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Thrombogenic Responses from Electrocured Tissue Adhesives

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Abstract

Electrocured tissue adhesives offer a new route towards addressing unmet surgical challenges in tissue fixation. We have recently developed an electrocuring adhesive (aka Voltaglue) by grafting carbene precursors on polyamidoamine (PAMAM) G5 dendrimers. The cationic adhesive can be cured through low-voltage activation and exhibits voltage and time dependent crosslinking, allowing some control over material and adhesive properties. Herein, we have evaluated the platelet adhesion and activation of the bioadhesives electrocured with applied voltages of 5V and 10V. The electrocured bioadhesives exhibited platelet resistant properties that was dependent on voltage magnitude.

Introduction

Surgical fixation of injured tissues is often required for proper healing and restoration of structure and function. Historically, the field of tissue fixation has relied on mechanical methods, including sutures, staples, and wiring [1]. The shortcomings of sutures and other mechanical fasteners have spurred the invention of adhesive technologies [2, 3]. The purported benefits of surgical adhesives mirror our common experience with everyday glues; instant fixation, ease of application without the labor of needles and thread. From a clinical perspective, they allow the incorporation of modern therapies, including drug delivery, hemostasis, and sealants. Others seek to expand the technology towards immobilizing various biomaterials, drug delivery depots, and medical implants [4-14]. Surgical adhesives commercialized or in development use varying methods to immobilize natural or synthetic materials through chemical bonding or molecular interlocking mechanism [15-20]. Despite these innovations, chemical bonding methods generally rely on two-part mixes and light curing. These methods are difficult to activate within laparoscopic or keyhole surgeries. In this regard, 1-pot surgical adhesive formulations that can be activated on-demand, on both dry and wet tissues are a current unmet clinical need. Electrocuring is an unexplored route towards addressing those surgical challenges [21]. Blood compatibility evaluation of electrocured adhesives is required for any future implant applications. Whole blood in contact with synthetic materials can induce surface mediated thrombosis via platelet adhesion, activation, and subsequent aggregation [22]. Platelet adhesion assays are first step to determine material blood compatibility [23]. In this work, 30% diazirine grafted G5 PAMAM was synthesized and electrocured with different applied voltages (5V and 10V) through a 3-carbon electrode chip. The electrocured bioadhesives are evaluated for platelet adherent and activation properties using human blood components.

Materials and Methods

Materials

Poly(amidoamine) dendrimer of 5th generation (G5 PAMAM, Mw=28.8 kDa) was purchased from Dendritech, Inc, USA. 3-[4-(bromomethyl)phenyl]-3-(trifluoromethyl)-diazirine (bromo-diazirine) was purchased from TCI, Japan. Poly-(lactic-co-glycolic acid) (PLGA 53/47) was procured from Sigma Aldrich, USA. Commercially available disposable screen printed Zensor electrodes were purchased from Zensor Research & Development, Taiwan. The 24 well polystyrene cell cultured plates were purchased from TPP Techno Plastic Products AG, Switzerland.

Synthesis of diazirine grafted PAMAM

Bromo-diazirine (371.7 mg) dissolved in methanol was added into the G5 PAMAM (in methanol) with an intended theoretical yield of 30% grafting of the total -NH_2 groups available. The reaction was carried out at room temperature for 24 hours with constant stirring in dark. Methanol was removed by a rotary evaporator followed by high vacuum (< 1 Torr). A pale yellow liquid of diazirine grafted PAMAM hydrogel bioadhesive (PD-30 further in text) was obtained. **Figure 1** shows the schematic illustration on synthesis of PD-30 from precursors of PAMAM and bromo-diazirine.

Electrocuring

PD-30 in 50% w/w PBS/bioadhesive was activated at different applied voltages of 5V and 10V using an Ivium potentiostat. The activation (alternatively electrocuring) was carried out over a disposable 3-carbon electrode chip (Zensor, Taiwan) as shown in **Figure 1**. The bioadhesive was activated by sandwiching the gel in between the Zensor electrode and a PLGA sheet (100 μm thick). After 10 minutes of activation, Zensor electrode and PLGA sheet were tested to failure (cohesive failure always observed) via lap-shear adhesion. PLGA sheets with electrocured bioadhesives were further evaluated for platelet adhesion and activation.

