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Fibrin Targeted Block Copolymers for the Prevention of Postsurgical Adhesions

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Abstract

Despite advances in surgical methods, postsurgical adhesions (PSA) remain a significant clinical challenge affecting millions of patients each year. These permanent fibrous connections between tissues result from the bridging of wounded internal surfaces by an extended fibrin gel matrix (FGM). Adhesion formation is a result of a systems level convergence of wound healing pathways, complicating the design of materials that could inhibit their occurrence. In this study, a systematic approach that identifies key material properties required for functional performance optimization was used to design a new fibrin-targeted PSA prevention material. A series of multifunctional polymers with varied molecular architectures was synthesized to investigate the effect of changing polymer structural parameters on the ability to disrupt the formation of an extended FGM. Initial studies in a murine adhesion model demonstrated a statistically significant reduction in the degree of PSA formation, demonstrating the potential value of this systematic approach.

Keywords

Post-surgical adhesion; Fibrin; in vivo; Block Copolymer; CREKA

Introduction

Each year, approximately 300,000 patients undergo hospital readmissions for the removal of postsurgical adhesions (PSA), soft tissue bridges which form as a result of surgical trauma ^{1,2} During healing, damaged tissue can become permanently attached to adjacent

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surfaces through the formation of a fibrous scar. Patients can experience abdominal or pelvic pain, intestinal obstructions, infertility, and increased difficulty in subsequent surgical procedures³⁻⁵. Based on the significant financial costs and the immeasurable cost in terms of patient discomfort, the need to eliminate or reduce the incidence and severity of PSA is apparent.

A key step in the formation of PSAs is the bridging of tissue surfaces by a fibrin gel matrix (FGM). Because the primary component of the FGM is fibrin, it is hypothesized that this protein is a preferential marker of pro-adhesive sites, providing a mechanism to direct the formation of a protective polymer layer. Indeed, the likely efficacy of an approach that disrupts the formation of an extended FGM has been demonstrated in several studies focused on the application of fibrinolytic agents to reduce PSA formation⁶⁻⁸. Because of their ability to break down existing fibrin clots, however, these treatments have been shown to increase the risk of serious complications, including hemorrhage⁹⁻¹¹.

In an effort to reduce PSA occurrence, we have applied a quality-by-design (QbD) approach¹² to develop a fibrin-targeted aqueous polymer that can bind to the FGM, and inhibit the early stages of PSA formation. The key elements to this approach include the linking of material properties to critical attributes and the identification of a design space that allows for future improvements and developments. This rational approach to material design facilitates the relationship between basic fundamental material science and practical commercialization. To identify the key parameters, a series of poly(ethylene glycol methacrylate-*b*-methacrylic acid) block copolymers was synthesized and subsequently functionalized with the fibrin targeting peptide, CREKA, to generate a variety of permutations of molecular architecture, as shown in Figure 1(a). These design parameters were then employed to probe the state space of the polymers' functional performance. Four independent variables were investigated, and their effects were evaluated in four response metrics (Figure 1(b)). 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.

Materials and Methods

Polymer Preparation

All polymers used in this investigation were prepared as has previously been reported and are described in Table 1^{13,14}. Briefly, protected poly(ethylene glycol methacrylate)-*b*-poly(methoxymethacrylate) block copolymers were synthesized using group transfer polymerization. After hydrolysis of the methoxy group, the polymers were functionalized with targeting peptides. Polymer solutions were prepared by dissolving polymer in Tris buffered saline (TBS) at the desired concentration and were stored at -20 °C prior to use.

Quartz Crystal Microgravimetric (QCM) Analysis

QCM was used to assess the ability of materials to protect fibrin surfaces from subsequent fibrinogen deposition, as has previously been described¹³. Shown schematically in Figure 2(a), the experimental protocol allowed the direct assessment of fibrin adsorption to surfaces. As a control, the same procedure was followed without the addition of polymer

solution or the subsequent buffer rinse. Each material was evaluated in a minimum of three experiments. By monitoring changes in the resonant frequency of the sensor crystal as it was exposed to these solutions, it was possible to measure both the relative amount of fibrinogen adsorbed to the surface and the rate of surface adsorption (Figure 2(b)). The maximum fibrinogen adsorption observed was determined using a least squares analysis in Excel®. In order to control for variations in sensor crystal response, the frequency change for the second fibrinogen adsorption step was then normalized to the first fibrinogen response and is reported as a ratio (Fg_2/Fg_1). The pseudo-first order kinetic half-life ($t_{1/2}$) of the second fibrinogen adsorption step was also extracted from these analyses.

