

# Isoreactive Manipulation of Bioadhesive Polymers Impacts Tissue-Specific Interactions

Jahid Ferdous<sup>1</sup>, Eva Romito<sup>1,2</sup>, Heather Doviak<sup>2,3</sup>, Alexandra Moreira<sup>1</sup>, Mark J. Uline<sup>1\*</sup>, Francis G. Spinale<sup>1,2,3,4</sup>, Tarek Shazly<sup>1\*</sup>

<sup>1</sup>College of Engineering and Computing, University of South Carolina, Columbia, SC, USA

<sup>2</sup>Cardiovascular Translational Research Center, School of Medicine, University of South Carolina, Columbia, SC, USA

<sup>3</sup>Department of Cell Biology and Anatomy, School of Medicine, University of South Carolina, Columbia, SC, USA

<sup>4</sup>WJB Dorn Veteran Affairs Medical Center, Columbia, SC, USA

Email: \*uline@cec.sc.edu, \*shazly@mailbox.sc.edu

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## Abstract

Bioadhesive polymers can serve as surgical sealants with a wide range of potential clinical applications, including augmentation of wound closure and acute induction of hemostasis. Key determinants of sealant efficacy include the strength and duration of tissue-material adhesion, as well as material biocompatibility. Canonical bioadhesive materials, however, are limited by a tradeoff among performance criteria that is largely governed by the efficiency of tissue-material interactions. In general, increasingly bioreactive materials are endowed with greater bioadhesive potential and protracted residence time, but incite more tissue damage and localized inflammation. One emergent strategy to improve sealant clinical performance is application-specific material design, with the goal of leveraging both local soft tissue surface chemistry and environmental factors to promote adhesive tissue-material interactions. We hypothesize that co-polymer systems with equivalent bioreactive group densities (isoreactive) but different amounts/oxidation states of constituent polymers will exhibit differential interactions across soft tissue types. We synthesized an isoreactive family of aldehyde-mediated co-polymers, and subjected these materials to physical (gelation time), mechanical (bulk modulus and adhesion strength), and biological (*in-vitro* cytotoxicity and *in-vivo* biocompatibility) assays indicative of sealant performance. Results show that while bioadhesion to a range of soft tissue surfaces (porcine aortic adventitia, renal artery adventitia, renal cortex, and pericardium) varies with isoreactive manipulation, general indicators of material biocompatibility remain constant. Together these findings suggest that isoreactive tuning of polymeric systems is a promising strategy to circumvent current challenges in surgical sealant applications.

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## Keywords

Bioadhesion, Dextran Aldehyde, Soft Tissue, Polymer, Surgical Sealant

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### 1. Introduction

Bioadhesive polymeric materials have an established history of medical use, with utilities ranging from acute induction of hemostasis in cases of trauma to augmentation of wound closure in surgical applications. [1] [2] [3] Irrespective of specific use, the safety and efficacy of these materials largely depend on sufficient adherence to soft tissue surfaces, adequate residence time at the site of application, and acceptable biocompatibility. [4] Clearly, the required bioadhesive strength, material degradation/erosion kinetics, and tolerable immune/inflammatory response will all vary by application, with internal application sites subjected to high mechanical loads presenting the greatest challenge.

Bioadhesive materials can be loosely divided into two categories which exemplify the current state of sealant technologies. On the one hand, there are numerous synthetic materials which adhere vigorously to the full range of soft tissues and persist at the site of application for long periods of time. Many of these materials are based on cyanoacrylate and its derivatives, wherein adhesive bonds with soft tissues are rapidly formed in the presence of trace water. [5] [6] [7] [8] [9] Although endowed with high bioadhesive potential, these materials and their degradation by-products induce significant inflammation, confer destructive compressive mechanical forces to underlying soft tissue, and are therefore mainly used in dermal applications. Conversely, polymers based on natural compounds, most notably fibrin, are biocompatible in the context of internal applications. [10] [11] [12] However, the bioadhesion strength of these formulations is minimal, and material degradation/erosion processes are accelerated by enzymatic activity. While cyanoacrylate- and fibrin-based materials are only a small fraction of proposed technologies, their inherent limitations/tradeoffs persist to various degrees across all materials considered for soft tissue sealant applications (**Table 1**) [13] [14].

To address the long-standing challenges limiting sealant use and efficacy, recent efforts have focused on tissue-specific material design. [15] [16] These studies have demonstrated that polymer-based adhesive bond formation with various soft tissues is concurrently modulated by the mode of chemical bond formation and the targeted tissue surface characteristics. Moreover, the sensitivity of polymer adhesion strength to increasingly bioreactive material formulations varies with target tissue type, suggesting that in addition to careful selection of bioreactive group chemistry, optimization of bioreactive group content available for adhesive bond formation must be done on a tissue-specific basis. Building upon this theme, recent studies have shown that polymer-based adhesion can significantly vary in the context of certain disease states, providing further impetus for application-specific material design. [17] [18] Clearly, the notion of de-

**Table 1.** Synthetic and natural surgical sealants. Surgical sealants are composed of a variety of base materials, including both synthetic and natural polymers. In general, current surgical sealants are limited by a tradeoff in performance criteria that is governed by the chemical mode and extent of adhesive interactions with soft tissues.