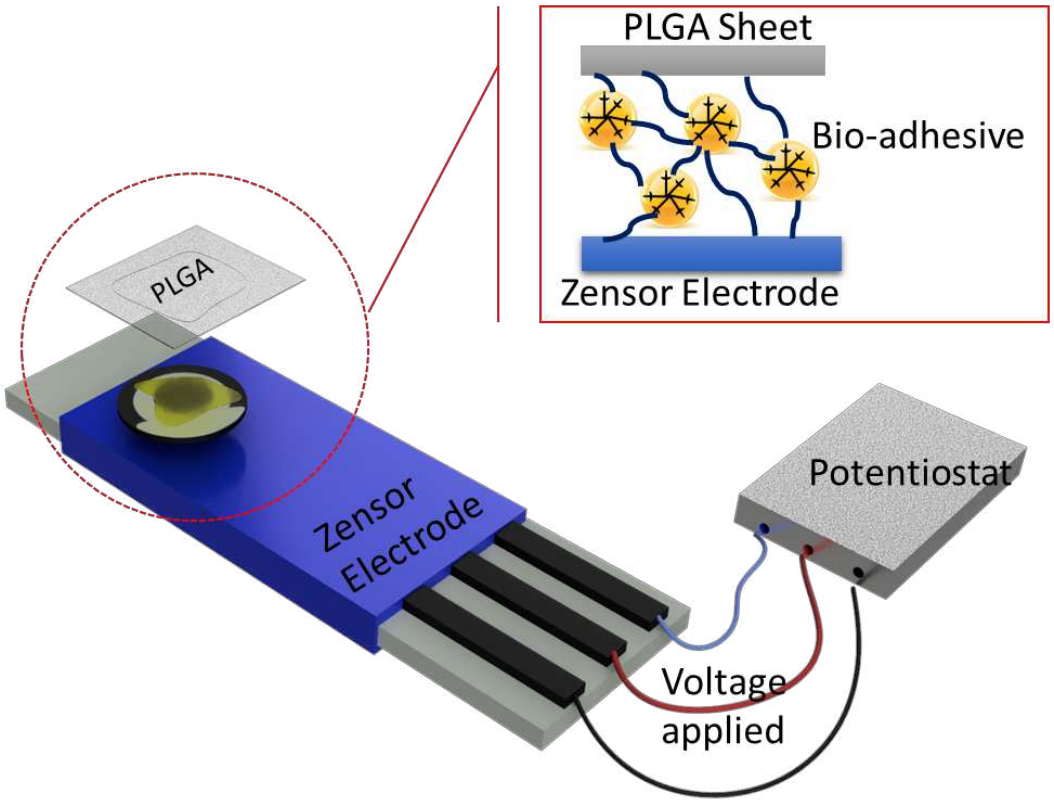

Figure 1. A schematic presentation showing the set up for the electrical activation of bioadhesive using Zensor electrode

Shear adhesion strength

The shear adhesion strength of PD-30 diluted in 1X PBS (50% w/w) was measured as per ASTM standard F2255–05. PLGA thin sheets were cut into 2x2 cm dimension and mounted on glass microscope slides, and Zensor electrodes were mounted on the other microscope slides using double sided tape. PD-30 (≈10 mg) was sandwiched between Zensor electrode and the PLGA sheet, which was then clamped using two paper binder clips and activated using 5 V, or 10 V for 10 minutes using a DC power supply (Agilent Technologies, USA). A tensile tester (Chatillon Force Measurement Products, USA) was used for lap-shear adhesive failure evaluation at 3 mm min^{-1} with a 10 N load cell. Maximum adhesive strength were recorded (n=3) for each applied voltage.

Human-derived platelet adhesion and activation evaluation

The electrocured bioadhesives were UV sterilized for 15 mins by exposing the adhesive surfaces to UV light in a sterilized cell culture hood. Fresh blood drawn from the cubital veins of healthy human volunteer was immediately mixed with 3.8 wt% of sodium citrate solution at 9:1(v/v) dilution ratio. The citrate mixed blood was centrifuged at 1000 rpm at 8 °C for 15 mins to obtain platelet-rich plasma (PRP). The PRP was further diluted with

