

## Novel Protein-Binding Phospholipid Polymer Hydrogels for Regenerative Medicine Applications

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### Statement of Purpose:

For various types of mucosal tissue injuries due to irritant chemicals or surgical removal of cancer, very few effective treatment approaches exist. The long-term goal of this study is to develop an approach for coating of injured mucosa with biocompatible phospholipid polymer layer and delivering stem cells that will ultimately differentiate into epithelial cells to regenerate the mucosal tissue. In pursuit of this goal, we are currently designing a layer-by-layer cell delivery approach using an activated phospholipid polymer, which forms a strong bond with biological tissue while it is also reactive with a second layer of a polymer hydrogel encapsulating stem cells and bioactive molecules for differentiation of the cells into the target lineage. More specifically, we are currently synthesizing and characterizing a copolymer of 2-methacryloyloxyethyl phosphorylcholine (MPC), *n*-butyl methacrylate (BMA), and *p*-vinylphenylboronic acid (VPBA), and *N*-hydroxysuccinimide oligo(ethylene glycol) methacrylate (PENHS) (PMBVS). It has been hypothesized that this NHS-containing polymer can form a strong bond with the tissue surface through the reaction of activated carboxylate groups and amino groups of tissue proteins and subsequent addition of poly(vinyl alcohol) (PVA) will form a hydrogel layer that allows for cell immobilization. Moreover, because of the low protein adsorption property observed on the MPC polymers, the encapsulated cells will be protected from undesired bioactive molecules present in the external environment.

### Methods:

#### Polymer synthesis

PMBVS was synthesized via radical random copolymerization of monomers, MPC, BMA, VPBA and PENHS dissolved in ethanol (0.5M) at 50:20:20:10 ratio in the presence of an initiator, azobisisobutyronitrile (1/100 of monomer concentration) at 60 °C for 6.5 hours.

#### Assessment of polymer function

The reactivity of PMBVS to proteins was evaluated using surface plasmon resonance (SPR) analysis with collagen-coated substrates. Briefly, a self-assembled monolayer (SAM) of carboxylic acid (–COOH) was prepared on gold-coated SPR substrates (ALTECH CO., LTD., Tokyo, Japan). Using the carbodiimide-NHS chemistry, the COOH-SAM was activated and type I collagen (100 µg/mL) was immobilized on to the surface of substrates. A solution (10 mg/mL) of PMBVS in phosphate buffered saline (PBS) was flowed over the collagen-coated substrate and the angle shift was measured for up to 25 minutes with SPR (SPR-670M, Moritex Co., Tokyo, Japan). A solution (10 mg/mL) of PMBV (without NHS

units) was used as a control. The total polymer adsorbed onto the surface was estimated from the resonance angle shift in ng/cm<sup>2</sup>.

### Results:

The results of <sup>1</sup>H-NMR spectroscopy revealed that the actual molar ratio in the product was 54/16/27/3 (MPC/BMA/VPBA/PENHS) and the yield was approximately 30%. When a solution of PMBVS in PBS (5.0 wt%) and the same volume of PVA (degree of polymerization = 1000) in PBS (2.5 wt%) were mixed at room temperature, gelation began to occur within 1 minute.

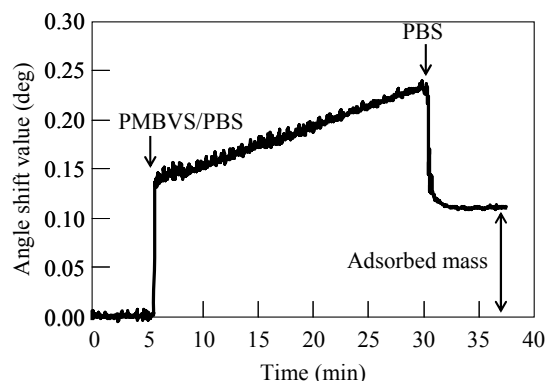


Figure 1. SPR result to demonstrate adsorption of PMBVS to collagen-coated substrates.

The results of SPR revealed that flowing a PMBVS solution over a collagen-coated substrate for 25 minutes led to a significance change in angle shift of 110 mDA, which was absent when a PMBV solution was flowed over a similar substrate surface. The amounts of polymer absorbed, which were estimated from the values of angle shifts, were 55 ng/cm<sup>2</sup> and less than 5 ng/cm<sup>2</sup> for PMBVS and PMBV, respectively. These results suggest that the addition of NHS units to the copolymer led to addition of the protein-binding feature.

### Conclusions:

The results of the present study provided evidence that the new formulation of phospholipid polymer, PMBVS exhibited a hydrogel forming property similar to the cell-encapsulating PMBV/PVA gel previously reported (Konno T, *Biomaterials*. 2007; 28: 1770-7). More important, the present study demonstrated that we have synthesized a protein-binding MPC polymer that can be used to coat biological tissue surfaces. Together, these results suggest that the new polymer synthesized in the present study will allow immobilization of hydrogel-encapsulated cells on tissue surfaces that need repair and regeneration. Further studies, however, are necessary to validate the efficacy of such a cell delivery system.