Physical Properties of Electrospun Gelatin Networks Compared with Fetal Membrane Tissues

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Statement of Purpose: Fully one-third of preterm births are due to rupture of the fetal membranes (the 'breaking of waters') prior to full term gestation. In most cases the reasons for this failure are unknown, and there is currently no clinical intervention. The fetus is typically delivered within 24-48 hours due to the risk of ascending infection from the vagina into the cervical canal. Some cases of preterm membrane rupture do occur after fetal surgery or amniocentesis, when the membranes have been purposefully transected.
The aim of this research project is to characterize the physical properties of intact fetal membranes and electrospun fibrillar networks that serve as candidate materials for a tissue engineered fetal membrane patch. The application is mechanically demanding, as the membranes stretch significantly during third trimester pregnancy and thus any patch material must be extremely stiff, strong, and tough.
Methods: Gelatin from porcine skin with a 300 g bloom strength at 12 wt. percent was dissolved in a 9:1 wt. ratio mixture of acetic acid and distilled water to create uncrosslinked fibers. Citric acid at 15 wt. percent and sodium hypophosphate (SHP) at 7.5 wt. percent were added to the gelatin, acetic acid and distilled water as crosslinking agents. All chemicals were obtained from MilliporeSigma aside from the acetic acid from VWR. The gelatin solutions were electrospun for 7 hours at a rate of 0.3 mL/hr and a working distance of 12.5 cm. Four categories of nanofiber mats were fabricated. These categories include as-electrospun—(NU), as-spun including crosslinking agents (NX), and heat-treated (150 °C for 4 hours) without (TU) and with (TX) crosslinking agents. The samples were held between 3D printed grips with circular openings and placed on the ZwickRoell 2.5 kN universal mechanical testing machine. Drumhead indentation testing to failure was conducted. The load and displacement at failure were used to calculate the elastic modulus E [1] and failure strength σ_f [2]. The same mechanical testing protocol was followed for de-identified fetal membrane samples obtained with full consent from Vidant Medical Center/East Carolina University (UMCIRB 12-002524). Tear toughness values were taken from previously published data on the same sample types [3,4] for comparison with the new elastic modulus and failure strength measurements.
Results: There were direct correlations between the elastic modulus and strength data for the four different conditions of electrospun networks (Figure 1). The samples without crosslinking agents (NU, TU) were stronger than crosslink groups (NX, TX) but the NX samples were substantially stiffer than the others. All electrospun samples were stiffer and stronger than the amnion. However, when the tear toughness values were considered, the TX group was the only set of electrospun samples that approached the tear toughness of real amnion, demonstrating that these three different properties—stiffness, strength, and toughness—do not necessarily track in the same manner in fibrous materials and tissues.

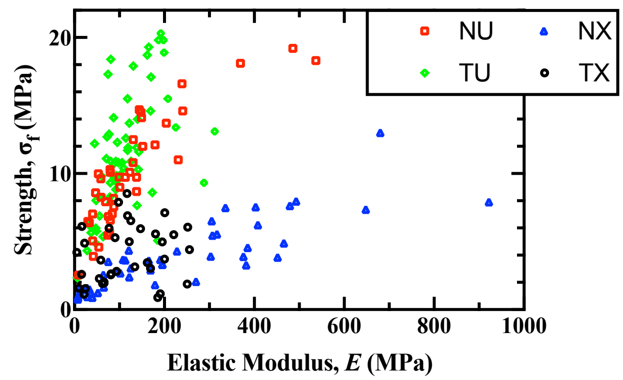

Figure 1: Failure strength plotted as a function of elastic modulus for four different types of electrospun gelatin scaffolds.

Conclusions: Heat-treated and chemically crosslinked (TX) gelatin scaffolds had smaller strength and stiffness than three other combinations of heat treatment and crosslinking, but far superior fracture tear toughness. For optimization of tissue engineering scaffolds or patches for fetal membranes, all three properties must be considered, as they do not trend in the same direction due likely to microstructural differences in each material .

Table 1: Property values from this work (E, σ_f) compared with prior fracture tests on the same materials [3,4].

Group	Elastic Modulus, E (MPa)	Strength, σ_f (MPa)	Tear Toughness, T (J m^{-2})
Amnion	14.8 ± 16.4	2.9 ± 2.9	~1–2*
NU	134 ± 118	10.1 ± 4.1	396 ± 240†
TU	113 ± 61	11.8 ± 4.7	373 ± 116†
NX	264 ± 265	3.6 ± 2.5	135 ± 44†
TX	115 ± 75	3.9 ± 2.1	791 ± 242†

* data from [4]
† data from [3]

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