Mechano-morphological Properties of Electrospun Micro/Nano-fibrous Vascular Scaffold of Protein/Polyglyconate Blends by Carbodiimide

Xing Zhang[1], Vinoy Thomas[2], Yogesh K. Vohra[2]
[1]Department of Biomedical Engineering, [2]Center for Nanoscale Materials and Biointegration (CNMB)
Department of Physics, University of Alabama at Birmingham (UAB), AL 35294, USA

Statement of Purpose: We studied the effect of carbodiimide/ethanol cross-linking on the mechanical and morphological properties of a novel tri-layer vascular graft composed of non-woven micro/nano-fibers via sequential electrospinning of gelatin (G), elastin (e) and polyglyconate (M) solution blends onto a small diameter (4 mm) rotating mandrel [1, 2]. Previous structural and morphological characterizations revealed that the new graft possesses porosity up to 80% [1]. In addition, i*n vitro* biodegradation study demonstrated that the graft lost nearly 50% of initial mass by the end of the 30-day aging period in hydrolytic medium [2]. Since the presence of proteins in the graft, we hypothesize that chemical cross-linking of proteins by 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) can improve the graft's mechanical performance and retain sufficient integrity for a longer period during *in vitro* biodegradation.

Methods: To fabricate the tri-layer tubular scaffold (Ge-GeM-GM), corresponding electrospun fibers were deposited onto a rotating stainless steel rod (4 mm of diameter and 400 rpm) layer by layer via sequential-spinning [2]. Then strips measuring 0.5 mm × 1 mm harvested from the graft were weighted, pre-wetted in ethanol and immersed in 200 mM EDC/ethanol solution for 18 h (*N*=3) and 36 h (*N*=3), respectively, for cross-linking. Afterward, the degree of cross-linking was determined using 2,4,6-trinitrobenzenesulfonic acid (TNBS) as described by Barnes *et al* [3]. After being hydrated in phosphate buffered saline (PBS) for 4 h, the cross-linked strips were subjected to micro tensile testing (INSTRON 5566, MA) with a loading rate of 0.3 N/min and scanning electron microscope (SEM).

Results: The degree of cross-linking is illustrated in Fig.1. The graft cross-linked for 18 h yielded an average degree of 7.67% ± 1.53% while the graft for 36 h 19.67% ± 2.08%.

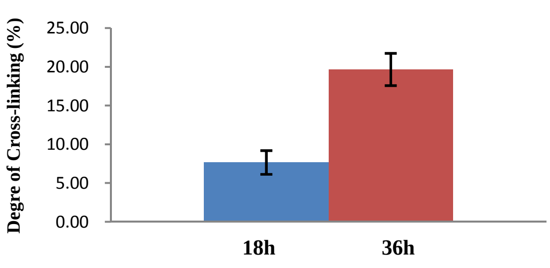

Figure 1 Degree of Cross-linking for 18 h and 36 h groups

The uncross-linked graft exhibited a maximum tensile stress of 0.61±0.05 MPa with a failure strain of 37±5%, and a tensile modulus of 3.02±0.14 MPa. In contrast, the graft cross-linked for 18 h yielded a stress of 0.87±0.34 MPa with a failure strain of 54±6%, and a tensile modulus of 4.02±1.28 MPa. The graft cross-linked for 36 h featured a stress of 0.72±0.10 MPa with a failure strain of 55±12%, and a tensile modulus of 3.27±0.40 MPa. Representative stress-strain curves are plotted in Fig.2.

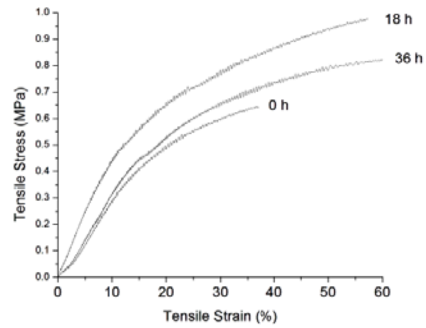

Figure 2 Representative stress-strain curves of grafts before and after cross-linking

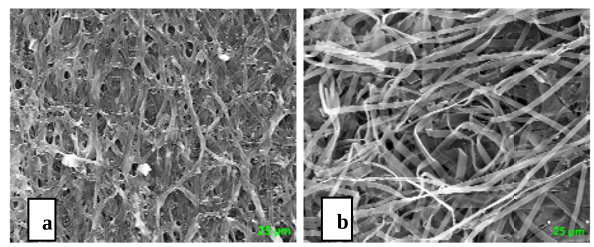

Figure 3 SEM Images of luminal layer: (a) after cross-linking (18 h) and (b) before cross-linking. Scale bar is 25 µm

Conclusions: A prolonged incubation is necessary to achieve high degree of cross-linking. SEM images (Fig.3) illustrate that the porosity and fibrous morphology of the graft is preserved after 18 h of cross-linking despite the occurrence of fiber gelation and fusion. Under hydrated condition, cross-linked grafts demonstrated higher tensile strength and tensile modulus as compared to un-cross-linked ones. The decrease in tensile stress of grafts cross-linked for 36 h compared against that cross-linked for 18 h is due to delamination of inner protein layer. The mixing of pure maxon fibers into the inner and middle layers via concurrent electrospinning could be used to prevent the delamination. The impact of mixed maxon fibers will also be discussed.

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References:

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