Classes of Sealants (Base Materials)	Potential Applications	Strengths	Weaknesses	Ref
SYNTHETIC MATERIALS				
Cyanoacrylate	Dermal applications; Wound closure; Hernia repair	Rapid polymerization; high adhesion strength	High toxicity of degradation by-products	23 - 25
Polyurethane	Orthopedic and renal surgery; Pancreatic occlusion; Vascular surgery	High elasticity; Moderate-high adhesion strength	Moderate toxicity of degradation by-products	26 - 29
Poly(ethylene glycol)	Cranial surgery; Spinal surgery; Retinal applications	Moderate adhesion strength; High biocompatibility; Soft tissue-like mechanical properties	Significant/uncontrolled swelling	30 - 32
NATURAL MATERIALS				
Fibrin	Hemorrhage control; Wound closure	High biocompatibility; High hemostatic potential; Rapid curing <i>in-situ</i>	Low adhesion strength; High cost; Risk of disease transmission	33 - 36
Albumin/glutaraldehyde	Vascular surgery; Cardiac surgery; Lung surgery	Moderate adhesion strength; Rapid cross-linking	Toxicity of cross-linking agent; Moderate biocompatibility	37 - 39
Collagen/Gelatin	Lung surgery; Vascular surgery; Gastrointestinal surgery	Low risk of disease transmission; Low cost; Moderate-high adhesion strength	Toxicity of crossing-linking agent; Moderate biocompatibility (depending on formulation)	40 - 42
Polysaccharides (including dextran and chitosan)	Lung surgery; Hemorrhage control	Tunable polymer microstructure; High biocompatibility; Hemostatic potential	Moderate adhesion strength	15;19 - 20; 43 - 44

signing soft tissue sealants for universal deployment is fading, whereas tissue- and application-specific approaches are gaining momentum.

It is well-established that increasing polymer reactive group content will promote bioadhesion, albeit to different degrees and saturation levels when applied to various soft tissue surfaces. [15] [16] These previous studies suggest that a natural variation among soft tissue surfaces may exist with regard to the availability of reactive groups targeted by sealants for adhesive bond formation. Such variance could emerge due to intrinsic differences in tissue surface composition, local environmental factors, and certain disease states. We postulate that bioadhesive co-polymer formulations with equivalent total reactive group content (isoreactive) but different reactive group distribution along constituent polymer chains will exhibit tissue-specific interactions. Moreover, because total reactive group content is associated with an increased inflammatory response, we expect that isoreactive design manipulations will not significantly impact material biocompatibility.

We synthesized a family of two-component, aldehyde-mediated bioadhesive materials composed of dextran aldehyde and chitosan polymers. In this experimental material system, both cohesive cross-linking within the material and adhesive cross-linking to local tissue surfaces are achieved through aldehyde-me-

diated imine bond formation. Within our series of experimental materials, the dextran oxidation state and solid content are simultaneously varied such that total aldehyde concentration is fixed, *i.e.* this is a family of isoreactive material formulations. We assess key sealant properties and biological response variables following application of these materials to multiple soft tissue surfaces, and evaluate the potential for isoreactive tuning of bioadhesive materials to enhance tissue-specific interactions

## 2. Materials and Methods

All animals were treated and cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all protocols were approved by the University of South Carolina's Institutional Animal Care and Use Committee.

### 2.1. Synthesis of Dextran Aldehyde-Chitosan Amine Co-Polymer

**Dextran oxidation** The synthesis of dextran aldehyde has been previously described. [19] Briefly, a 10 wt.% dextran solution (average molecular weight of 40 kDa, 0.12 mol saccharide rings, Sigma no. 31389) was oxidized via dissolving the powder in deionized (DI) water. The dextran solution was subsequently mixed for five hours at room temperature with a previously prepared sodium periodate solution, which was also prepared in DI water with concentrations ranging from 5 - 15 wt.%. Resultant solutions were equally portioned into multiple dialysis membrane tubes (molecular-weight cutoff of 3500 Da), and dialyzed in DI water for five days. Following dialysis, aqueous solutions were extracted, frozen with liquid nitrogen, and lyophilized for six days yielding oxidized dextran.