Microplate Turbidity Analysis

A microplate assay was employed to monitor the reaction of a surface-bound fibrin with solution phase fibrinogen after interaction with various materials. This technique facilitated the investigation of the kinetics of the fibrin gel propagation and of the structure of the resulting gel. Previous studies have demonstrated that surface-formed fibrin layers retain approximately five active thrombin molecules per fibrinogen molecule¹⁵. These surfaces, then, can react with solution-phase fibrinogen to form a fibrin gel on the surface. A stable gel surface was prepared in Costar high binding 96-Well EIA/RIA plates. Fibrinogen (50 μ L, 2.0 mg/mL), thrombin (20 μ L, 2.5 U/mL), and calcium chloride (10 μ L, 100 mM) were added to each well, incubated for 5 hours at ambient conditions, and rinsed twice with 200 μ L of TBS. Subsequently, a polymer solution (50 μ L, 0.10 mg/mL in TBS, $n = 4$) was added. Finally, 150 μ L of fibrinogen (0.5 mg/mL in TBS) was added to each well, and the UV absorbance was monitored at 350 nm every two minutes using a Cary-50 spectrophotometer with microplate reader (Varian, Santa Clara, CA). The initial rate of increase in UV absorbance was used to probe the rate of fibrin propagation from the gel surface.

Cellular Attachment

In order to test the anti-cellular adhesive properties of these materials, a cell culture study was carried out using the mouse mesenchymal D1 cell line (ATCC CRL-12424) in fibrin-coated 24-well tissue culture plates. This pluripotent cell line was selected due to its strong adherence to charged surfaces and wound healing components (e.g., collagen I and fibrin)¹⁶⁻¹⁸. Cell attachment studies were performed using a modification of a previously published procedure¹⁴. In each well, 152 μ L fibrinogen solution (13.2 mg/mL) were mixed with 40 μ L $CaCl_2$ (100mM) solution and 8 μ L thrombin (5U/mL) solution and stored overnight at 4° C. After rinsing 3 times with sterile TBS, polymer solutions were added (200 μ L, 0.10 mg/mL in sterile TBS) and incubated at 37 °C for 90 minutes. Wells were then triple rinsed with sterile TBS to remove unbound polymer, seeded with 250,000 D1 cells in 500 μ L Dulbecco's Modified Eagle Medium (DMEM, HyClone Laboratories) containing 10% fetal bovine serum (GIBCO/Invitrogen), and incubated at 37 °C for two hours. After incubation, unattached cells were removed from the wells by triplicate rinses with sterile TBS. Attached cells were lysed by sonication in high salt solution (0.05 M NaH_2PO_4 , 2 M NaCl, and 2 mM EDTA) and analyzed for DNA content via Hoechst 33258 staining^{19,20}. Untreated fibrin gels were used as controls, and a minimum of 6 replicates were studied for

each material. Representative images of the cells were obtained using an inverted phase contrast microscope.

***In Vivo* Postsurgical Adhesion Model**

The ability of materials to suppress postsurgical adhesions was determined in a published murine model previously used in mechanistic adhesion formation studies²¹. All methods were approved and performed in accordance with the guidelines set by the University of Kentucky Institutional Animal Care and Use Committee. While a variety of methods of adhesion induction are available²²⁻²⁹, previous work has demonstrated that a “double injury” wound induction method allows for standardization of the type and extent of adhesions³⁰. Administration and subsequent scoring of animals were done in a blinded fashion to control for operator bias.

Six week old female specific germ free (SPF) inbred BALB/c mice (weight 20g) were obtained from Harlan laboratories (SD Harlan, IN) and acclimatized for 10 days prior to the experiment. In this work, a “double injury” wound induction involved peritoneal excision/window formation and peritoneal abrasion. To minimize operator's variability, all surgical procedures were performed by the same experienced surgeon.

