Crosslinked Carboxymethylcellulose Hydrogels: Versatile Platforms for Studying Cellular Behavior in 3D Biomaterials

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Purpose: Efforts to engineer complex tissue therapies will be dramatically improved by a better understanding of how cells interact with their dynamic 3D environments. Though we understand much about the roles of soluble factors and cell interactions, we have only recently begun to appreciate the roles of spatial organization and scaffold stiffness. Progress in this field is slowed because we lack appropriate materials to study stiffness-dependent cell behaviors as they occur in 3D environments. Thus, our goal is to develop new 3D biomaterials that are designed to a) mimic the properties of soft tissues, b) enable studies of stiffness-dependent cell processes, and c) provide novel mechanisms for manipulating key biomaterial physical properties. We present the development, characterization, and application of carboxymethylcellulose (CMC) gels as tunable platforms for investigating cell response in 3D biomaterials. CMC, a hydrophilic cellulose derivative, is an inexpensive, viscoelastic, biocompatible carbohydrate that is specifically degradable by cellulase. Cellulase is not expressed by mammals; yet the degradation of CMC by cellulase is a biocompatible process. Thus, as a uniquely degradable biomaterial, we are investigating processing tools to enable flexibility in CMC gel design. We present the synthesis and characterization of crosslinked CMC gels and two basic applications: cell-adhesive scaffolds and microspheres for encapsulation and gel porogen applications. Systems of interest are neural regeneration and in vitro models of breast cancer; however, these versatile CMC-based gels could be implemented in a variety of systems where tunable gel scaffolds are required.

Methods: Reagent source was Sigma-Aldrich or else noted. *CMC Modification:* CMC (90 kDa) was rendered photoreactive by modification with aminoethylmethacrylate (AEM) and ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC) (2 molar excess to CMC carboxylates) in pH 8.5 buffer for 2 h [1]. The CMC-methacrylate product was dialysed, dried and stored frozen. Modification was verified with ^{1}H-NMR [2] and a carboxylate assay [3]. *Gel Synthesis and Characterization*: CMC-methacrylate (0.5-16%) was dissolved alone or with 8 kDa polyethylene glycol dimethacrylate (PEG-DM; 4-12%; [1]) in phosphate buffered saline containing 0.05% Ciba Irgacure 2959 and crosslinked by 365 nm UV light for 1-5 min. To measure the impact of polymer and crosslink density on gel properties, we performed swelling and rheological analyses, characterized gel porosity (i.e., effective diffusivity of bovine serum albumin [2,4]) and measured gel degradation by cellulase [3]. *Cell Response:* To render the gels adhesive to fibroblasts, acrylate-PEG-RGD (1-6 μmol/ml) was covalently bound into the gels during crosslinking [5]. NIH3T3 fibroblasts were seeded on 2D or within 3D gels. Biocompatibility was measured by a proliferation assay (Promega) and cytotoxicity staining (Invitrogen). Cell adhesion on 2D gels was quantified with phase microscopy and spreading in 3D gels was verified with FITC-phalloidin F-actin stain (Invitrogen). *Microspheres*: To synthesize CMC microspheres, a PBS solution of 1-3% CMC-methacrylate with 0.1% ammonium persulfate and 0.1% TEMED was sonicated in oil for 1-2 h during crosslinking and then the spheres were washed with hexane and acetone-water mixtures. Sphere diameters were measured with a Coulter Multisizer III and phase microscopy.

Results/Discussion: Physical characterization of CMC gels revealed that increased CMC content in copolymer gels increases swelling, porosity and degradation while decreasing gel stiffness (**Fig 1**). Specifically, for gels with 12% total polymer, those with 8% CMC-methacrylate had increased swelling, porosity (De/Do) and degradation rate compared to gels with 5% CMC. The trend for gels with 20% polymer is consistent: gels with increased CMC:PEG have a lower shear modulus and higher porosity. (Swelling and degradation studies of 20% gels are ongoing.) These results suggest that a increased ratio of CMC:PEG correlates with decreased crosslink density. Cell studies indicate that crosslinked CMC gels support cell adhesion and viability in 2D and 3D culture. Microspheres have been synthesized of average diameter 1.2±0.5 μm (**Fig 2**). Current work focuses on utilizing CMC microspheres to process macroporous PEG gels, expanding gel physical property range by varying polymer molecular weight, and investigating cellular response to changes in 3D gel physical properties.

Conclusions: We show that crosslinked CMC gels span a physical property range relevant for studies of cell response in soft 3D biomaterials, offer unique advantages as tunable and processable scaffolds, and thus hold promise for a variety of applications where 3D scaffolds are required.

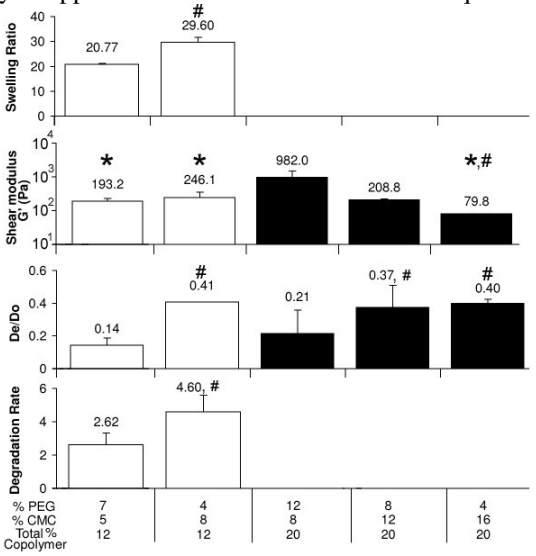
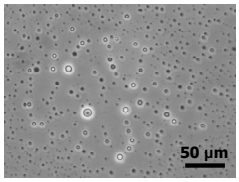


Fig 1 (above). Gel Physical Properties. D_e/D_o, effective diffusivity of encapsulated BSA in the gel normalized by the diffusion of BSA in water (i.e., 9.1e10^{-7} cm^2/s). Degradation rate, % mass loss of gel per h in 0.02 u/ml cellulase. Statistical significance: # vs 5% CMC/7% PEG; * vs 12% CMC/8% PEG. ***Fig 2 (right). CMC Microspheres*** (phase microscopy).



References: [1] L Lombardo et al., in prep. [2] J Leach et al *Biotechnol Bioeng* 2003, 82:578. [3] H Kosters & H deJongh *Anal Chem* 2003, 75:2512. [4] J Leach et al. *Biomaterials* 2005, 26:125. [5] D Hern & J Hubbell *JBMR* 1998; 39:266; J Leach et al. *JBMR* 2004, 70:74.