**Aldehyde content** Aldehyde content was determined via a previously described titration method. [20] Briefly, oxidized dextran powder (125 mg) was fully dissolved in 10.0 mL of NaOH (0.25 M) at 40°C. Following a brief cooling period, 15.0 mL of HCl (0.25 M), 50 mL DI water, and 1.0 mL of 0.2 wt.% phenolphthalein were added to the solution. A titration with NaOH (0.25 M) was then performed, with the endpoint indicated by a solution color change from clear/yellow to purple/violet. Aldehyde content was then calculated based on the titration endpoint. All variants of dextran aldehyde synthesized for these studies were characterized by the above protocol.

**Chitosan synthesis** A 2 wt.% chitosan solution (average molecular weight of 340 kDa, Sigma Aldrich) and 1% acetic acid solution were prepared and mixed at room temperature for 5 hours. The mixing period yielded a viscous homogenous solution, which was subsequently degassed and stored at room temperature until use.

**Isoreactive co-polymers** Four batches of dextran aldehyde with a range of percent oxidations were selected for subsequent studies. Given the aldehyde content of each batch (determined above), the wt.% of polymer required to form aqueous solutions with equivalent total aldehyde group content (isoreactive) was

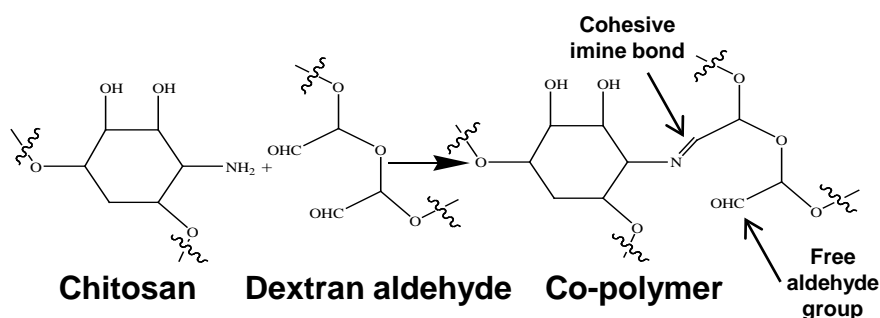
calculated. Four isoreactive dextran aldehyde solutions (A-D) were then prepared via completely dissolving the appropriate amount of oxidized dextran in DI water.

Co-polymer material systems were formed with dextran aldehyde solutions (A-D) in combination with the prepared chitosan solution. In all co-polymer systems, the aldehyde group density (of the dextran component) was 3-fold higher than the amine group density (of the chitosan component). To facilitate co-polymer cross-linking, dextran aldehyde and chitosan solutions were loaded into a dual-chamber syringe equipped with a 12-step mixing tip. Upon injection and controlled mixing, constituent polymers react via imine bond formation to yield a solid co-polymer (Figure 1). When these materials are injected onto soft tissue surfaces, the relative excess of aldehyde groups promotes concurrent and analogous imine bond formation with tissue-present amine groups.

## 2.2. Bulk Material Properties

**Gelation time** The gelation time of each co-polymer formulation is defined as the time required for solid globule formation following a 100  $\mu\text{L}$  injection onto a glass surface maintained at 37°C. The injected material was continuously agitated with a magnetic stirring rod, and solid globule formation was visually determined.

**Compressive modulus** Cylindrical test samples (diameter = 9.5 mm and height = 6.25 mm) were prepared via co-polymer injection into a silicon mold. Samples were allowed five minutes to cross-link, after which they were carefully removed from the mold. A uniaxial mechanical testing system (Bose® Biodynamic Test Instrument, Minnetonka, MN) configured for unconfined compression testing was used to apply a ramped displacement (5 mm total displacement; displacement rate of 0.005 mm/sec) to each sample. Force and displacement data were continuously recorded (data acquisition rate of 20 points/sec) using an integrated system software (Wintest®, Minnetonka, MN). The mechanical behavior of these materials was assumed to be linear, elastic, homogeneous, and isotropic,



**Figure 1.** (a) Dextran aldehyde. The oxidation states of dextran aldehyde variants were determined via a colorimetric titration, and ranged from approximately 24% - 72% in the present study. (b) Bioadhesive co-polymer formation. Amine groups on chitosan polymers cross-link with aldehyde groups on oxidized dextran polymers via imine bond formation. Aldehyde groups which remain free after co-polymer network formation are available for analogous bond formation with tissue-present amines.

and the materials were modeled as incompressible solids due to the high water content. In the context of these assumptions, recorded mechanical data were processed to yield true stress versus strain curves and ultimately calculate the compressive elastic modulus (E) of each test sample (*i.e.* slope of the stress-strain curve).

### 2.3. Adhesive Material Properties

The morphology and mechanical strength of tissue-material interfaces formed between isoreactive co-polymer formulations (A-D) and select porcine soft tissue surfaces (aortic adventitia, renal artery adventitia, renal cortex, and pericardium) were quantified to reflect adhesive material properties. For the following *ex-vivo* studies, soft tissues were harvested from 7 - 12 month old swine and completed protocols within 2 hours of animal sacrifice.