Mice were anesthetized by intramuscular injection of ketamine hydrochloride (80 mg/kg) and xylazine (10 mg/kg) with addition of isoflurane inhalation as required. After pre-operative preparation (shave and disinfection), a midline laparotomy incision was created from the xiphoid caudally to bladder reflection of parietal peritoneum caudally. Then, both peritoneal surfaces of the lateral abdominal wall were exposed. Two wound sites were assigned for the evaluation. The first, a 1 cm peritoneal excision/window, was created on the right side of the animals' lateral abdominal wall approximately 1 cm from midline incision. The second wound site was generated by gauze abrasion. As abrasion has been considered to be the most difficult method to standardize, the injury was administered with abrasive swipes (×5) with equal strength to improve the consistency of injury^{31,32}. This abrasion was performed on the left side of the animals' lateral abdominal wall approximately 1 cm lateral to midline incision. The surgical site was closed with 4-0 Vicryl suture in full thickness closure. Prior to complete closure, 0.25 ml of saline or polymer was delivered into the peritoneum. To control for bias, sample administration was randomized by coin flip and blinded to the surgeon (n=8). After 2 weeks, adhesion formation was assessed and scored. The abdomen was opened with an inverted U-shaped laparotomy incision. Adhesion Scoring Group classification (extent, severity and degree of adhesions) was determined in a blinded fashion by two independent observers (Table 2)^{13,33}.

Statistical Analysis

Unless stated otherwise, all values are reported as mean ± standard deviation (n = 3). In order to understand the impact of the molecular architecture on the various *in vitro* performance parameters evaluated, a hierarchical statistical analysis was conducted using the materials described in Table 1. As described in Figure 3, a series of statistical tests was performed using Minitab 16 in order to identify structural parameters with a statistically significant effect on each response. First, all factors and responses were analyzed in a

general linear model using multivariate analysis of variance (MANOVA). Potentially important factors, as indicated by Pillai $p < 0.1$, were identified. Each response was then subjected to analysis of variance (ANOVA) testing with a generalized linear model and the impact of each factor on the response was evaluated. For each model, residual plots, including a normal probability plot, a frequency plot, and standardized residual plots were generated and used to verify the validity of the assumptions made in the general linear model. Statistical significance in the *in vivo* adhesion scores was determined using the Wilcoxon rank-sum test.

Results

Quartz Crystal Microgravimetric (QCM) Analysis

As shown in Figure 2(c) and in Supplemental Figure 1, the molecular architecture of the polymer had a dramatic effect on the degree of fibrinogen accumulation. Some of the materials, including the peptide control, the larger PEG block, lower shorter PEG chain (P4) conjugates, and the poly(methacrylic acid) (PMAA) conjugate PMAA-L, exhibited significant levels of inhibition of the fibrinogen adsorption ratio. Several of the unconjugated polymers (PMAA, P2, and P4) increased the second fibrinogen adsorption ratio dramatically (116%, 384%, and 195%, respectively). The low level peptide conjugates of the long PEG chain polymers (P1-L and P3-L) demonstrated no observable suppression of fibrinogen adsorption. The two most significant structural parameters that lead to an improvement in the ability to suppress fibrinogen adsorption were increasing PEG chain length ($p = 0.012$) and the number of targeting peptides ($p < 0.001$). The untargeted PMAA and P2 polymers exhibited apparent reductions in the rate of fibrinogen adsorption. The half-life of this adsorption was most significantly affected by the PEG chain length ($p = 0.011$), by the number of PEG chains ($p = 0.025$), and by the number of peptide groups conjugated to each polymer molecule ($p < 0.001$). As shown in Supplemental Figure 2, however, the variation among the samples is very small in most cases. It is also apparent from Figure 2(c) that the rate of fibrinogen deposition is strongly correlated with the maximum amount of fibrin deposited on the surface.

Microplate Turbidity Analysis

As shown in Figure 4(a), the rate of change in UV absorption (350 nm) was measured by fitting the initial absorbance data with a linear equation and determining the slope of the resulting line. The value of this rate varies with the structure of the barrier material, as shown in Figure 4(b) and in Supplemental Figure 3. While most of the polymer samples appear to have slowed the kinetics of fibrin propagation from the observed control value of $3.9 \pm 0.7 \times 10^{-3} \text{ min}^{-1}$, the phospholipid (EPC) increased the rate of fibrin propagation to $5.9 \pm 1.9 \times 10^{-3} \text{ min}^{-1}$. The rate of fibrinogen propagation was a strong function of the number of peptide groups; however, the impact of the other structural parameters investigated was not evident.

