity at the injury site; however, a lack of mechanical loadings on the tendon causes scar tissue proliferation and adhesions that hinder natural tendon function. Although stability to the injured tendon is necessary, mobility is critical, as mechanical stimulus associated with motion of the healing tendon decreases the post operative adhesions formation and increases strength.

After tendon injury, the body initiates a cascade of events distinguishable by their cellular and biochemical processes. The repair sequence progresses through three well-known stages: tissue inflammation, cell proliferation, and remodeling [2–4]. Remodeling stage begins 6–8 weeks after tendon injury. This phase is illustrated by a decrease in cellularity (reduced matrix synthesis and type III collagen), and an increase in type I collagen synthesis. Type I collagen fibers are structured longitudinally along the axis of the tendon for the mechanical strength of regenerated tissue [5-6]. During later phases of remodeling, interactions between the collagen structures lead to higher tendon stiffness and, consequently, greater tensile strength; however, the repaired tissue never achieves the physical properties of natural tendon.

The biomechanical characteristics of tendon during repair process have been studied widely [7-9] and these reports have shown that current procedures of tendon repair produce a tissue with biomechanical properties that are inferior to those of natural tendon. Efforts at improving the biomechanical properties of repaired tissue have led to the new therapies, including tissue-engineering techniques, followed by bio mechanical testing of the regenerated tissue. The characteristics of tendon during repair process may also be correlated at the molecular level with the mechanical properties of collagen fibers, i.e., viscoelasticity[10-11]. Tendons do not usually rupture or fail under normal circumstances; therefore, it is more appropriate to measure their physical properties within the linear region. The rate of elongation to which the tendon is subjected modulates the amount of load

transferred. Therefore, functional parameter design must be based on in vivo function, rather than on a comparison of parameters such as maximum force and stiffness. This method to tendon repair may better define the biomechanical properties of regenerated tissue.

Poly (lactic-co-glycolic acid) (PLGA), a bio degradable polymer, has become increasingly popular for fixation of bony and ligamentous structures in orthopedic surgery [12,13]. As a bioabsorbable implant, it undergoes hydrolysis in the body to produce its original monomers, glycolic and lactic acids. These monomers are by-products of metabolic pathways under normal physiological conditions. Since the body handles these two monomers effectively, there is minimal systemic toxicity associated with PLGA for biomedical applications. Numerous devices have been developed based mainly on polymers prepared from glycolic (polyglycolide) and lactic (polylactide) acids[14,15] . However, the use of bioabsorbable membranes in tendon repair surgery has been sparsely documented.

Following the implantation of any bioabsorbable device, a proliferation of fibrous tissue occursalong with material from the degrading implant. Furthermore, the tissue forms a composite membranous structure called a neo membrane that can be exploited in guiding tissue re generation [16]. A recent report showed that enveloping a tendon injured site with a poly glycolide membrane produced no specific effect on either scar formation or the reunion of the transected tendon ends as compared with no enveloped controls [17].

However, scar formation is reportedly unavoidably associated with the healing of surgically rejoined Achilles tendons, particularly during the first postoperative weeks in other reports[18-20].Although the potential adverse effects and benefits of the use of bio absorbable materials for tendon repair have been discussed in some scientific reports, no consensus has been reached on this matter, especially concerning the management of acute Achilles tendon ruptures [21,22].

Previous study [23] introduced the advantages of electrospun PLGA/collagen/drug nanofibrous membranes forwound dressing. The bio absorbable membranes are no longer a scaffold material, but are functionally active in human fibroblast responses, and effectively accelerate tissue repair. This study investigates nanofibrous membranes in tendon repair. Histologic examination of the repaired site and bio mechanical testing of the healing tendon are investigated using two augmentation methods and compared to those without augmentation.

MATERIALS AND METHODS

Fabrication and Property of the Membrane

The PLGA used in this study is a commercially available material (Resomer RG 503, Boehringer, Ingelheim, Germany) with a 50:50 lactide: glycolide ratio and intrinsic viscosity of 0.4. Type I collagen from bovine Achilles tendon and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) were purchased from Sigma-Aldrich (Saint Louis, MO). The physical property of the membrane is discussed in a previous study [23].