PBS in a 1:1 (v/v) ratio. Diluted PRP (60 µL) was dispensed onto the sterilized electrocured bioadhesives (sample dimension 1cm^2) in a 24 well tissue culture plate. The PRP treated adhesive surfaces are incubated at 37 °C for 1 hour in a 5% CO_2 cell culture incubator. After 1h of incubation, the adhesive surfaces are gently washed in PBS (three times). The adherent platelets were fixed with 3% v/v glutaraldehyde (prepared in PBS) and kept overnight at 4 °C. After fixation, the samples were washed in PBS (three times) and subjected to serial dehydration with 10%, 25%, 50%, 75%, 90%, and 100% ethanol (10 mins each). The bioadhesive surfaces were dried and coated with platinum for 40 seconds prior to SEM observation. The samples were observed under a SEM (JEOL 6360, Japan) for platelet adhesion. The results were interpreted qualitatively (observation using SEM photomicrographs) and quantitatively (calculating the average number of adhered and activated platelets per mm^2 of the adhesive surface). The quantitative results were estimated considering three representative SEM photomicrographs under similar magnification of 2000X at three different areas of the sample. The distribution of different shape platelets were explained in terms of resting stage (non-dendritic) and activated (pseudopodial or dendritic) platelets [24].

Results and discussion

Bioadhesive hydrogel PD-30 was synthesized by grafting carbene precursors on polyamidoamine dendrimers [21]**.** The carbene precursor of 3-[4-(bromomethyl)phenyl]-3-(trifluoromethyl)-diazirine or bromo-diazirine was used as an electrocuring functional group, capable of crosslinking on-demand. In present synthetic procedure, the diazirine grafting percentage was controlled to maximum of 30%, in order to retain the water miscible properties of the PAMAM [25]**.** The prepared bioadhesive hydrogel was crosslinked via electrocuring using a Zensor electrode (**Figure 1**). The degree of crosslinking was controlled under varied electrocuring voltage. PD-30 was electrocured with two different applied voltages such as 5V and 10V to prepare the bioadhesives of different degree of crosslinking. The tissue adhesive properties of PD-30 were studied on PLGA substrates, a commonly implanted biomaterial. **Figure 2A** shows the load vs displacement at different voltages. The profiles showed viscoelastic properties after electrocuring with an increase in shear modulus from 5V to 10V. The maximum shear adhesion stress was measured and the mode of failure was also observed. After curing for 10 minutes at different voltages, the hydrogel exhibited a mean lap shear strength of (2.0±0.2)N/cm^2, and (2.9±0.1)N/cm^2 for 5V and 10V, respectively (**Figure 2**). Inspection of the surfaces after the failure indicated an electrocured bioadhesive layer on the surfaces of both PLGA and Zensor electrode, which was an indication of cohesive failure as opposed to adhesive failure at the interface. Control experiments showed no signs of adhesion with only viscous material properties present.

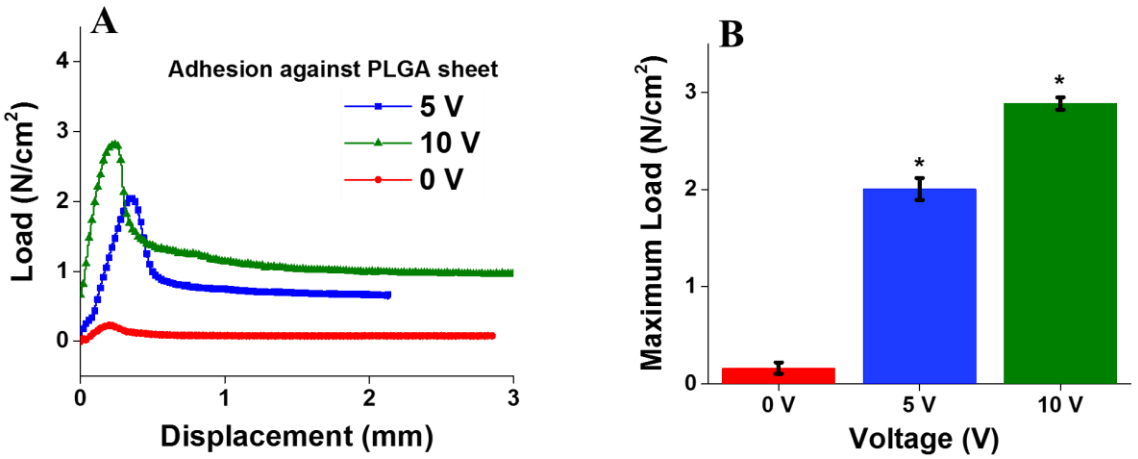

Figure 2. Lap-shear adhesion strength t. Load vs displacement curve of electrocured PD-30 bioadhesives using 1X PBS at a concentration of 50 wt% (A), and a comparison of maximum load values obtained across all tested voltages (0V, 5V, and 10V) after 10 minutes of electrical activation (B). *Significant at $p < 0.05$.