Cellular Attachment

The results of the cellular attachment study, shown in Figure 5 and in Supplemental Figure 4, indicate that many of the materials significantly reduced the attachment of cells to the

model fibrin surfaces. The majority of the PMAA polymers resulted in a significant reduction of cellular attachment, as did most of the long PEG chain polymers. A comparison of the tissue culture polystyrene (TCP) versus the fibrin control wells, shown in Figure 5(a), is very instructive. The fibrin control showed normalized cellular attachment of 1.00 ± 0.09 , while the TCP control had cellular attachment values of 0.30 ± 0.09 . The longest polymer chain resulted in a large decrease in the level of cellular attachment. While many of the polymers resulted in a significant reduction in the number of cells attached to the fibrin surface, no statistically significant correlation between polymer structure and performance was observed.

In Vivo Post Surgical Adhesion Model

All treatment and control animals survived the surgery with no apparent side effects. At the termination of the experiment, all animals had gained weight, suggesting the absence of major complications. As can be seen in the images in Figure 6(a), the formation of adhesions varied greatly among the animals. When adhesions were seen, they were found to bridge between the abdominal wall and intestinal surface and between the buried suture knot and the abdominal defect. Adhesions in the control group were filmy to opaque with little to no obvious vascular structures, these were typically strongly adhered and required dissection to separate the surfaces. In the treatments groups, adhesions remained mostly filmy with little to no dense fibrous material. These adhesions were notably easy to separate, with simply lifting of the surface being sufficient to detach the adhesion. Data for two observers was averaged for each animal, and the results are presented in Figure 6(b). The degree of PSA formation was reduced by 16%, ($p=0.036$).

Discussion

The goal of this study was to identify the specific molecular structure to function relationships hypothesized to be important in the ultimate *in vivo* performance of the material. *A priori*, one can theorize that the specific molecular properties, including block lengths, number of targeting groups, and overall molecular weight, are likely to have competing effects upon the observed functional behavior (e.g., binding affinity, anti-adhesive ability, and stability). It is not clear, however, which of these molecular variables will dominate final material properties. An enhanced understanding of the materials facilitate an informed selection for initial *in vivo* testing and by developing a rich catalog of data from the onset, it becomes possible to further optimize the design during future translational and scale up aspects of the research.

While fibrin would not be an effective target in all pro-adhesive settings (e.g., cauterization or implant induced fibrosis), fibrin represents an ideal proof-of-concept target due to its known involvement in the wound healing cascade and to the existence of a known fibrin-specific targeting pentapeptide, CREKA³⁴⁻⁴⁰. 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. Designed to be applied as an aqueous irrigation solution during surgery, this treatment strategy may possess distinct advantages over current PSA prevention methods for several reasons. First, application will

not require any modification to surgical technique or extend the duration of surgery. Second, unknown or inaccessible tissue damage can be treated. Finally, the potential for adverse interactions with the wound healing process is minimized.

In the assessment of the fibrinogen accumulation ratio, a metric hypothesized here to correlate specifically with anti-adhesive capacity, it was anticipated that longer PEG chains, longer polymer chains, higher PEG chain density, and a higher density of targeted peptide units would provide increased protection against the adsorption of fibrinogen to surface-bound fibrin layers. These predictions were confirmed in most cases; in fact, the high molecular weight polymer with long PEG chains and a high level of CREKA (P3-H) resulted in the complete suppression of the second fibrin adsorption step. Compared to the rest of the polymer architectures, this structure appears to represent a balance of surface affinity (due to the high peptide content) and protective ability (due to the long PEG chains). The difference observed between the short polymer chains and long polymer chains was also notable. The shorter PEG chains were much less effective at inhibiting fibrinogen adsorption, potentially as a result of the action of the PEG groups on the peptide units. Interestingly, some non-targeted polymers (e.g., PMMA and P3) resulted in an increase in the fibrinogen accumulation ratio. This increase suggests that certain materials may even potentiate the formation of the FGM, thereby enhancing PSA formation. This effect emphasizes the importance of complete *in vitro* characterization prior to *in vivo* evaluations.

While the relatively minor impact of the polymer structure on the kinetics of fibrinogen deposition was surprising, the fact that the majority of the polymer samples slowed the propagation of the fibrin gel in the turbidity analysis suggests that the mechanism of fibrinogen deposition and polymerization was unaffected by the polymer. The rapid kinetics seen with EPC is likely a result from the simultaneous growth of phospholipid lamella within the fibrin gel matrix structure. Since both of these structures can scatter light, such a mechanism would lead to the more rapid turbidity increase.