Study Group

Forty-eight New Zealand white rabbits were used in the study and were allotted to the (1) primary suture, (2) Enveloped Augmentation of Nanofibrous Membrane (EANM), and (3) Interposition Augmentation of Nanofibrous Membrane (IANM) groups. In the EANM group, a 1 cm × 1 cm PLGA membrane was encapsulated around the site of Achilles tendon transaction after the primary suture was placed; in the IANM group, a 1 cm × 1 cm PLGA membrane was placed between the sites of Achilles tendon transaction before the primary suture.

Animals were sacrificed at 2, 4, 8, and 12 weeks (4 in each group, 12 at each time point). The defected Achilles tendons were surgically transected with a length of 40 mm for histology and mechanical analysis.

Surgical Procedure

Under proper anesthetic and sterilized technique, the Achilles tendons of the bilateral lower legs were surgically transected 20mm proximal to the postero inferior corner of the calcaneus and then re approximated with a 4-O polydioxanone suture using Kessler stitches. In the right leg, the repair site was augmented with the enhanced nanofibrous membrane. The skin was closed with No. 4 Dexon.

Histologic Examination of the Repair Site

To examine the histology and immune histochemistry of the repair site, all samples were stained with either hematoxylin and eosin (H&E) or with antibodies to bovine type I collagen (which was surgically implanted), and rabbit type I collagen (which is present during late stages of the healing process). The regeneration of the transected tendons and the cellular components of the scar tissue were studied histologically. Maturation of the scar tissue was evaluated by the number of fibroblasts and capillaries and the volume of collagen in the healing tendons.

Biomechanical Testing of the Tendons

The tendons used for mechanical analysis were tested immediately after transection. The tendon was sutured using a No. 2 Ethibond suture in a whipstitch. The other side of the tendon was clamped and then posted in a material testing system (MTS) to measure the maximal pullout load. The tendon was loaded at a displacement rate of 1 mm/s until the defected site separated or the tendon ruptured at its normal substance. The load-time curve was recorded by the MTS software and the maximum pullout load, pullout site, and failure mode were noted for each specimen.

Constitutive Equation

This study uses the Maxwell-Wiechert model for analysis, in which a spring and dashpot are used to simulate the elastic and viscous components of the stress/stain response.

The (elastic) spring obeys the following relations for tensile and shear stress:

$$\sigma = E \cdot \varepsilon \; \tau = G \cdot \gamma.$$

The dashpot (the viscous component of the response) obeys the relations for tensile and shear stress below:

$$\sigma = \eta \cdot \dot{\varepsilon} \; \tau = \mu \cdot \dot{\gamma}.$$

The Maxwell model consists of a spring and dashpot in series.

Assuming constant area, the equilibrium of forces equation gives

$$\sigma = \sigma_1 = \sigma_2.$$

Similarly, the deformation equation is

$$\varepsilon = \varepsilon_1 + \varepsilon_2,$$

Which can be rewritten as

$$\dot{\varepsilon} = \frac{1}{E} \cdot \dot{\sigma}_1 + \frac{1}{\eta} \cdot \sigma_2,$$

Giving

$$\dot{\varepsilon} = \frac{1}{E} \cdot \dot{\sigma} + \frac{1}{\eta} \cdot \sigma,$$

The governing equation of the Maxwell model. In this study, the tendon was loaded at a regular displacement rate of 1 mm/s and the length of the tendon was surgically transected with fixed 40 mm. Thus,

$\dot{\varepsilon}$ = 1 (mm/s) * 1/40 (mm) = 1/40 (1/s),

giving

$$\dot{\sigma} + \frac{E}{\eta}\sigma = \frac{E}{40},$$

and from the above,

$$\sigma = \frac{\eta}{40}\left[1 - e^{-\frac{E}{\eta}t}\right].$$

Using MATLAB 2010, a curve was fit to the equation to find the values of E and η.

Statistics and Data Analysis

Data were collected from the samples and analyzed by a one-way ANOVA. Differences were considered statistically significant for p values less than 0.05.

Results

Histology Analysis

Figures 1–3 show the histology analysis of models in the primary suture, EANM, and IANM groups, respectively. The H&E stain results show granulation tissue formation and inflammatory cell infiltration in all groups at the 2nd and 4th weeks. The defected tendons healed gradually in all groups with newly synthesized fibrous tissue and sparse inflammatory cells at the 8th and 12th weeks. Rabbit type I collagen increases, as expected, during tendon repair in all three groups. In the EANM and IANM groups, bovine type I collagen (surgically implanted) was found in all models at the 2nd and 4th weeks and dissolved by the 8th and 12th weeks.