The thrombogenic responses from the electrocured bioadhesives were evaluated using platelet adhesion and activation test and were compared with the responses from the surface of a commonly implanted biomaterial, PLGA. Figure 3 (a-f) shows the SEM photomicrographs of adhered platelets over the surfaces of PLGA and PD-30 bioadhesives electrocured at 5V and 10V. SEM images revealed that the adhered platelets over the surfaces of PLGA and bioadhesives (electrocured with different voltage) showed the distribution of platelets of different morphology such as dendritic (activated) and non-dendritic (resting stage). As observed from the SEM images, most of the platelets adhered to the surfaces of electrocured PD-30 were of dendritic in morphology. Irrespective of the curing voltage, a higher number of platelets were adhered on the surface of electrocured bioadhesives compared to PLGA. The results indicated that electrocured PD-30 bioadhesives have higher thrombotic potential than PLGA. Figure 3 g shows the quantitative analysis on the number of adhered and activated platelets over the surfaces of PLGA, and PD-30 bioadhesives electrocured at 5V and 10V. The quantitative results demonstrated that the number of adhered and activated platelets were significantly reduced when cured at higher voltage. The bioadhesive electrocured with 10V displayed less platelet adhesion and activation compared 5V. Furthermore, the degree of activation (the percentage of activated platelets to the total number of adhered platelets: **Figure 4**) was also influenced in a similar trend. This indicated that the platelet resistant properties of the electrocured PD 30 can be improved with electrocuring at high voltage. Lap-shear adhesion data suggest more crosslinking at 10V. More crosslinking would expose less terminal amines and serve to prevent interactions of fibrinogen adsorption and subsequent platelet adhesion and activation [26]**.** The surface of pure PLGA had 60% less adhesion and 20% less activation.

