Synthetic Polymers Hydrogels for Cell Encapsulation and as Cell Supports.

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INTRODUCTION AND OBJECTIVE

Stem cell research is increasingly focussing on the effects of the extracellular matrix (ECM) on key properties such as maintenance of pluripotency, expansion, and specificity during expansion differentiation. Synthetic extracellular matrix mimics may provide tunable ECMs that are pathogen-free, fully defined, and can enable larger-scale production. At the same time, synthetic ECMs can play other roles in helping to move stem cells from the research stage to the clinic, including direction of differentiation of encapsulated cells, and protection of transplanted cells from the hosts' immune system (Figure 1).

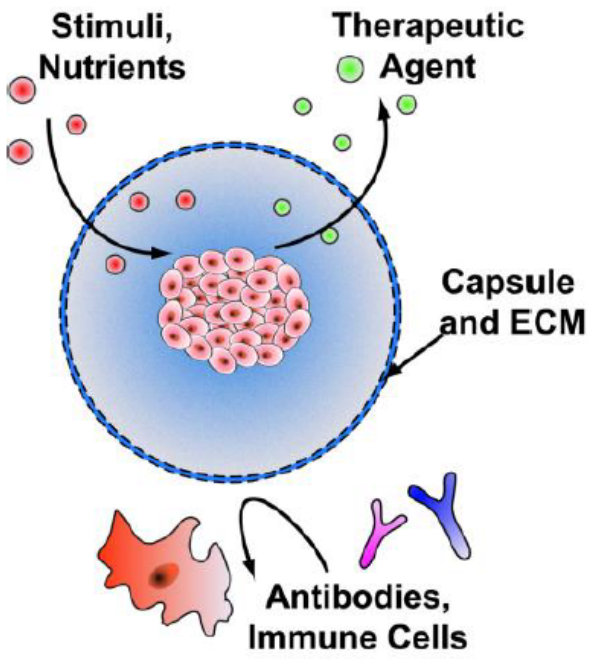

Figure 1. Polymer Capsules for Cell Immuno-Isolation

This talk will highlight our approach to synthetic polymers that may be used to form crosslinked hydrogels for both stem cell research and therapeutic cell encapsulation.

MATERIALS AND METHODS

Poly(methylvinylether-alt-maleic anhydride) (PMMAn) was purchased and heat-treated to reform the fully anhydride form as described previously (Gardner 2010).

Diels-Alder modified PMMAn was prepared by reaction of aliquots of PMMAn dissolved in acetonitrile, with furfurylamine (diene component) and 2-(2-aminoethyl) maleimide (dienophile component), followed by hydrolysis of residual anhydride groups. Degree of crosslinking was followed by magic angle spinning ^{1H} NMR in D_2O.

Copolymers of aminopropylmethacrylamide (APM) and hydroxypropylmethacrylamide (HPM) were prepared by RAFT controlled radical copolymerization. Copolymers of APM and methacrylic acid (MAA) were prepared by free radical copolymerization, and characterized by potentiometry, and temperature controlled transmissivity.

Polymer films were prepared by LbL deposition of reactive polymers, as well as by bulk deposition of premixed polymer solutions, followed by curing and chemical post-modifications.

RESULTS AND DISCUSSION

Our work is build on the premise that synthetic extracellular matrices can be designed on the basis of fairly common co-polymer backbones that are modified to include key functions such as the ability to attach fluorescent labels, form covalent crosslinks, and control both hydrophobic/hydrophilic balance and charge distribution.

These polymer backbones include polymethacrylic acid that was modified by copolymerization of fluorescent comonomers, as well as electrophilic comonomers such as methacryloyloxyethyl acetoacetate (MEAA). The resulting reactive polyanions were combined with polyamines such as poly-L-lysine to form crosslinked hydrogel beads and capsules (Mazumder 2009).

Poly(methylvinylether-alt-maleic anhydride) (PMMAn), (Figure 2) also serves as a versatile scaffold. Here, some of the anhydride groups were hydrolysed to form a water-soluble polymer that retained enough anhydride groups to form covalent crosslinks upon combination with poly-L-lysine-coated alginate beads. Animal experiments showed minimal immune responses, which was attributed to both the covalent immobilization of the poly-L-lysine, and the excess anionic charges generated by hydrolysis of residual anhydride (Gardner 2010, Gardner 2012).

This talk will review recent work on these synthetic ECM mimics, including results on crosslink stability

and opportunities for decrosslinking, and film formation.

The talk will also describe our current work involving pairs of diene and dienophile modified PMM that can undergo mutual crosslinking through the bioorthognal Diels-Alder reaction.