**Interfacial morphology** To facilitate visualization of the tissue-material interface, fluorescently labeled co-polymer formulations were prepared via 0.5 wt % inclusion of fluorescence (6-fluorescein-5-carboxyamido hexanoic acid, Invitrogen) into the chitosan component as previously described. [15] Co-polymers (100  $\mu$ L total volume, via dual-chamber syringe and mixing tip) were injected onto soft tissue cylindrical biopsy specimens (8 mm diameter and 1 mm thickness), and allowed to cross-link at room temperature under static conditions for five minutes. Samples were subsequently snap frozen, cryo-sectioned (20  $\mu$ m), and labeled with a nuclear dye (DAPI, Vector Laboratories) to further delineate the adhered material from the underlying soft tissue surface. Quantitative fluorescent microscopy was used to measure the fluorescence emanating for a predefined and consistently sized material region juxtaposed to the tissue surface (2 mm along the tissue-material interface, extending 1 mm into the material bulk). The mean regional fluorescence (N = 6) for each combination of material and tissue type was computed and normalized with respect to the mean bulk fluorescence of that application scenario (tissue-material pair). This relative metric reflects the degree of material continuity between the bulk co-polymer and the soft tissue surface, *i.e.* the continuity of the adhesive interface.

**Adhesion strength** Co-polymer adhesive mechanics were quantified using a previously described testing methodology. [19] [21] Briefly, tissue-material-tissue constructs were formed using two cylindrical biopsy specimens (8 mm diameter and 1 mm thickness) of a given tissue type, between which co-polymer (100  $\mu$ L) was injected. Constructs were carefully mounted within a mechanical testing system (Bose® Biodynamic Test Instrument, Minnetonka, MN) configured for uniaxial tensile testing. A compressive setting force (1 N) was applied to the constructs for a five minute period, followed by application of a ramped tensile displacement (0.05 mm/s) until the occurrence of failure. Integrated system software (Wintest) continuously acquired load and displacement data, which were later processed to yield the ultimate true stress of the construct. The ultimate stress serves as an indicator of the adhesive strength of the co-polymer to the targeted soft tissue surface.

## 2.4. Material Biocompatibility

The biocompatibility of isoreactive co-polymer formulations (A-D) was assessed via *in-vitro* cytotoxicity studies and *in-vivo* sub-cutaneous implantation studies. While neither method is directly relevant to specific sealant applications, these studies provide general indications as to whether isoreactive design manipulations within the co-polymer system will likely impact material biocompatibility.

***In-vitro cytotoxicity*** Primary rat fibroblasts (~7e4 cells/mL) were seeded on to 24 well plates and cultured to confluence using standard media (*Cell Applications*, Inc.). Each well plate was then drained of media to facilitate direct injection of co-polymers (100  $\mu$ L) onto the cell monolayer. Materials were allowed five minutes for cross-linking, after which fresh culture media was replenished within each well plate. Following a 48 hour incubation period, a neutral red uptake (NRU) assay (Sigma Aldrich) for cell viability/cytotoxicity was performed. The assay consist of a two hour co-incubation of cells/materials with the supravital dye (neutral red), a washing treatment, and subsequent quantification of absorbance. Obtained absorbance measurements were normalized with respect to control wells (identical cell cultures with no material exposure) and reported for each co-polymer formulation (A-D).

***In-vivo studies*** Sterile sample preparations of co-polymer formulations A-D were prepared for subcutaneous implantation in adult male Sprague Dawley rats (180 - 220 g, *Charles River Labs*). A randomized pattern of five discrete subcutaneous dorsal implantation sites was assigned to each rat (n = 12). Each of four implantation sites was assigned one co-polymer formulation (A-D), wherein a 100  $\mu$ L injection was sterily delivered. The fifth implantation site was used for a sham procedure (100  $\mu$ L saline injection). After 7 days, the rats were sacrificed and tissue was harvested for histological and molecular assays. For histological studies, tissue samples were fixed in 4% formalin, sectioned (20  $\mu$ m thickness), and stained with hematoxylin and eosin (H & E). Histological images (40X) were subjected to blind scoring, where the inflammatory cells present in four randomly selected regions (25 mm<sup>2</sup> regions, total area of 100 mm<sup>2</sup> per slide) were counted and summed. Additional tissue samples collected from each implant site (n = 3 per material & sham group) were snap-frozen upon acquisition and later used to quantify local interleukin (IL) levels. Tissue samples were thawed, homogenized, and analyzed using the Bio-Plex Pro Assays Quick Guide (Bio-Rad), enabling quantification of local IL-1 $\beta$ , IL-2, and IL-6 concentrations (assay sensitivity of 0.8 - 2.0 pg/mL).