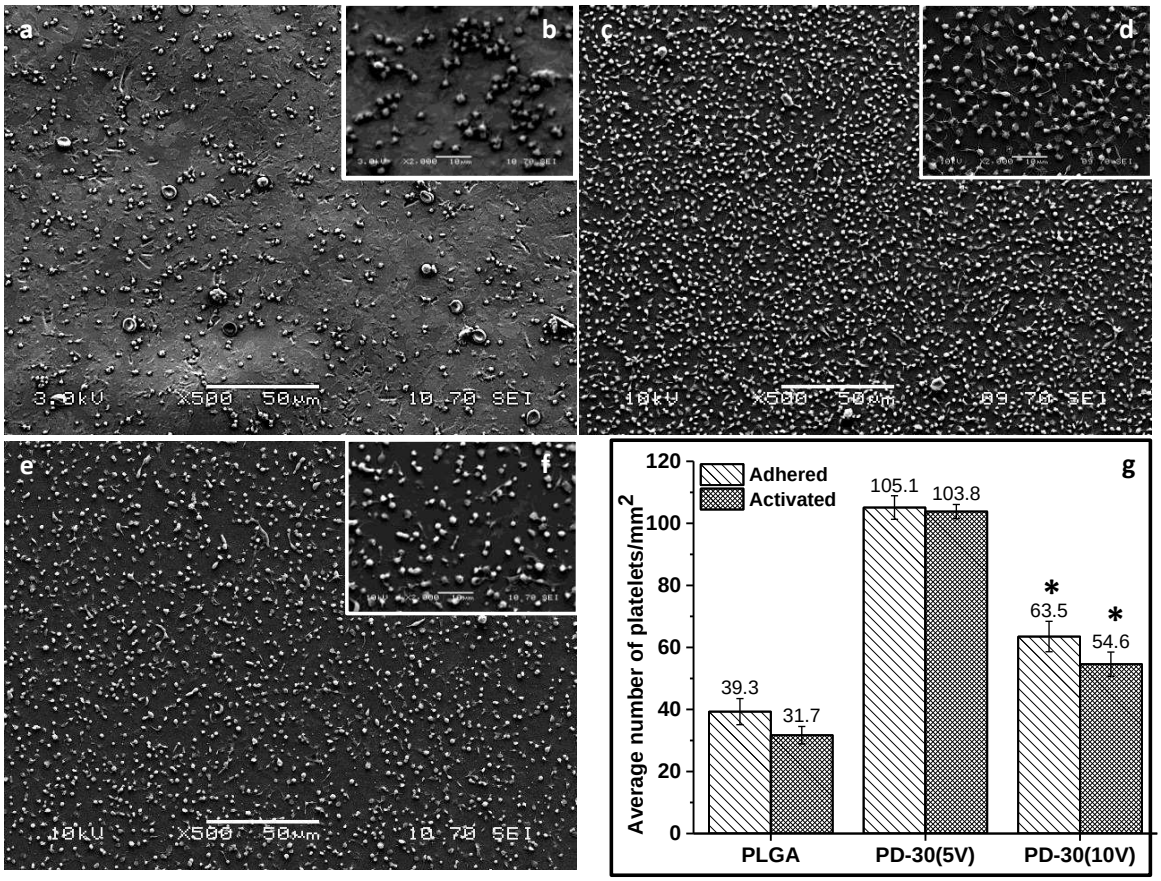

Figure 3. SEM photomicrographs (a-f) of the adhered platelets over the surface of PLGA (a, b) and electrocured PD-30 at 5V (c, d) and 10V (e, f), and the quantitative analysis of the adhered and activated platelets over different bioadhesive surface (g). *Significant at $p < 0.05$ among the similar comparison groups (adhered/activated); a, c, e: low magnification and b, d, f: high magnification SEM photomicrographs

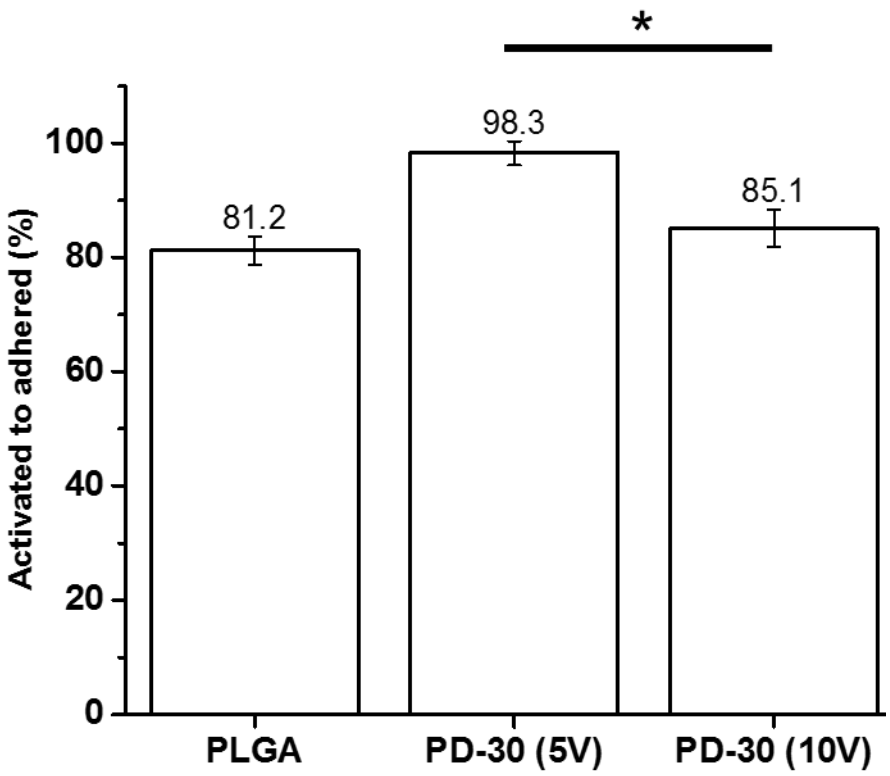

Figure 4. The degree of platelet activation in terms of the number of activated platelets to the total number of adhered platelets/mm^2 surface of the PLGA and bioadhesives electrocured with 5V (PD-30 (5V) and 10V (PD-30 (10V). *Significant at $p < 0.05$.

Conclusion

In conclusion, electrocured bioadhesives exposed to human-derived platelet rich plasma have more platelets adhered and activated when compared to a PLGA thin film. However, the adherence and activation could be significantly modified by the magnitude of voltage activation. The on-demand crosslinking and platelet activation could be promising for *in vivo* implant applications. Moreover, the dendrimer bioadhesive opens the possibility for further chemical refinement with blood modifiers towards more application specific therapies.

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