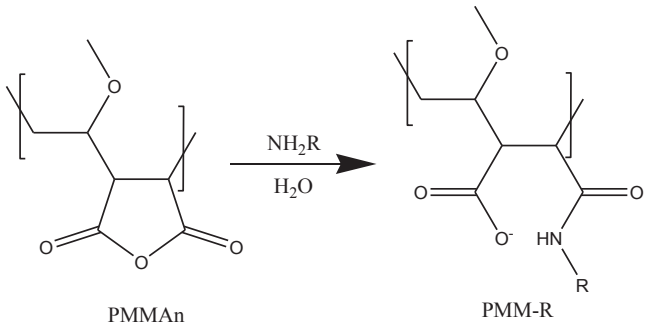

Figure 2. Forming Diene and Dienophile-modified polyanions from a common PMMAn precursor

The resulting Diels-Alder modified PMMAn polymers may be combined with conventional alginate technology to form spherical Diels-Alder-crosslinked beads (Figure 3), used as reactive injectables, or used as 2D and 3D film formers.

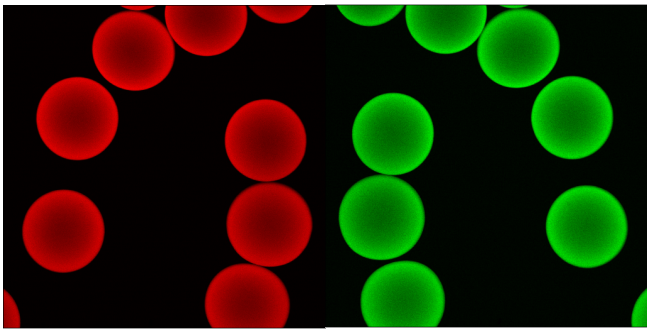

Figure 3. Diels-Alder Crosslinked PMMAn Gels

Finally, the talk will describe amine-functional copolymers designed to replace poly-L-lysine as polycation/polynucleophile in hydrogel forming processes used today (Figure 4).

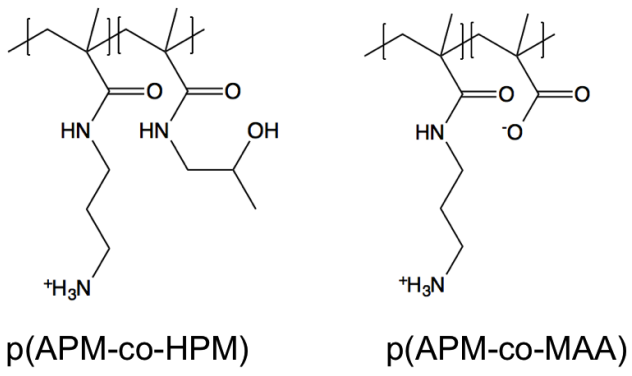

Figure 4. Cytocompatible Amine-functional Copolymers

These copolymers have charge densities varying from cationic through neutral to net anionic, while retaining nucleophilic amines useful for crosslinking with a variety of polyelectrophiles including PMM.

Controlled polymerization using RAFT has been used to prepare narrow MW PAM/HPM copolymers, and the effect of MW and composition on cytotoxicity evaluated using MTT and Alamar Blue assays.

We are exploring the use of these polymers to form homogeneous hydrogel films in 96 well plate format, to enable screening experiments for stem cell development. One of the key issues in use of multi wall plates is the formation of meniscii due to different surface tensions between well surfaces and the polymer solutions.

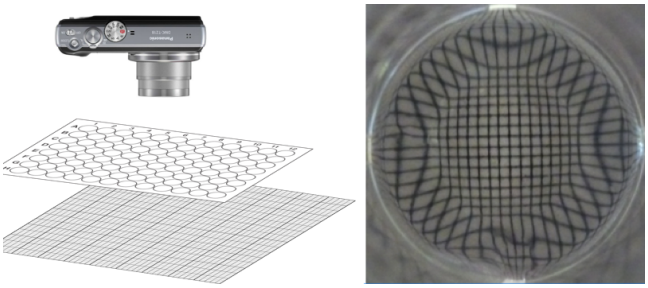

Figure 5. Optical Profiling of Meniscii in Multiwell Plates coated with Polymer Hydrogel.

In addition, the internal charge compensation in the p(APM-co-MAA) polyampholytes results in solubilities that can be controlled through small changes in pH, salinity, and temperature, permitting control over coacervation of these reactive copolymers.

CONCLUSION

Synthetic polymer hydrogels can serve as extracellular matrices in a variety of roles related to stem cell research.

REFERENCES

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ACKNOWLEDGMENTS

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