## 2.5. Statistical Analysis

Obtained data were analyzed using Mann-Whitney tests for significance between groups and Wilcoxon rank tests for pair-wise comparisons within groups, with groups defined by co-polymer formulation (experimental groups) or included as controls (sham procedure for *in-vivo* studies). Differences were considered to be significant if *p*-value < 0.05.

### 3. Results and Discussion

#### 3.1. Co-Polymer Bioreactive Group Content and Linear Distribution Parameter

Isoreactive material synthesis yielded four dextran aldehyde-chitosan co-polymer formulations (A-D) that facilitate investigation of the proposed material design strategy (Table 2). Specifically, the dextran aldehyde components of these formulations were endowed with equivalent total aldehyde content ( $2.3 \times 10^{20}$  groups/mL), but differed in terms of oxidation states/solid content. In all formulations, the chitosan component was identical, and provided an amine group density of  $7.6 \times 10^{19}$  groups/mL. Therefore, in all co-polymer formulations, the ratio of aldehyde: amine groups were 3:1. The relative concentrations of these groups ensure that aldehydes are available for both cohesive bond formation within the co-polymer network and adhesive bond formation with tissue-present amines. The selection of a 3:1 reactive group ratio was motivated by previous findings with an analogous material system that show a notable decline in biocompatibility with increasing free aldehyde content [19].

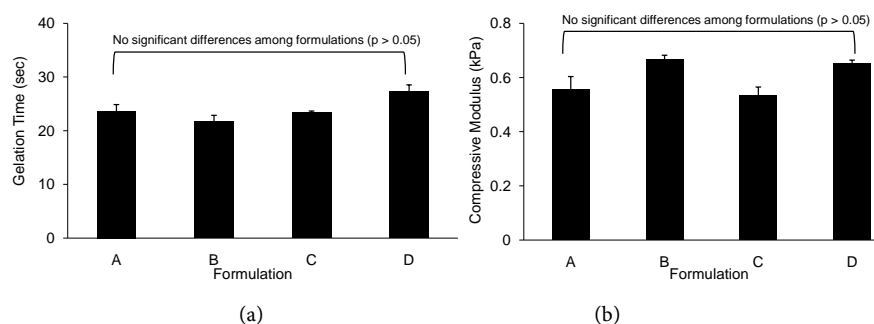
#### 3.2. Bulk Property Response to Isoreactive Design Manipulations

Assays were conducted to determine if key intrinsic properties for surgical sealant applications vary in response to isoreactive manipulation. Specifically, the impact on co-polymer gelation kinetics (mean time for liquid-solid phase transition under controlled component mixing conditions) and the compressive elastic modulus (determined via unconfined uniaxial compression testing of cylindrical material samples) were determined. Among the examined co-polymer formulations, no significant differences were found in either mean gelation times or compressive moduli (Figure 2). These findings suggest that for these base material constituents (40 kDa dextran and 340 kDa chitosan) prepared within experimental range of dextran oxidations and solid contents, molecular mobility and steric effects within the forming co-polymer networks were similar, leading to similar rates and degrees of cross-linking.

**Table 2.** Co-polymer formulations. Four variants of dextran aldehyde (A-D) with titrated percent oxidation and solid contents were synthesized for this study. The solid content of each dextran aldehyde polymer within the delivery solution was tuned such that all formulations contained an equivalent aldehyde group density prior to mixing with a 2% chitosan solution. The aldehyde: amine ratio within the resultant cross-linked co-polymer systems was therefore equivalent (3:1) in all formulations.

Co-Polymer Formulation	Dextran Aldehyde				Chitosan			Co-Polymer
	Molecular Weight (kDa)	Percent Oxidation (%)	Solid Content (%)	Aldehyde Content (#per mL)	Molecular Weight (kDa)	Solid Content (%)	Amine Content(#per mL)	Reactive Group Ratio (CHO:NH <sub>2</sub> )
A	40	24.3	10.8	$2.30 \times 10^{20}$	340	2	$7.63 \times 10^{19}$	3
B	40	42.1	6.32	$2.30 \times 10^{20}$	340	2	$7.63 \times 10^{19}$	3
C	40	53.5	4.93	$2.30 \times 10^{20}$	340	2	$7.63 \times 10^{19}$	3
D	40	71.3	3.63	$2.30 \times 10^{20}$	340	2	$7.63 \times 10^{19}$	3



