Mechanically Tunable Hyaluronan-Based Hydrogels for Dental Pulp Stem Cell Growth

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Statement of purpose: Hyaluronic acid (HA) is a natural polysaccharide found most abundantly in the extracellular matrix (ECM) of connective tissues and it facilitates cell proliferation and migration. It has structural, lubricating, and wound healing functions in the human body and can be degraded naturally by body's natural processes. Therefore HA has being increasingly studied as scaffolds for tissue engineering in recent years. However, HA polymer alone has limited mechanical strength and is not suitable for broad tissue engineering applications. The purpose of this study is to design hyaluronic acid (HA) based hydrogels using different MWs of HA and different amount of 2-aminoethyl methacrylate (AEMA) crosslinker to obtain HA scaffolds with tunable mechanical properties to promote dental pulp stem cell (DPSC) growth.

Methods: A serial of hyaluronic acid macromers were synthesized by conjugating (AEMA) to hyaluronic acid with different molecular weights (16k-270k) and different degree of substitutions (12.5-25%). The synthesized macromers were further polymerized into hydrogels through UV polymerization technique using Irgacure 2959 as a photo initiator and UV exposure intensity of 360-390 mW/cm^2 for 10 min. The storage and loss moduli of the hydrogels were measured using a rheometer. The compression moduli of the HA hydrogels and their degradation in water with time were measured using a dynamic mechanical analyzer (DMA). The cytotoxicity of the synthesized macromers to DPSCs was studied by using MTT assay. Further, DPSCs were encapsulated into the hydrogels *in situ* during the photo-polymerization and trypan blue cell staining assay was used to evaluate the DPSC cell survival inside the hydrogels.

Results: HA-AEMA macromer (shown in Fig. 1) was successfully synthesized and its chemical structure was confirmed by ^1H NMR. Fig. 1 shows the storage moduli of the HA hydrogels synthesized from HA –AEMA macromers with different MWs of HA and different amount of AEMA. With 25% degree of AEMA substitution, there is no difference of the storage moduli between the hydrogels made of 16k and 18k HA, and between those made of 66k and 270k HA. However, the storage modulus of the hydrogels made of 66k HA was one time higher than those of the hydrogels made of 16k and 18k HA. With the same MW of HA at 66k, the 66k-25 hydrogels had significant higher storage modulus than the 66k-12.5 hydrogels, which is about 24 times. The reason is probably due to the higher amount of the AEMA degree of substitution in the former hydrogels (25% vs. 12.5%). The DMA measurement results demonstrated that the compression moduli of the 66k-25 HA hydrogels decreased with time for one month, probably due to the hydrolytic degradation of the hydrogels with time. The cell viability results of DPSCs in the presence of HA-AEMA macromers determined by MTT assay suggested that the macromers were not toxic to DPSCs. The DPSCs encapsulated inside the 18k-25 and 270k-25 HA-AEMA hydrogels during the hydrogel synthesis process were able to survive for at least 24 h based on tryptan blue staining study.

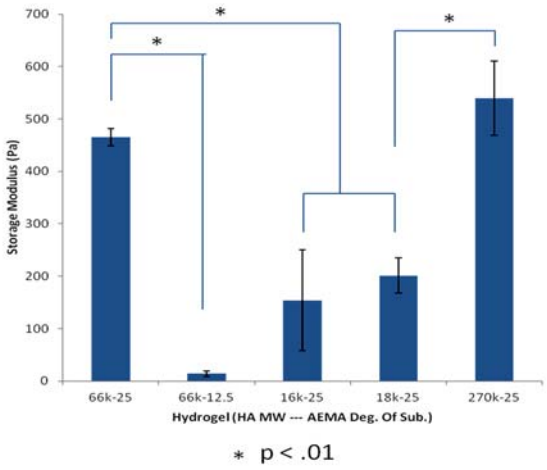

Fig. 1. Storage modulus comparisons based on MW of HA, and AEMA degree of substitution.

Conclusions: HA hydrogels containing AEMA can be developed with tunable mechanical properties by controlling the MWs of HA and the AEMA degree of substitution to promote DPSC growth. Further studies will include evaluating the potential of the designed HA hydrogels for differentiating DPSCs into different cell lineages.