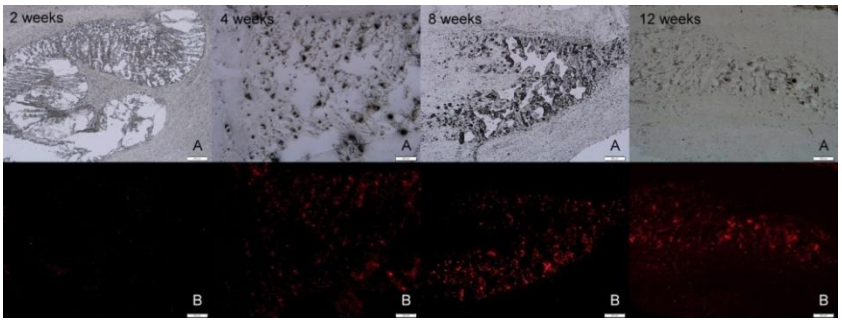

Figure1. *Histology analysis 2, 4, 8, and 12 weeks postoperative for models in the primary suture group. Samples were stained with (A) H&E and (B) rabbit type I collagen antibody.*

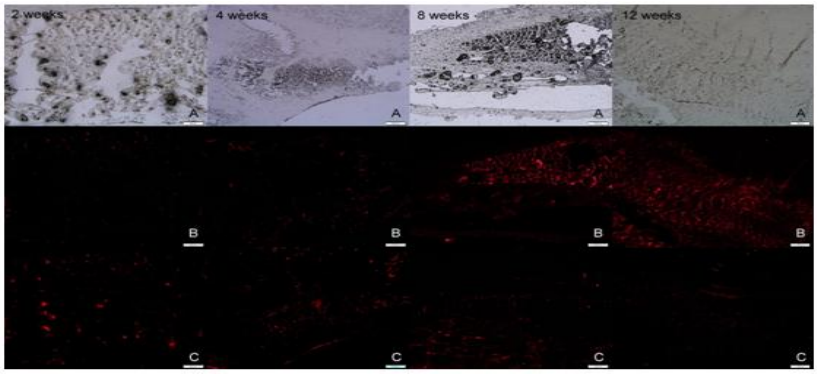

Figure2. *Histology analysis of 2, 4, 8, and 12 weeks postoperative for models in the EANM group. Samples were stained with (A) H&E, (B) rabbit type I collagen antibody, and (C) bovine type I collagen antibody.*

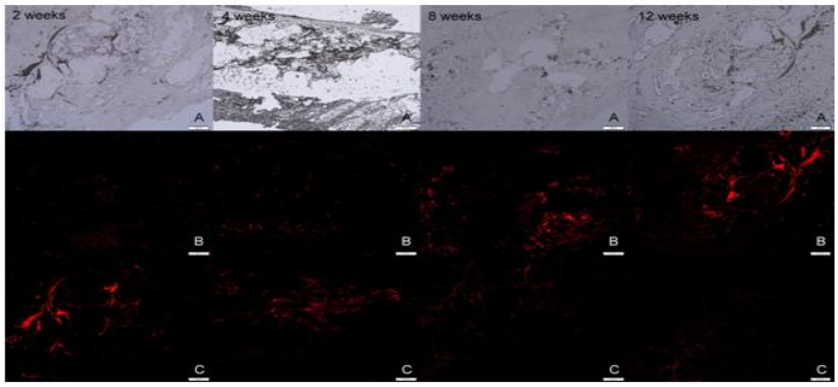

Figure3. *Histology analysis of 2, 4, 8, and 12 weeks postoperative for models in the IANM group. Samples were stained with (A) H&E, (B) rabbit type I collagen antibody, and (C) bovine type I collagen antibody.*

Biomechanical Analysis

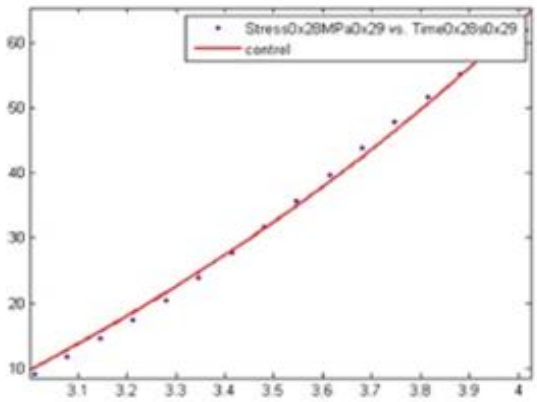

Figure4. *The curve fit for the control tendon. Sum of squared errors (SSE): 228.3, R-square: 0.9502, Adjusted R-square: 0.9467, root mean square error (RMSE): 4.038.*

