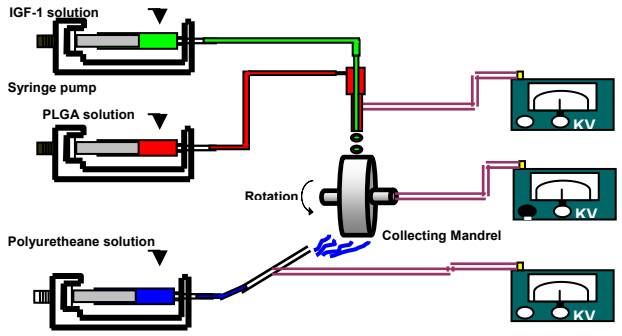
Rapid Fabrication of Growth Factor Releasing, Anisotropic and Elastic Scaffolds for Soft Tissue Engineering

Feng Wang[1], Zhenqing Li[1], Jianjun Guan[1]
[1] Department of Materials Science and Engineering, The Ohio State University, Columbus, Ohio 43210

Statement of Purpose: Engineering soft tissues is one of the most demanding and challenging applications in tissue engineering. Successful soft tissue engineering is largely dependent on the scaffolds. The scaffolds possessing soft tissue-like mechanical properties, emulating the nanofibrous structure of the extracellular matrix (ECM), and having structural anisotropy and mechanical properties similar to the target tissues, are attractive as they might provide a native-like environment for cells to adhere, grow and differentiate, and facilitate ECM production and neotissue development. When implanting a cellularized scaffold in vivo, a major concern is that cell survival may be limited. One of the effective approaches is to controlled release cell prosurvival growth factor. In this study, we have generated scaffolds that can sustained release IGF-1, a growth factor with capability to enhance survival of many cell types including mesenchymal stem cell (MSC). We developed an one-step technique that can rapidly fabricate IGF-1 releasing scaffolds by simultaneously electrospinning polymer nanofibers, encapsulating IGF-1 into microspheres and electrospraying microspheres. To mimic mechanical properties of the soft tissues, a flexible and relative strong biodegradable polyurethane was used. By controlling fabrication conditions, aligned scaffolds can be obtained to mimic structural anisotropy of the soft tissues (Scheme 1). We report here the fabrication and characterization of such scaffolds in terms of their morphology, mechanical properties, IGF-1 release kinetics and bioactivity, and ability to support mesenchymal stem cell survival/growth.



Scheme 1. Schematic drawing of the apparatus used for simultaneous fabrication of growth factor loaded nanofibrous scaffold with anisotropy.

Methods: Polyurethane was synthesized by using polycaprolactone as soft segment, 1,4-diisocyanatobutane as hard segment and peptide Ala-Ala-Lys as chain extender [1]. The synthesized polyurethane was elastase sensitive and has been shown to accelerate tissue remodeling when implanted in vivo [2]. The setup shown in Scheme I was used to fabricate IGF-1 loaded, nanofibrous, anisotropic and flexible scaffolds. The fabrication process generally lasts for 3 h to yield a scaffold with thickness ~200 μm. Biological activity of the released IGF-1 was assessed in terms of its ability to stimulate the growth of smooth muscle cells (SMCs). Human MSCs were seeded on the scaffolds to evaluate the effect of IGF-1 on cell survival/growth under normal and hypoxia conditions.

Results: Successful encapsulation of protein was confirmed by fluorescent images of FITC-BSA loaded PLGA microspheres where FITC-BSA was found to locate within the microspheres. The microspheres were seen to distribute uniformly in the aligned polyurethane scaffolds. Degree of fiber alignment was greater than 70%. The scaffolds showed anisotropic mechanical properties with tensile strength and modulus at orientation direction greater than those at perpendicular direction. The scaffolds were flexible and relatively strong with tensile strength ranging from 3.0-10.6 MPa, modulus ranging from 1.9-7.4 MPa and breaking strength around 80% at the orientation direction, depending on the PLGA microspheres density. Scaffolds were elastase sensitive and PLGA addition did not significantly change elastase-sensitivity. The IGF-1 exhibited a three-stage release profile, a fast release in the first 3 days, a slow release between 3 and 21 days, and a fast release after 21 days. The released IGF-1 preserved bioactivity during the 4-week release period as confirmed by its ability to stimulate SMC proliferation. MSCs were seeded on the surface of the scaffolds with different IGF-1 loading and found that IGF-1 accelerated the MSC growth during a 7-day culture period, with higher IGF-1 loading showing greater stimulation effect. The MSC seeded scaffolds were also cultured in hypoxia (1% O_2) and nutrient starvation (1% FBS) conditions for 3 days, the scaffolds with higher IGF-1 loading was found to have higher cell survival.

Conclusions: we present here a technique that can rapidly fabricate growth factor loaded elastic scaffolds by simultaneously electrospinning polyurethane fibers, electrospraying growth factor encapsulated microspheres. The released growth factor was found to retain bioactivity. The scaffolds demonstrated their ability to stimulate MSC proliferation. The developed fabrication technique may be used for fabrication readily implantable scaffolds. The scaffolds may be used for engineering various anisotropic soft tissues such as blood vessels and cardiac muscle.

References:

1. Guan J, et al. Biomacromolecules, 2005; 6: 2833-2842.
2. Guan J, et al. Pharm Res, 2008; 25:2400-2412.