ROS, pH and temperature responsive hydrogels for stem cell therapy

Hong Niu, Xiaohong Hu, Zheng Huang, Zhaobo Fan, Yu Dang, Chao Li, Jianjun Guan
Department of Materials Science and Engineering, The Ohio State University

Statement of Purpose: Stem cell therapy has been considered as a promising approach to regenerate ischemic tissues such as ischemic heart, limb and brain. These tissues are characterized by high reactive oxygen species (ROS) content that compromises cell survival. Protecting cells from ROS attack may improve cell survival. One of the major limitations to deliver stem cells into ischemic tissues is low cell retention. To overcome these issues, we developed hydrogels that are ROS sensitive so as to protect the encapsulated cells. The hydrogels were also designed to have high gelation rate at the pH of ischemic tissues in order to increase both gel and cell retention in tissues.

Methods: Materials used for synthesis were N-isopropylacrylamide (NIPAAm), 2-Hydroxyethyl methacrylate (HEMA), 4-(hydroxymethyl)-phenylboronic acid pinacol ester, acryloyl chloride, diethyl propylmalonate, and benzoyl peroxide (BPO). The ROS sensitive acrylic monomer (AAcPB) was synthesized by the reaction of 4-(hydroxymethyl)-phenylboronic acid pinacol ester and acryloyl chloride. The pH-sensitive monomer (PAA) was synthesized according to the procedure by Ferrito et al. [2]. The hydrogel was then synthesized by copolymerization of NIPAAm, HEMA, AAcPB and PAA (molar ratio 77/10/8/5 and 75/10/10/5) via free radical polymerization. ^{1}H-NMR spectrum was used to confirm the structure of synthesized monomers and polymers. Physical properties of the hydrogels such as gelation time, water content were characterized following our previous established procedures [1]. The ROS responsiveness was firstly confirmed by the different chemical structure with and without the presence of H_2O_2. Furthermore, the degradation rates, lower critical solution temperature (LCST) w/o H_2O_2 were examined. Similarly, the pH responsiveness was verified at different pH, ranging from pH=6.5 to pH=8.0. Also, the responsive kinetics to H_2O_2 and pH was studied. *In vitro*, the mesenchymal stem cells (MSCs) were encapsulated in the hydrogels and cultured for 7 days with growth medium containing 100 mM H_2O_2. Cells encapsulated in the ROS insensitive hydrogel [3] were used as control. dsDNA content was measured by a Quant-iT PicoGreen dsDNA Assay Kit. To image cells, they were stained with live cell tracker CMFDA before seeding. To test *in vivo* biocompatibility, the hydrogels were injected subcutaneously into mice. After 4 weeks, the samples were collected and processed for Hematoxylin and Eosin (H&E) and F4/80 stainings.

Results: Chemical structure of the synthesized hydrogels was confirmed by ^{1}H-NMR spectra. Molar ratios of the components were consistent with the feed ratios. The hydrogels showed much higher degradation rate in H_2O_2 solution than in PBS, demonstrating that the hydrogels were ROS responsive. NMR spectra showed that -PB signals were disappeared in the final degradation product. The gelation time of the hydrogel solutions at pH=6.5 was faster than at pH=7.4. A substantial decrease in the LCSTs was found for the hydrogel solutions when decreasing pH. The hydrogel solutions can be readily injected through 26G needles at 4°C. MSC growth in the hydrogels was characterized by dsDNA content (for live cells). The results showed that cell number in the hydrogels increased during a 7-day culture period (**Figure 1**). When cultured in the medium supplemented with H_2O_2, cell survival was significantly higher in the ROS responsive hydrogels than in the control hydrogel without ROS responsiveness. These results demonstrated that the ROS responsive hydrogels have the potential to protect encapsulated stem cells when implanted in the ischemic tissues. To examine in vivo biocompatibility, the hydrogels were implanted subcutaneously using mouse model. H&E and F4/80 staining results showed that the hydrogels did not induce substantial inflammation response.

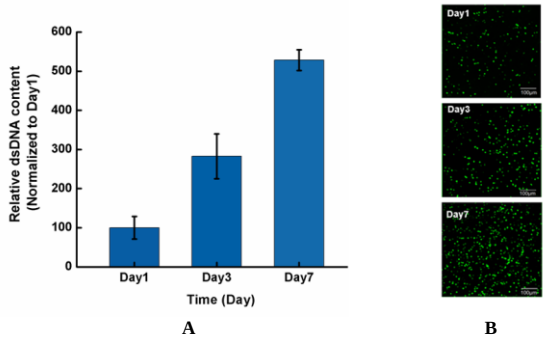

Figure 1. (A) dsDNA content of MSCs encapsulated in hydrogels; (B) Live cell staining of MSCs in hydrogels for a 7-days culture under normal condition

Conclusions: Triple-responsive hydrogels were developed and applied in stem cell therapy for tissue engineering. The hydrogels were injectable and capable of both pH sensitive and ROS sensitive. MSCs encapsulated in these hydrogels were capable of growth, proliferation and differentiation. Especially, the cell death rate in the hydrogels was significantly suppressed under H_2O_2 environment when compared with APLA hydrogels. *In vivo* tests further confirmed the non-toxicity and biocompatibility of the hydrogels.

References:

[1] Z. Li et al, Biomaterials. 2012; 33: 5914-5923.
[2] M. Ferrito et al, Macromol. Synth. 11 (1992) 59–62.
[3] Xu Y et al. Acta biomaterialia, 2015, 26: 23-33.