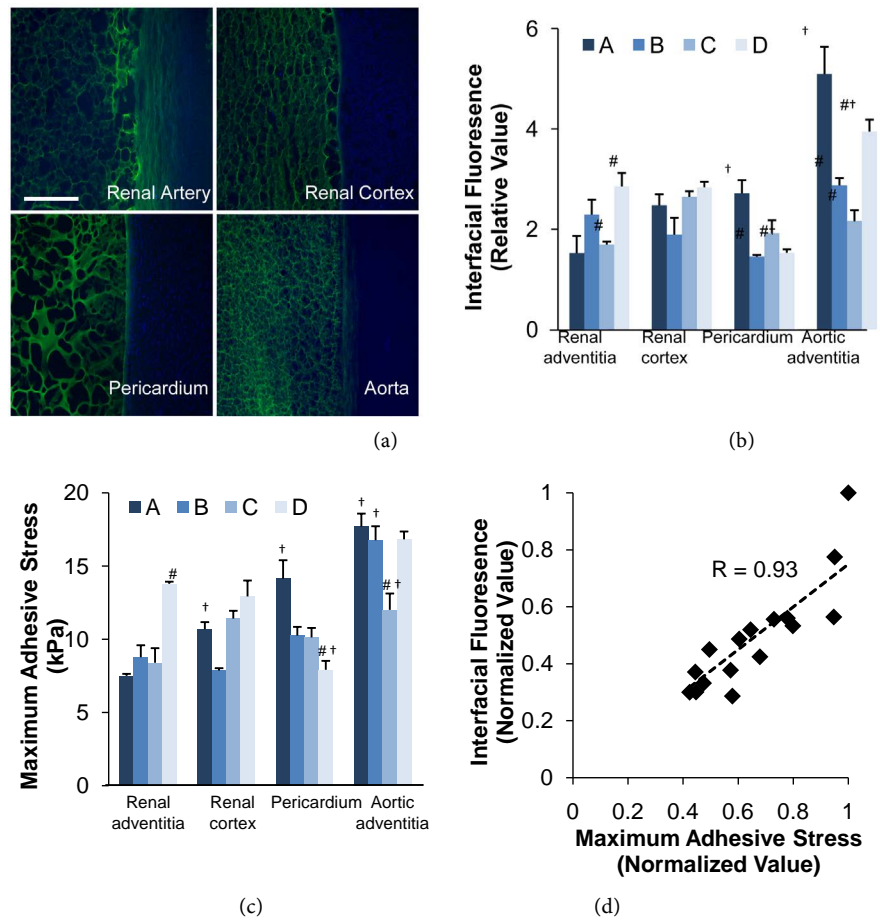


**Figure 2.** Bulk material properties. (a) Gelation time. Co-polymer gelation time was defined as the time to solid material formation following controlled component injection from a dual-chambered syringe equipped with a mixing tip. Co-polymer (a)-(d) gelation times ranged from 21 - 28 seconds, with no significant differences or discernable trends with respect to dextran aldehyde percent oxidation/solid content; (b) Compressive modulus. The compressive modulus of each co-polymer was determined via unconfined compression testing of cylindrical samples. Co-polymer (a)-(d) compressive moduli ranged from 0.50 - 0.65 kPa, with no significant differences or discernable trends with respect to dextran aldehyde percent oxidation/solid content.

### 3.3. Tissue-Specific Adhesion Response to Isoreactive Design Manipulations

The adhesive interactions between co-polymer formulations and a range of soft tissue surfaces were assessed in terms of tissue-material interfacial continuity and maximal adhesion strength. The soft tissue surfaces considered were the aortic adventitia, renal artery adventitia, renal cortex, and pericardium, all of which are potential targets for clinical sealant applications (**Figure 3(a)**). Significant differences in adhesion, manifested by both interfacial continuity and maximum adhesion stress prior to interfacial failure, were found among co-polymer formulations when applied to each tissue type. For example, interfacial continuity with the aortic adventitia exhibited a nonmonotonic dependence on dextran percent oxidation/solid content, and was maximal with application of formulation A (lowest percent oxidation). Conversely, interfacial continuity on renal cortex applications was insensitive to explored isoreactive manipulation (**Figure 3(b)**).

Similar tissue-specific responses were found when adhesion was assessed from a mechanical perspective (**Figure 3(c)**). Interestingly, among the co-polymer formulations tested, the maximal adhesion strength to each tissue type occurred when the dextran percent oxidation was either its lowest (formulation A, aortic adventitia and pericardium) or highest (formulation D, renal artery adventitia and renal cortex) value. This finding suggests that with respect to the defined mode of isoreactive variation, the optimal value for bioadhesion to each of these tissue types falls outside of the range covered by our experimental material system. Moreover, direct correlation ( $R = 0.86$ ,  $p$ -value  $< 0.005$ ) between tissue-material interfacial fluorescence and maximal adhesion strength supports the interchangeability of these response variable for assessment of tissue-material adhesion (**Figure 3(d)**).



