Synthesis of thermo-responsive, protein reactive copolymer for cartilage tissue engineering

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Delivery of cells and biofactors with injectable hydrogels is a promising approach for different tissue engineering applications such as cartilage repair (Kulshrestha et al., 2010). The immense interest to injectable hydrogels is due to their tunable physico-chemical, swelling and mechanical properties (Annabi, Mithieux, Weiss, & Dehghani, 2009, 2010). In this approach, the polymer solution is loaded with chondrogenesis cells (such as chondrocyte or stem cells) and injected to the cartilage defected site. Thermogelation process is an ideal and benign scheme for *in situ* gelation of polymers by increasing the temperature to above the lower critical solution temperature (LCST) of copolymer (Sanchez & Tsinman, 2011).

PNIPAAm based copolymers are widely used for synthesis of thermoresponsive injectable hydrogels (Guan, Hong, Ma, & Wagner, 2008). Lack of cell motif sites however is the main drawback of this class of biomaterials. Naturally derived proteins were physically mixed to promote the biological properties of PNIPAAm based copolymers. Physical mixture of natural and synthetic polymers however might lead to formation of scaffold with non-uniform microstructure. To address this problem, proteins can be chemically conjugated to synthetic polymer to form homogenous hybrid hydrogels.

The aim of this study was to synthesize a functionalized, and thermoresponsive copolymer which can form covalent bonds with proteins to form hydrogel at physiological condition.

Material and method: D,L-lactide, stannous 2-ethylhexanoate ($Sn(OCt)_2$), N-isopropylacrylamide (NIPAAm), and N-acryloxysuccinimide (NAS) were used as received. 2-Hydroxyethyl methacrylate (HEMA) was used after distillation under reduced pressure. Poly(NIPAAm-co-NAS-co-(HEMA-PLA)-co-PEG) copolymer, denoted as PNPHO, was synthesized by free radical polymerization. Known amount of monomers were dissolved in tetrahydrofuran and polymerization was conducted for 24 h to acquire desirable molecular weight and yield. The precursors were then precipitated in diethyl ether, filtered, and dried under vacuum.

Results: The synthesis of PNPHO copolymer was confirmed with ^{1}HNMR spectra with evidence of proton peaks for each monomer, as shown in Figure 1.

The bioconjugation of copolymer with naturally derived copolymer was assessed by using elastin as a model protein. ATR-FTIR spectra were used to confirm the chemical conjugation between elastin and copolymer. As shown in Figure 2, the copolymer exhibited characteristic peak at 1812 cm^{-1} associated with succinimide group. After the conjugation of elastin, this peak disappeared completely, indicating the participation of elastin in the condensation reaction with succinimide group. This result confirmed the formation of covalent bond between PNPHO and protein which can be used to create bioactive hydrogels with uniform microstructure and suitable mechanical strength.

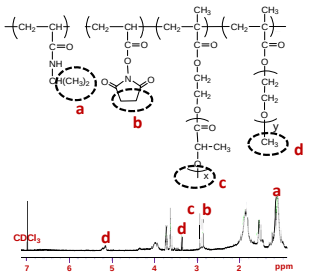

Figure 1. ^{1}HNMR spectra of PNPHO copolymer (a for NIPAAm, b for NAS, c for PLA/HEMA, and d for PEG).

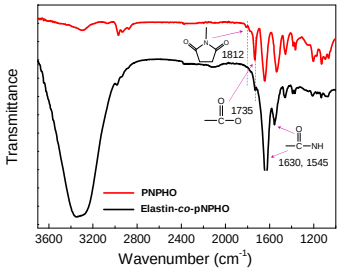

Figure 2. FTIR Spectra of copolymer and conjugate hydrogel.

The conjugated copolymer and elastin solution (PNPHO-elastin) exhibited thermoresponsive behavior and formed hydrogel by increasing temperature to 37 °C. The formed hydrogel possessed good mechanical properties and structural integrity.

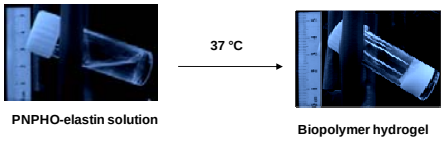

Figure 3. Thermogelation behavior of PNPHO-elastin solution by increasing temperature to 37 °C.

Conclusion: A functionalized, thermoresponsive copolymer was synthesized. This copolymer formed covalent bonds with naturally derived proteins such as elastin. The conjugate system was converted to hydrogel with good structural integrity by increasing the temperature of solution from room temperature to 37 °C. The designed copolymer in this study might be a good biomaterial for cartilage repair due its thermoresponsive properties and bioconjugation capacity.