Reversible Addition-Fragmentation Chain Transfer in the Synthesis of Biodegradable Poly(2-hydroxyethyl methacrylate) Hydrogels for Cardiac Tissue Engineering Scaffolds

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Statement of Purpose: Cardiac tissue engineering faces many challenges, including the development of a biomaterial that can transition from *in vitro* culture to *in vivo* delivery. Hydrogels composed of poly(2-hydroxyethyl methacrylate) (pHEMA) are an appropriate starting point due to their biocompatibility, tissue-like physical properties, and flexibility in architecture. For use in cardiac tissue engineering, pHEMA hydrogels must support cardiomyocyte growth *in vitro* and degrade *in vivo* to soluble, nontoxic products. Reversible addition-fragmentation chain transfer (RAFT) can incorporate such properties by (1) introducing functional groups for protein immobilization to optimize cell attachment, (2) synthesizing a degradable pHEMA containing ester segments in the backbone and (3) maintaining tight control of molecular weight to ensure solubility and renal clearance of the degradation products.

Methods: Chemicals purchased from Sigma Aldrich, St. Louis, MO unless otherwise stated. RAFT chain transfer agent (CTA) 4-cyano-4-(dodecylsulfanylthiocarbonyl) sulfanyl pentanoic acid (DTPA) was synthesized according to the literature[1]. Degradable CTA containing poly(caprolactone) (PCL) was synthesized from PCL diol (MW ~530) and DTPA with N,N'-diisopropyl carbodiimide and 4-dimethylaminopyridine in dichloromethane to form PCL-DTPA. PHEMA linear polymer and crosslinked hydrogels were prepared with CTA in the presence of azobisisobutyronitrile, ethylene glycol and dimethylformamide at 60° C under nitrogen. CTAs and linear polymers were analyzed by ^{1}H NMR on a Bruker AVance series instrument at 300 MHz (Bruker BioSpin, Billerica, MA). Additional carboxyl moieties were introduced by copolymerization with methacrylic acid (MAA). Equilibrium water content and swelling ratio were measured. Elastic modulus was measured for dog-bone shaped hydrogel samples on an Instron 3340 series mechanical tester (Instron, Norwood, MA). Collagen Type I (BD Biosciences, San Jose, CA) was immobilized by 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) / N-hydroxysuccinimide (NHS) chemistry. Cytotoxicity of hydrogel eluent to NIH 3T3 fibroblasts was evaluated by morphology and MTT assay. Attachment and viability of cardiomyocytes differentiated from H7 human embryonic stem cells (hESC) were evaluated for contractile ability, by hematoxylin and eosin staining, and for the expression of cardiac Troponin T (cTnT) using immunohistochemistry (Clone 13-11, ThermoFisher Scientific, Waltham, MA).

Results: Two RAFT chain transfer agents were synthesized for the polymerization of pHEMA and pHEMA-*co*-MAA. DTPA was used to synthesize linear polymer at 5-100 kDa. ^{1}H NMR spectra of purified, low molecular weight chains (5-20 kDa) show characteristic peaks from DTPA and pHEMA in ratios similar to theoretical calculations. A degradable macroCTA agent capable of polymer extension at both ends was synthesized and used to polymerize pHEMA. 1H NMR analysis of linear polymers prepared by PCL-DTPA confirmed the presence of PCL and DTPA in the purified polymer. Hydrogels of controlled chain length were prepared by RAFT polymerization with DTPA, typically having 10 kDa chains at 4.5 mol% crosslinking. High water content (48.5%; 70.5% w/ 5 mol% MAA)) and swelling ratio (2.0; 3.4 w/ 5 mol% MAA) were measured, similar to that of standard pHEMA hydrogels. Tensile modulus was measured at ~320 kPa for pHEMA homopolymers that is in the range of living tissue. Collagen I was immobilized to hydrogel surfaces to enable cell attachment. Virgin and modified RAFT polymerized hydrogels were determined to be noncytotoxic. NIH 3T3 fibroblasts cultured in eluent of hydrogels exhibited no reduction in MTT activity; versus 99% reduction of latex positive control eluent. Human ESC-derived cardiomyocytes attached and spread on collagen-modified surfaces of RAFT pHEMA and maintained contractile function for the two weeks tested. Histological analysis showed cardiomyocytes with intact nuclei, healthy morphology and expression of cTnT, a component of the contractile apparatus.

Conclusions: RAFT was successfully applied to the controlled polymerization of pHEMA hydrogels. The solubility limit of linear pHEMA is ~10 kDa, and incorporation of PCL at the center of the chain doubles the chain length, enhancing mechanical properties and allowing for adequate renal clearance following *in vivo* degradation. PHEMA hydrogels are resistant to protein adsorption, so carboxyl moieties were incorporated and functionalized by the immobilization of collagen I. Carboxyl groups and EDC/NHS chemistry were chosen for protein attachment to prevent chain-chain crosslinks that could alter mechanical properties and interfere with degradation. Viability of cardiomyocytes seeded onto RAFT pHEMA hydrogels was confirmed by functional and histological analyses. The noncytotoxicity, ability to support cell growth and function, and degradability support the candidacy of RAFT polymerized pHEMA hydrogels as a cardiac tissue engineering biomaterial. Future studies will address the preparation of 3D architectures necessary for the culture of cardiomyocytes and subsequent implantation as a cell-based therapy of myocardial infarction.

References: 1. Moad, G. Polymer. 2005; 46: 8458-8468.