**Figure 3.** Adhesive material properties. (a) Qualitative interfacial morphology. Fluorescently labeled co-polymer formulations exhibit differential morphology when adhered to soft tissue surfaces, manifested as sparse and porous interfacial regions in comparison to the bulk material. Scale bar = 1 mm and applies to all images; (b) Quantification of interfacial fluorescence. The mean fluorescent signal emanating from predefined tissue-material interfacial regions (2 mm along the tissue-material interface, extending 1 mm into the material bulk) were quantified and normalized with respect to the bulk material. Interfacial regions significantly differed ( $p < 0.05$ ) in terms of various co-polymers formulations (a)-(d) adhering to a given soft tissue surface as well as a given co-polymer formulation adhering to different surfaces; (c) Adhesive mechanics. The maximal stress prior to tissue-material interfacial failure was quantified via uniaxial tensile tests on tissue-material-tissue constructs. The maximal adhesive stress significantly differed ( $p < 0.05$ ) in terms of various co-polymers formulations (a)-(d) adhering to a given soft tissue surface as well as a given co-polymer formulation adhering to different surfaces; (d) Correlation between morphology and mechanics. Metrics to quantify interfacial morphology (B above) and mechanics (c above) exhibited a strong positive correlation ( $R = 0.93$ ,  $P < 0.05$ ), suggesting that adhesive strength is governed by the material continuity within the interfacial region. # indicates significant difference of formulation (b) (c) or (d) vs. (a) with a given tissue; † indicates a significant difference of a given formulation interacting with renal cortex, pericardium, or aortic adventitia vs. the renal adventitia.

### 3.4. Cell and Tissue Response to Isoreactive Design Manipulations

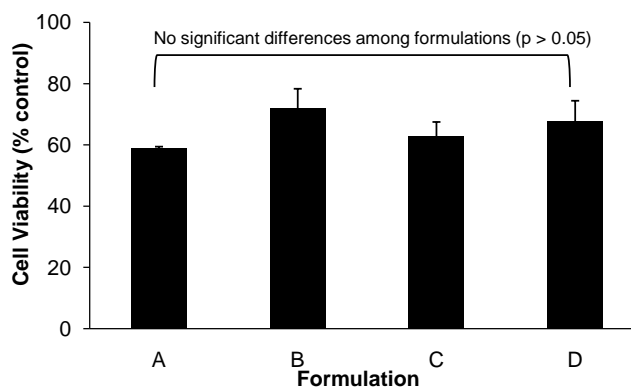
Assays to determine the cytotoxic effects of formulations A-D demonstrated that co-polymer formulations are similarly tolerated by the cell culture monolayer.

All formulations maintained greater than 58% viability of the control wells, with no significant differences in cytotoxicity among the material formulations (Figure 4). Moreover, no trend in cytotoxicity with respect to titrated polymer design variables (oxidation state and solid content) emerged among the formulations tested, suggesting that isoreactive manipulations have no discernable impact on this aspect of material biocompatibility.

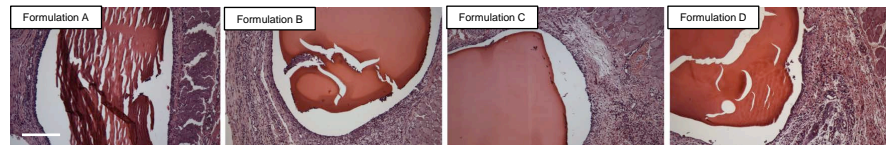
While cytotoxicity assays suggest reasonable and consistent material biocompatibility, complementary subcutaneous implantation studies were undertaken to quantify and compare the *in-vivo* tissue response to isoreactive co-polymers. Obtained results demonstrate no significant elevations in inflammatory cell count relative to sham, and no dependence on dextran oxidation state/solid content was observed among the formulations tested (Figure 5). Tissue samples extracted from each implant site were subjected to a molecular cytokine assay, in which cytokine markers of inflammation were quantified with a rat-specific multiplex array. Relative to the sham procedure, no significant differences in cytokine concentrations were found with co-polymer implantation, and once again no trend in cytokine expression with respect to isoreactive manipulation (Figure 6). Taken together, these findings demonstrate the general biocompatibility dextran-chitosan co-polymers, and more importantly support the hypothesized insensitivity of biocompatibility to isoreactive design manipulation.

### 3.5. Study Limitations

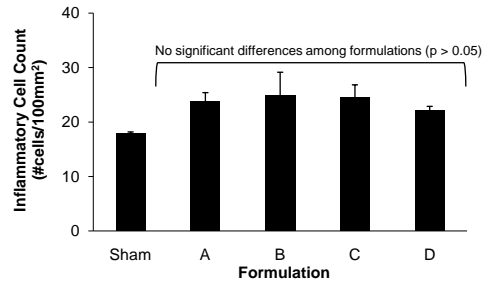
There are several study limitations that should be considered upon interpretation of our findings. First, we have not directly shown that the surface-present biochemical groups (amine groups) targeted for adhesive bond formation in fact have different densities/spatial distributions among tissue surfaces. While beyond the scope of the present study, the tissue-present amine group distribution could be quantified with the use of functional atomic force microscopy (fAFM). [16] [22] Indeed, future studies using fAFM on soft tissue and material surfaces for the purpose of quantifying and comparing the spacing/density of the



**Figure 4.** *In-vitro* cell response. The cytotoxicity of co-polymer formulations was quantified via neutral red uptake (NRU) assay formulations following direct material application to fibroblast cultures. There were no significant differences or discernable trends with respect dextran aldehyde percent oxidation/solid content.