The mechanical properties of normal tendon were first analyzed with the MTS and the maximum failure load was noted at 150.23N. Figure 4 shows the curve fit to the properties of the control tendon. Assuming that the cross-sectional area of the tendon is 2 mm^2, with 95% confidence-bounded coefficients, the curve-fitting result gives control values of E= 1956MPa (1489, 2424) and η= 1.379e+004Pa-s (-2.978e+004, 5.736e+004).

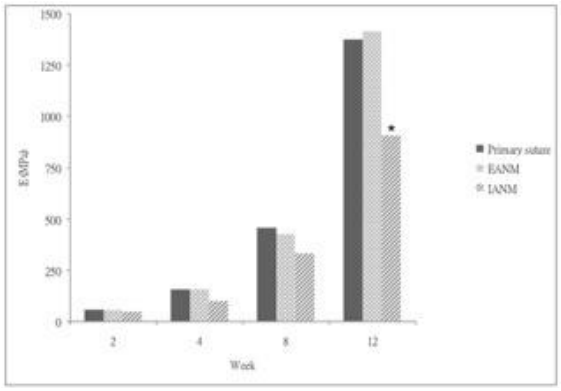

Figure5. *Comparison of the average elastic module values E across the three groups at different time points (* p< 0.05).*

Figure 5 compares the average elastic module value E of models in the three groups at the 2nd, 4th, 8th, and 12th weeks, respectively: 56.6, 155, 455, and 1376.2 MPa in the primary suture group; 55.17, 155.5, 425.5, and 1412.9MPa in the EANM group; and 47.09, 99.4, 329.4, and 908MPa in the IANM group. The average value of the IANM group was significantly lower than those in the other groups at week 12. At no point during this investigation did the average elastic module values reach the normal control value.

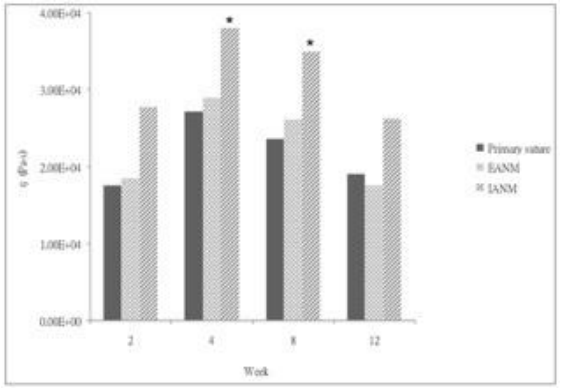

Figure6. *Comparison of the average viscous module value η across the three groups at different time points (* p< 0.05).*

Figure 6 shows the average viscous module values η across the three groups. The average value at the 2nd, 4th, 8th, and 12th weeks, respectively, is 1.75E+04, 2.72E+04, 2.36E+04, and1.90E+04Pa-s in the primary suture group; 1.84E+04, 2.90E+04, 2.61E+04, and 1.75E + 04Pa-s in the EANM group; and 2.77E+04, 3.81E+04, 3.50E+04, and 2.63E+04Pa-s in the IANM group. The average values of models in the IANM group were significantly higher than those in the two other groups at the 4th and 8th weeks. All average viscous module values were greater than the normal control value at all points in the investigation.

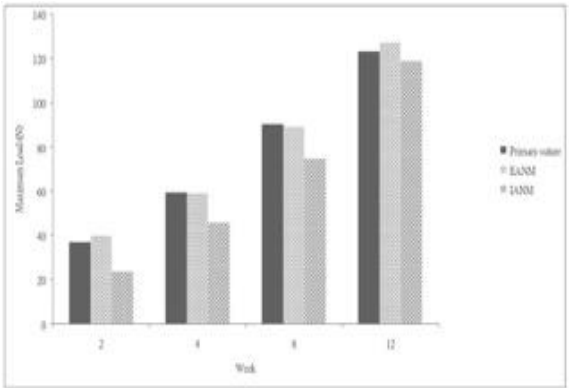

Figure7. *Comparison of the average maximum failure load value N between models in the three groups at different time points.*

Figure 7 depicts the average maximum failure load value N of models across the three groups at the 2nd, 4th, 8th, and 12th weeks, respectively: 36.93, 59.55, 90.4, and 123.1N in the primary suture group; 39.58, 59.14, 89.22, and 127.15N in the EANM group; and 23.64, 45.81, 74.44, and 118.56 N in the IANM group. The average maximum load failure value did not differ significantly between the three groups and was lower than the normal control value at all points in this investigation.