Interestingly, the PMAA polymers were surprisingly effective at reducing the observed cellular attachment. All of the materials appeared to reduce this response compared to the untreated fibrin control. This finding indicates that the materials have the likelihood of functioning well as adhesion barriers, suggesting that all of the materials adsorbed to the fibrin gel surface and can reduce cellular attachment through either steric hindrance or charge-based repulsion.

The results obtained from the *in vitro* experiments reveal several important trends in the relationship between the molecular architecture of the polymers and the ability of these materials to suppress fibrin deposition and fibrin gel propagation. PEG chain length appears to be an important determinant of performance for the materials. This result is in good agreement with previous studies that indicate when coupled to a surface, maximal protein adhesion resistance occurs with longer ($M_N = 5,000$) PEG chains⁴¹. This length of PEG chain appears to form a thick hydrated layer with a large exclusion volume and high steric stabilization and presents few binding sites for protein interactions^{42,43}. The number of pendant PEG chains in each polymer molecule does not appear to be a major factor in performance. Although the level of polymer adsorption was not explicitly measured in these

experiments, this lack of dependence on the concentration of PEG chains probably resulted from the formation of a surface layer with complete PEG coverage. Once this level of surface saturation was achieved, maximum surface protection for that specific polymer composition was obtained. In most experiments, the number of peptide units conjugated to the polymer was a strong predictor of the material's ability to affect the propagation of the fibrin gel matrix. Polymers conjugated with high levels of CREKA inhibited the degree of subsequent fibrinogen adsorption and reduced the rate of fibrin propagation. This supports the hypothesis that this peptide can be used to direct these polymers to fibrin surfaces in order to provide protection.

The results of these *in vitro* experiments were used to select a material for *in vivo* testing. Observed trends suggest that materials with long polymer chains, long PEG chains, and high levels of peptide conjugation would result in enhanced performance. While it did not have a significant effect in any of the observed responses, it is expected that longer polymer chains will provide a surface barrier with enhanced durability. As a result of the observed trends and its excellent performance in the experiments, the high molecular weight polymer with long PEG chains and the highest level of peptide conjugation (P3-H) was selected as the most promising candidate for use in animal testing. Indeed, the decrease in separation force that was required to detach adhesions in the treatment group over the control group suggests that this material approach may hold some promise as a PSA treatment therapy. While the current study cannot conclude fibrin targeting is directly responsible for this result, such a decrease would be expected with the proposed material as its primary function is to reduce the adhesion of the fibrin gel matrix to surrounding tissues. However, the overall limited degree of effectiveness suggests much more development is required before the success of this approach is realized. Such a result is expected based upon the lack of full prevention of cellular adhesion and fibrin propagation in the *in vitro* studies.

From this preliminary study, it is not possible to know the exact mechanism(s) for the low effectiveness of the polymers. Likely possibilities include insufficient polymer MW, PEG chain length, and number of adhesion groups, as theorized from the *in vitro* data resistance. The presence of adhesions at the suture knot suggests that fibrin targeting alone is not enough to inhibit PSAs at these sites, requiring the discovery of more robust targets. Further, residence time at the site of adhesion, poor timing of administration at establishment of the FGM, and the unknown impact of the material on the cells infiltrating the FGM are all possible candidates for consideration. Future work will center on the elucidation of these potential effects.

Conclusion

In this work, we developed a novel fibrin-targeted block copolymers for the prevention of postsurgical adhesions. The most important aspects of molecular architecture identified were the length of pendant PEG chains and the number of targeting peptides conjugated to each polymer molecule. These results were used to select a material with the highest likelihood of success *in vivo*. The validity of this approach was confirmed by the reduction in the degree of PSA formation observed after treatment with this material. Combining the functional understanding of the materials' behavior gained from the *in vitro* experiments with the

results of these initial animal tests, it is possible to tailor a material likely to exhibit improved protection from the formation of PSA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Schematic representation of polymer architecture and experimental design
a) Targeted block copolymer structure. **b)** Design variables (factors) and responses used in the investigation of polymer architecture and performance.

