Polymer Hydrogels for Cell Encapsulation

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INTRODUCTION AND OBJECTIVES

Cell-based therapy for endocrine disorders is a branch of regenerative medicine aimed at developing cells that express hormones or enzymes missing in a patient. Key examples include cells that express insulin in response to glucose levels, and that can be transplanted into diabetics. Polymer hydrogels have emerged as a critical tool for both stem cell differentiation, and the immuno-isolation needed to bring such therapeutic concepts to clinical reality. Classic hydrogels such as alginate-polylysine-alginate (APA) capsules were often based on electrostatic interactions, while currently there is a drive towards covalently crosslinked hydrogels that may serve both as immuno-isolation matrices, and indeed as synthetic extracellular matrices for stem cell development.

We develop self-crosslinking polyelectrolyte pairs for use in both areas. These polyanions and polycations form liquid or gelled polyelectrolyte complexes (PECs), and that crosslink through reaction between their functional groups, as illustrated in Figure 1. These reactive polymer pairs may be coated onto calcium alginate cores to form crosslinked hydrogel capsules and beads with good cell and host compatibility. Key to the approach is the covalent immobilization of the inflammatory polycations, and hydrolysis of residual electrophiles to carboxylates that enhance the net anionic charge of the hydrogels.

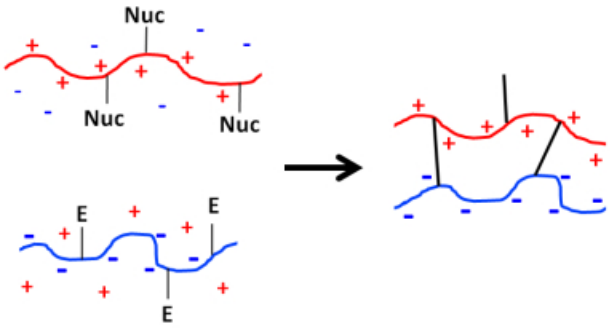

Figure 1: Covalent crosslinking of PECs.

Other versions involve zwitterionic polymers and polyamphiphiles, or use Diels-Alder chemistry for crosslinking. This talk will present the design of these gel formers, and some results from mouse models, conformal cell coatings and hydrogel films.

MATERIALS AND METHODS

Materials: Reactive polymers are prepared by thermally or photochemically initiated free radical copolymerization of reactive monomers with polar comonomers, in inert organic solvents such as tetrahydrofuran or dimethylsulfoxide (DMSO). Examples are copolymers of vinyldimethylazlactone (VDMA) with methacrylic acid (MA) or 2-methacryroyloxyethyl phosphorylcholine (MPC). Another example involves partial (50%) hydrolysis of commercial poly(methylvinylether-alt-maleic anhydride) (PMM) to form a watersoluble polyanion still containing 50% reactive anhydride groups.

The reactive polyanions are paired with watersoluble polyamines including commercial poly-L-lysine (PLL), copolymers of aminopropyl-methacrylamide (APM) with hydroxypropyl-methacrylamide (HPM), and copolymers of aminopropylmethacrylamide with anionic comonomers produced by free radical copolymerization in ethanol.

Reactive polymer pairs intended to crosslink by Diels-Alder chemistry are formed by modifying PMM as common backbone with 10 mol% of aminoethylfurane (diene) and aminoethylmaleimide (dienophile).

Capsule formation: Surface-crosslinked calcium alginate capsules containing C2C12 murine model cells are prepared by extruding a suspension of 2x6^10 murine C2C12 models cells/mL in 1.6w% sodium alginate (70:30 G:M, UMV, Novomatrix) into a calcium gelling bath. The resulting capsules are coated with polyamine and reactive polyanion, which electrostatically assemble at the capsule surface and spontaneously crosslink to form a permanent hydrogel shell. Alternatively, one or both of the reactive polymers can also be added to the sodium alginate cell suspension, leading to formation of core-crosslinked network beads that persist after removal of calcium.

Film formation: These reactive polymers can also form micron-range hydrogel films by either layer-by-layer deposition onto flat substrates, or by bulk deposition of a self-gelling mixture.

Characterization: Polymers are routinely labelled with fluorescein or rhodamine to enable mapping their distribution within capsules and films by confocal microscopy. Mechanical properties are determined using capillary aspiration and single bead uni-axial compression. Cell viability is tested using MTT and live/dead assays. Host compability is tested through the degree of fibrotic overgrowth after explantation from mouse peritoneal cavities, as well as by

measuring the levels of inflammatory cytokines in tail vein blood samples.