Discussion

Most biological materials obey Hooke's law over a limited range of stresses and strains. If a load is applied for a long period, then elongation of the material continues. Materials in which stress and strain are time dependent are said to be viscoelastic. For example, if you stretch an elastic band and continue to hold it at the stretched length, nothing much happens. In contrast, because a tendon is viscoelastic, it eventually changes. This behavior, defined as "creep," is unique to tendons and ligaments and likely stems from the behaviors of the collagen and other materials that they are composed of. Under continuous strain, the tendon responds with decreased stress, a behavior known as the stress relaxation response. Tendons' ability to relax directly relates to the amount of strain they

undergo; more strain means a greater relaxation response.

Tendons do not behave as inextensible material, but act as biological springs that can stretch elastically, storing and releasing energy during locomotion and regulating muscles' mechanical performance [24]. The mechanical properties of tendons have been extensively studied using isolated animal or human material undergoing elongation to failure [25], but few reports exist on the mechanical properties of tendons under maximal physiological load, and most of these refer to animal material testing.

In the study, fit the viscoelastic behavior of tendons into a constitutive equations model, the Maxwell- Wiechert model. An in vitro investigation was carried out, using an MTS and curve-fitting, to determine the time-independent elastic and time-dependent viscous behavior of tendons. These results show that the elastic module increased over time, though no tendons in three groups reached the elasticity of the control tendon by the 12th week. The tendons in the IANM group had the lowest elastic module at week 12 of the three groups. The viscous modules reached their maximum value in the 4th week in all tendons; these values were higher than that of the control tendon over the whole study. Likewise, the tendons in the IANM group had significantly higher viscous modules than the other tendons at the 4th and 8th weeks. The interposition of the nanofibrous membrane seems to alter the normal biophysiology of tendon healing. However, from the maximum load result, tendons in all three groups showed no significant differences over the entire investigation. No tendon in any group reached the yielding load of the control tendon at 12th week postoperatively, which is the same as the reports in the literature [26,27].

Management of large tendon defects can present a challenge to the orthopedic surgeon, though tendon augmentation can provide an effective management option[28,29]. Biological scaffolds are protein-based ECMs that are usually derived from human or animal connective tissues[30]. Advantages of biological scaffolds include a well-defined 3D surface protein microstructure (allowing host cell integration), and natural porosity (providing space for host cell attachment, proliferation, and migration and assisting gas and metabolite diffusion). Biological scaffolds can quickly interact with host tissue and induce new tissue formation faster than synthetic scaffolds.

In the current study, however, nanofibrous membranes seem to not improve tendon healing when compared with the tendons in the primary suture group. Host type I collagen is affected by the donor type I collagen, ie, delay proliferation, from the immune histology results. The standard host type I collagen in the primary suture group proliferates at the 4th week after repair surgery. However, in the EANM and IANM groups, the host type I collagen proliferates at the 8th week after repair surgery, which may explain our biomechanical analysis results.

Since being defined in 1988, tissue engineering has offered great potential in treating difficult injuries. Tissue engineering has sought to enhance biologic activity by delivering cells and/or a scaffold to a repair site to augment healing [31,32]. In the future, we will seed differentiated and multipotential stem cells on scaffold carriers to form engineered tissue constructs. To enhance their properties in culture, we will stimulate these constructs with chemical and mechanical signals. Advances in tendon repair may soon yield a cell-based product that will markedly advance the repair of soft tissue injuries [33–35].

In this study, an electrospun biodegradable nanofibrous matrix for tendon healing is introduced. However, in vivo results indicate that the membranes did not significantly affect tendon healing with the two augmentation techniques tested. Future experimentation is necessary to address this issue before the membrane is qualified for clinical application. However, these results show that the nano fibrous membranes were functionally active in human fibroblasts. By adopting the electro spinning technique, we can manufacture bio degradable biomimetic nanofibrous ECMs for long-term drug delivery.

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