Figure 2. Quartz crystal microgravimetric analysis of fibrinogen adsorption ratio and kinetics
a) Schematic representation of experiment. **b)** Representative QCM sensorgram of fibrinogen suppression study. After establishing a baseline in TBS, fibrinogen, thrombin, bovine serum albumin (BSA), polymer, and fibrinogen solutions were introduced into the system sequentially. As indicated by the shaded regions, a TBS wash was employed between solution introductions. The frequency shift (Δf , blue) and energy dissipation change (ΔD , red) were monitored continuously throughout the experiment. **c)** Representative QCM results for native and targeted polymers. The fibrinogen accumulation ratio (Fg_2/Fg_1) is shown in blue, while the half-life for the second fibrinogen adsorption step is shown in red. * For P3-H, no subsequent fibrinogen adsorption was seen, so no kinetic value is reported. Complete data for all materials tested is presented in Supplemental Figure 1 and Supplemental Figure 2.

Figure 3. Statistical analysis

a) *Schematic representation of statistical analysis protocol.* Statistically significant factors were first identified by MANOVA. Subsequent ANOVA testing of each response was used to determine impact of each significant factor on each response. b) *Summary of statistical effects.* Factors with significant ($p < 0.05$) effect are indicated. No significant effects were observed for chain length, and no significant factors were observed for cellular attachment.

Figure 4. Fibrin gel propagation rate

a) Linear kinetics of fibrin gel propagation. The absorbance change of fibrin gels were monitored for approximately 10 minutes after the addition of additional fibrinogen. The protected surface (red) had a 33% reduction in the rate of fibrin polymerization as compared to the untreated control (blue). **b)** Representative fibrin gel propagation results for native and targeted polymers. Complete data for all materials tested is presented in Supplemental Figure 3.

Figure 5. Cellular attachment assay

a) Representative images of cells attached to untreated fibrin and to the polystyrene control. All fibrin samples appeared to support a larger density of cells as a result of the 3-dimensional fibrin network. **b)** Normalized cellular attachment data from DNA fluorescence assay. Most polymer samples reduced the level of cellular attachment compared to the control. Complete data for all materials tested is presented in Supplemental Figure 4.

Figure 6. Results of in vivo adhesion assay

a) Comparison of animals with no adhesions and with dense adhesion formation. The control animal (left) had a large fibrous adhesion between the large intestine and the abdominal wall. No adhesion was seen the animal from the treatment group (right). **b)** Adhesion scores for animals treated with saline (blue) with animals treated with targeted polymer P3-H (red). While there may be a slight increase in the extent of adhesion formation, both the severity and degree of adhesions were reduced in the treatment group.

Table 1

Summary of Materials

	Code	Functional Group	Number of Functional Groups	Chain Length	Number of MA Units	Number of PEG Units	MA/PEG Ratio	PEG Chain Length	Molecular Weight
Polymer Samples	P1	-	-	18.4	15.9	2.5	6.5	1,100	4,300
	P1-L	CREKA	2.3	18.4	15.9	2.5	6.5	1,100	9,300
	P1-H	CREKA	6.7	18.4	15.9	2.5	6.5	1,100	14,400
	P2	-	-	18.2	16.0	2.2	7.3	300	2,200
	P2-L	CREKA	2.4	18.2	16.0	2.2	7.3	300	7,300
	P2-H	CREKA	7.4	18.2	16.0	2.2	7.3	300	12,800
	P3	-	-	30.9	26.7	4.2	6.4	1,100	7,300
	P3-L1	CREKA	2.2	30.9	26.7	4.2	6.4	1,100	14,800
	P3-H	CREKA	6.2	30.9	26.7	4.2	6.4	1,100	21,000
	P4	-	-	22.1	17.5	4.6	3.8	300	3,300
	P4-L	CREKA	2.3	22.1	17.5	4.6	3.8	300	8,700
	P4-H	CREKA	7.1	22.1	17.5	4.6	3.8	300	14,200
Control Samples	PMAA	-	-	18.3	18.3	0.0	N/A	N/A	1,600
	PMAA-L	CREKA	1.9	18.3	18.3	0.0	N/A	N/A	5,800
	PMAA-H	CREKA	5.8	18.3	18.3	0.0	N/A	N/A	10,800
	NP				No Polymer Control				
	CREKA				Free Targeting Peptide				
	EPC				L- α -phosphatidylcholine				

Table 2
Adhesion Scoring Group Classification for Adhesion Assessment

Score	Extent of Adhesion	Severity of Adhesion	Degree of Adhesion
0	None	No adhesions	No adhesions
1	1-25%	Filmy, avascular	Adherent tissue separate with gentle traction
2	26-50%	Vascular and/or opaque	Tissue layer separation requires moderate traction
3	51-75%	Cohesive attachment	Tissue layer separation requires sharp dissection
4	76-100%		