Development of novel injectable biodegradable elastomers for *in situ* tissue regeneration

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Statement of Purpose: Injectable materials are increasingly being explored for various biomedical applications such as drug delivery and tissue engineering due to their site-specific nature[1]. Injectable materials have advantages of being able to take the shape of a defect, avoiding the need for patient specific scaffold prefabrication. In addition to this, desired drugs and cells can be encapsulated in the injectable materials with a homogeneous distribution for cell and drug delivery in vivo. We acknowledge the need for an injectable biomaterials with 1) controlled mechanical and degradation properties; 2) short *in situ* crosslinking time in physiological conditions; 3) bioactive cues for improved cellular response in terms of cell proliferation and differentiation, and tissue growth; 4) in-situ forming porous structure for tissue infiltration and vascularization; and 5) excellent biocompatibility in terms of the material itself as well as any leachable material and the degradation product. Herein, we report a novel citric acid-derived biodegradable injectable polymer, Poly (poly (ethylene glycol) maleate citrate) (PPEGMC). The structural analysis, physio-chemical properties, bulk modification with biomolecules, cytocompatibility of networks, cell encapsulation, foreign body response of Poly (poly (ethylene glycol) maleate citrate) (PPEGMC) have been thoroughly investigated.

Methods: poly(ethylene glycol) (PEG 200), Citric acid, Maleic acid, Acrylic acid, Ammonium persulfate (APS), Tetramethylethylenediamine (TEMED) and RGD are purchased from Sigma-Aldrich (Milwaukee, WI) and used as received. Citric acid, PEG 200, Maleic acid were allowed to polycondensed at 135º C under nitrogen flow. Average molecular weight, chemical composition, and functionality of the pre-polmers were characterized by MALDI-MS, FTIR and ^{1}H-NMR. The pendant hydroxyl and carboxylic groups containing water-soluble pre-polymer were further covalently linked with RGD sequences using EDC/NHS chemistry. Pre-polymers were dissolved in water and crosslinked into elastic networks using APS and TEMED in presence acrylic acid under 365 nm ultraviolet light. Tensile mechanical properties of the crosslinked polymers were evaluated using a MTS Insight II mechanical tester. In vitro degradation of the polymers were evaluated in PBS at 37º C. Cytotoxicity of the prepolymers and the degradation products were evaluated by NIH-3T3 fibroblast culture. Cell viability and cell attachment were evaluated by culturing cells on and in the in-situ forming polymer networks. Foreign body response of PPEGMC was evaluated by injecting the in situ forming gel subcutaneously in 7-week-old Sprague-Dawley rats until totally absorbed by the body.

Results: The average molecular weights of PPEGMC were in the range of 1000-1500 Da. The pronounced peak in FTIR spectra within 1690–1750 cm^{-1} suggests the presence of carbonyl (C=O) groups from the ester bond and pendent carboxylic acid. The shoulder peak of lower wavelength at 1650 cm^{-1} proved the presence of double bond from maleic acid. Hydrogen bonded hydroxyl functional group showed absorbance as a broad peak centered at 3570 cm^{-1}. ^{1}H-NMR spectra confirmed that the composition of the polymers could be controlled by the feeding ratios of the monomers. Tensile mechanical tests on polymer samples showed that PPEGMC is a soft and highly elastic polymer with no permanent deformation. RGD chemistry was successfully incorporated to the prepolymer prior to crosslinking. Cytotoxicity evaluation demonstrated that RGD modified PPEGMC is "cell friendly". Foreign body response of the injected PPEGMC confirmed that this material could be crosslinked in situ within 3 minutes after being injected into the back of rats. Histology revealed that the polymers only induced slight inflammation and did not trigger tissue necrosis till they are fully absorbed in the body.

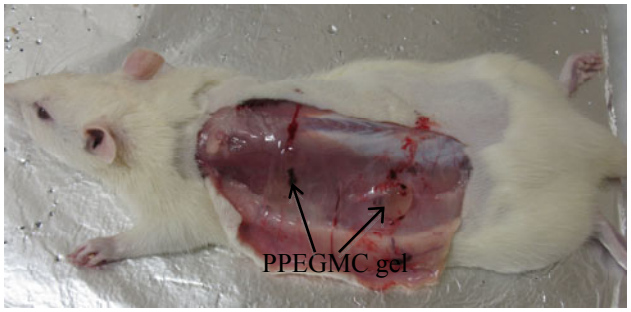

Figure 1: PPEGMC was injected subcutaneously and crosslinked in situ.

Conclusions: In this work, we have developed a new class of citric acid-derived injectable, synthetic, elastomeric, biodegradable polyesters that could be crosslinked under mild conditions within a short period of time in vivo. The water soluble pre-polymers can be injected to the area of interest to in-situ forming a biodegradable porous network with or without cell/drug. Available –COOH and –OH chemistries from the citrate units of the polymers can also be conjugated with other biomolecules for biofunctionalization. PPEGMC can be used as an injectable platform biomaterial for tissue engineering and drug delivery applications. We are under active investigation for large-bone defect regeneration using the newly developed injectable PPEGMC biomaterials.

References:

1. Gyawali, D, Tran RT, Guleserian KJ, Tang LP, Yang J. *Journal of Biomaterials Science: Polymer edition, 2009,* **in press**.