RESULTS AND DISCUSSION

Coating of partly hydrolyzed poly(methylvinylether-*alt*-maleic anhydride (PMM_{50}) onto calcium alginate beads previously coated with poly-L-lysine (PLL), leads to amide crosslinks between the polyamine and the anhydrides in PMM_{50} (Gardner 2011a). Crosslnking is followed by spontaneous hydrolysis of residual anhydrides to yield, in minutes, the highly anionic and crosslinked capsules shown in Figure 2.

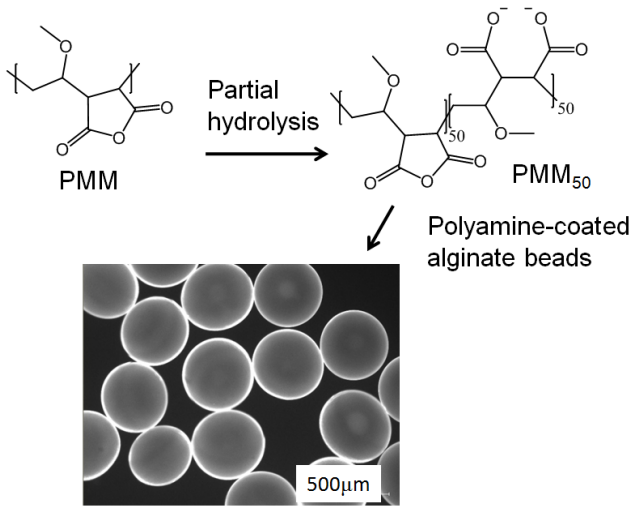

Figure 2: Covalently crosslinked hydrogel shells formed by amide reaction between PMM_{50} and PLL.

Copolymers formed from vinyldimethylazlactone with methacrylic acid, acrylamide, dimethylacrylamide, and 2-methacryroyloxyethyl phosphorylcholine (MPC), with VDMA content between 50 and 85%, show a rich spectrum of watersolubility and rate of hydrolysis. High VDMA content copolymers with methacrlic acid and MPC in particular, are promising candidates as crosslinked hydrogel formers due to their reduced rate of hydrolysis of residual electrophilic azlactones (Figure 3, Gardner 2011b).

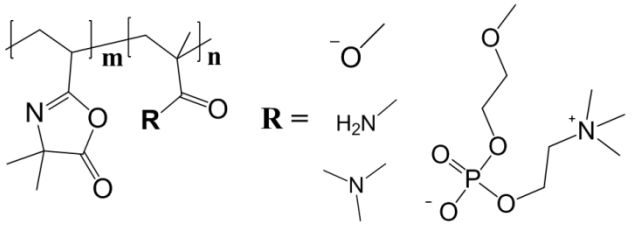

Figure 3: Copolymers of VDMA with anionic and neutral comonomers.

We currently focus on developing lower cationic charge density polyamines such as poly(aminopropyl-methacrylamide-co- hydroxypropylmethacrylamide) with APM:HPM ratios of 50:50, 25:75, and 10:90. These synthetic chitosan analogs are seen as having lower inflammatory potential in bioapplications than PLL, in analogy to Chaikofs' PEG-modified PLLs.

In addition, we are exploring the use of nucleophilic polyampholytes based on copolymers of aminopropylmethacrylamide with methacrylic acid. In the resulting copolymers the cationic charge is neutralized by anionic comonomer, whoile preserving its ability to crosslink with reactive polyanions as described above. The resulting hydrogel networks are expected to retain the good antifouling properties usually asociated with polyampholytes.

We are also exploring croslinking chemistry not reliant on nucleophiles. Poly(methylvinylether-alt-maleic anhydride) modified with furane, and maleimide groups, can be combined with calcium alginate templates to form Diels-alder crosslinked hydrogels that persist after removal of the calcium alginate template (Figure 4). These sets of reactive polymers have the advantage of slow crosslinking, permitting formation of thin films as well as capsules and other bulk gels after one-shot deposition of the reactive mixture.

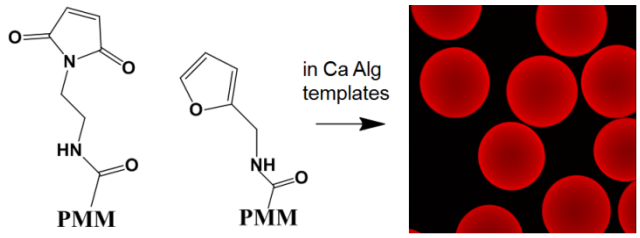

Figure 4: Diels-Alder crosslinked capsules.

CONCLUSIONS

The above reactive polymers form the basis for the development of synthetic extracellular matrices for use in encapsulation and immuno-isolation of therapeutic cells, as well as possibly for formation of 2D and 3D cell supports for stem cell research.

REFERENCES

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