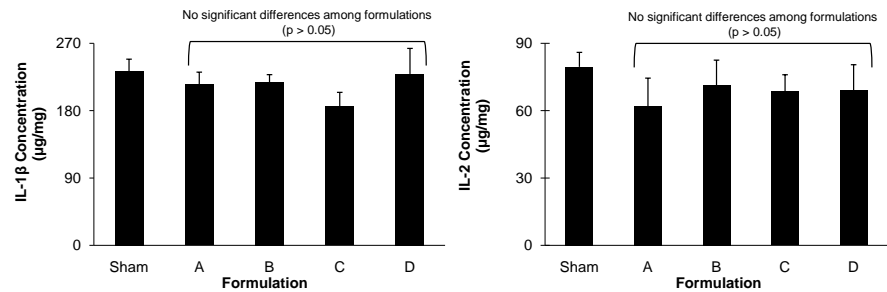


(a)



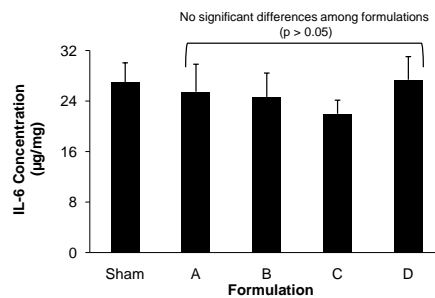
(b)

**Figure 5.** *In-vivo* inflammatory response. (a) Implant site. Co-polymer implants (light pink) are clearly visible with H & E staining and remain intact 7 days after subcutaneous dorsal implantation in rats. Scale bar = 1 mm and applies to all images; (b) Inflammatory cell count. The number of inflammatory cells local to co-polymer implant was not significantly elevated with respect to sham, and there were no significant differences or discernable trends with respect to dextran aldehyde percent oxidation/solid content.



(a)

(b)



(c)

**Figure 6.** Cytokine activity. Select interleukin (IL) concentrations (IL-1 $\beta$ , IL-2, and IL-6) in local tissue were quantified 7 days after subcutaneous dorsal implantation of co-polymers in rats. No significant elevations occurred with respect to sham, and there were no significant differences or discernable trends with respect to dextran aldehyde percent oxidation/solid content.

relevant (reciprocal) reactive groups would provide a means to directly test the proposed approach to enhance bioadhesion. Second, while bioadhesion strength

and interfacial morphology were assessed in a tissue-specific manner, we only provide general measures of material biocompatibility (*in-vitro* cytotoxicity and *in-vivo* tissue response following subcutaneous implantation). Moreover, the time point of the *in-vivo* studies (7 days post implantation) may have failed to detect the peak of the inflammatory response, which would likely occur upon material erosion and by-product generation. More comprehensive evaluation of the proposed design strategy, specifically the insensitivity of biocompatibility to isoreactive design manipulation, requires assessment of local tissue response in various implant scenarios and over the complete residence time of the material.

#### **4. Conclusion**

The aim of this study was to evaluate a novel approach for tissue-specific design of surgical sealants. Specifically, we investigated the potential for isoreactive tuning of polymer design variables to enhance tissue-material adhesion without compromising biocompatibility. Using an experimental aldehyde-mediated copolymer system, we were able to demonstrate that for select tissue types, isoreactive titration of constituent polymer oxidation state and solid content impacts bioadhesion in a tissue-specific manner, and conversely do not impact generalized indicators of material biocompatibility. These findings imply that for a given clinical application (targeted tissue type), isoreactive tuning of a surgical sealant can be optimized such that adhesion is maximized while material biocompatibility remains at a baseline level that is determined by other factors (most notably the overall bioreactive group content of the material). Although only demonstrated in our experimental material system and with a limited number of soft tissue types, we expect that this design concept can be extended to a broad range of bioadhesive materials that target a specific surface-present chemical group for adhesive bond formation.

#### **5. Declarations**

##### **Ethics Approval and Consent to Participate**

All animals were treated and cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all protocols were approved by the University of South Carolina's Institutional Animal Care and Use Committee.

##### **Competing Interests**

The authors declare that they have no competing interests

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##### **Authors' Contributions**

MJU, FGS, and TS conceived and designed all studies. JF, ER, AM synthesized

and characterized all co-polymers. AM, HD, FGS performed and analyzed implantation studies. JF, MJU, FGS, and TS prepared the manuscript. All authors read and approved the final manuscript.

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