Synthesis and Characterization of a Photocrosslinkable Collagen-Hyaluronan Interpenetrating Network for Neural Tissue Engineering: An ECM mimic

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Statement of Purpose: Tissue engineering focuses on the creation of biodegradable 3D scaffolds that not only serve to mimic extracellular matrix (ECM) but which also help to provide stimuli to direct growth and formation of desired tissue. In this study, we have synthesized a photocrosslinkable interpenetrating polymeric network (IPN) of collagen and hyaluronic acid (HA) for neural tissue engineering. IPNs made in this way will share the characteristics of each network, will allow cell adhesion, migration and proliferation and will also have improved mechanical strength.[1] IPNs are different from conventional crosslinked gels, as in IPNs, collagen and HA are not crosslinked to each other; both the polymers are present in network form and the two networks are present together (Fig.1). These IPNs were compared with semi-IPNs, in which only collagen was present in network form and HA chains were entangled in collagen gel. Both, HA and collagen have great potential in tissue engineering as they are the main structural components of ECM. Here in this study, we have made a novel gel which shows the combinatorial properties of both the polymers and thus it can be of great interest in tissue engineering. These IPNs were characterized and were compared with semi-IPNs. Cell compatibility studies were done with two different types of neural cells, PC12 cells and Schwann cells.

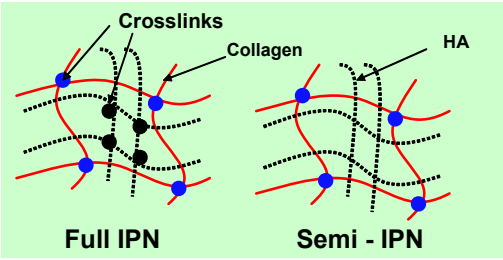

Figure 1: Schematics of collagen-HA IPNs and Semi-IPNS. In IPNs both, collagen and HA, are present in network form while in semi-IPNs, only collagen is crosslinked and HA is entangled in the first polymer's network

Methods: Photopolymerizable HA (GMHA) was synthesized by modifying it with glycidyl methacrylate as described previously.[2] IPNs of collagen and GMHA were synthesized by mixing GMHA and collagen. The resulting solution was mixed with 10x DMEM and sodium bicarbonate-Hepes-0.5M NaOH in 8:1:1 ratio and then allowing collagen fibrillogenesis at 37°C.[3] The resulting semi-IPN was converted into an IPN by exposing it to UV to promote HA crosslinking. The IPN was characterized using rheology, swelling studies, SEM, HAase degradation studies; these properties were compared to those for semi-IPNs. SEM was used for morphological characterization and to evaluate the efficiency of molecular interpenetration and stabilization in IPNs compared to semi-IPNs. Viscoelastic properties of gels were determined by rheology experiments which provide a measure of stiffness and degree of crosslinking. Finally, cell culture studies were performed to determine the adhesion and viability of cells on the IPNs. Since the study is mainly focused on neural tissue engineering, PC12 cells and Schwann cells adhesion was studied on these IPNs.

Results/Discussion: IPNs of collagen and HA were successfully synthesized. Rheology results demonstrated that the storage modulus for the IPNs was much higher than that for the semi-IPNs, showing better mechanical properties and increased stiffness. Further, the modulus increases with increasing HA concentration because of increased crosslinking. Swelling studies confirmed that IPNs swelled less than semi-IPNs indicating more crosslinking. SEM images also supported the fact that IPNs have more crosslinks and thus they are mechanically stronger than semi-IPNs. Cell adhesion studies were further performed with IPNs to assess whether they can support cell adhesion and proliferation. Both PC12 cells and Schwann cells were shown to adhere to these IPNs; live-dead stain assay confirmed the presence of viable cells.

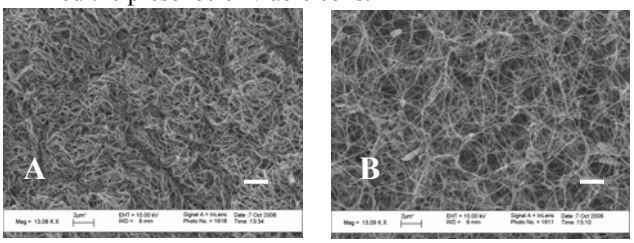

Figure 2: SEM images of A) Collagen GMHA IPNs, and B) Semi-IPNs. IPNs show more crosslinked and stiffer structure than semi-IPNs (scale bar: 2 μm)

Conclusions: IPNs of collagen and HA were synthesized and characterized. These IPNs can be attractive candidates for tissue engineering as they have multiple polymers, each present in crosslinked network form in the presence of the other polymeric network. Collagen and HA have great potential alone as biomaterials, and combining their properties can give a hydrogel that is mechanically stronger, selectively degradable and which mimics the natural ECM chemistry of the body.

References:

1) Hasirci et.Al. J Biotechnol. 2001;86:135-150.
2) Leach et Al. Biotechnol. Bioeng. 2003;82(5):578-589
3) Deister et Al. J Neural Eng. 2006;3:172-179.