

Fibrin Targeted Block Co-polymers for the Prevention of Postsurgical Adhesions

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Statement of Purpose: Despite advances in surgical techniques and prevention technologies, postsurgical adhesions (PSA) remain a significant clinical challenge affecting millions of patients each year[1]. These permanent fibrous connections between tissues result from the bridging of wounded internal surfaces by an extended fibrin gel matrix (FGM). The formation of these adhesions is a result of a systems level convergence of wound healing pathways, complicating the design of materials that could inhibit their occurrence. Because deposition of fibrin is a primary event in the wound healing process, it is hypothesized that fibrin would make an ideal marker of pro-adhesive sites, thereby providing a mechanism to direct the formation of a self-forming protective polymer layer. Application of fibrin-homing polymer molecules during the initial stages of wound healing should interrupt the formation of this extended FGM and serve as an effective means to prevent PSA.

To identify the key molecular parameters that dictate barrier function, a series of poly(ethylene glycol methacrylate-*b*-methacrylic acid) (PEG-PMA) block copolymers was synthesized and subsequently functionalized with targeting peptides (CREKA) to generate a variety of permutations of molecular architecture. These design parameters were then used to probe the state space of the polymers' functional performance. Four independent variables were investigated, and their effects were evaluated in four response metrics. The effect of changes to these structural variables was assessed by measuring the performance of the materials *in vitro* prior to undertaking *in vivo* testing.

Methods: All polymers used in this investigation were prepared as has previously been reported[2,3]. Quartz crystal microgravimetry was used to directly identify polymer capacity to inhibit fibrin deposition. Briefly, gold coated quartz surfaces were modified with a fibrin layer. Polymer solutions were then flowed over the crystal, followed by a washing step and a new fibrinogen solution (1mg/ml). The mass of fibrinogen deposition was then monitored. Cell binding studies were also performed.

In vivo studies were conducted based upon a previously published murine model[4]. In 7 week female BALB/c mice, a "double injury" wound induction involved peritoneal excision/window formation and peritoneal abrasion. Prior to complete closure, 0.25 ml of saline or polymer (1mg/ml) was delivered into the peritoneum. To control for bias, sample administration was randomized by coin flip and blinded to the surgeon. After 2 weeks, adhesion formation was assessed and scored. Adhesion Scoring Group classification (extent,

severity, and degree of adhesions) was determined in a blinded fashion by three independent observers using a previously reported scoring rubric[5].

Results: Polymers conjugated with CREKA were able to suppress fibrin deposition in QCM studies, demonstrating the ability to reduce the adhesiveness of a model wound surface as compared to the No polymer (NP) and pure CREKA controls (Figure 1). Studies also demonstrated that only diblock PEG-PMA targeted block copolymers both inhibit fibrin deposition and cell attachment *in vitro*.

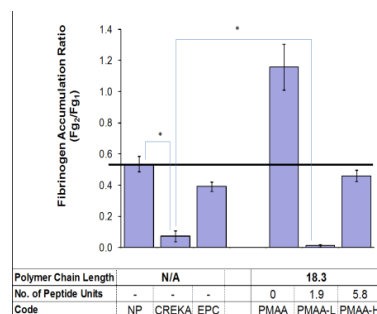


Figure 1. Fibrinogen adsorption ratios as a function of blocking material. Adsorption ratio was calculated as the ratio of frequency decreases in the fibrin coating step prior to and after the blocking step.

Best performing *in vitro* polymers were then assayed in an *in vivo* animal study. All mice behaved normally and showed similar weight gains, suggesting no significant adverse effects of the polymer administration. A statistical improvement in the degree of adhesions was observed (2.4 ± 0.13 vs. 2.8 ± 0.06 for control group, $n=6$, $p=0.015$). Statistical evaluation of the polymer state space identified key material properties that can be tuned for further improvement of the anti-PSA targeted polymer approach.

Conclusions: Fibrin targeted block copolymers demonstrated the ability to suppress fibrin deposition and cellular adhesion. A complex relationship between material properties and performance was identified, providing insights into the further development. A single infusion of a blocking polymer was able to reduce the degree of observed adhesions at sites of peritoneal injury but not at synthetic sites (e.g., adhesions to the suture knot), suggesting the potential use of targeted polymers as post-surgical adhesion suppressing agents.

References:

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