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Non-aqueous, tissue compliant carbene-crosslinking bioadhesives

Ankur Harish Shah^a, Oleksander Pokholenko^a, Himanshu Sekhar Nanda^b, Terry W.J. Steele^{a,}*

^aSchool of Materials Science and Engineering, Division of Materials Technology, Nanyang Technological University, Singapore 639798.

^bDepartment of Mechanical Engineering, PDPM-Indian Institute of Information Technology, Design and Manufacturing (IIITDM)-Jabalpur, Dumna Airport Road, Jabalpur-482005, MP, India

Corresponding Author: Terry W. J. Steele (e-mail: wjsteele@ntu.edu.sg)

Abstract

Surgical adhesives are an attractive alternative to traditional mechanical tissue fixation methods of sutures and staples. Ease of application, biocompatibility, enhanced functionality (drug delivery) are known advantages but weak adhesion strength in the wet environment and lack of tissue compliant behaviour still pose a challenge. In order to address these issues, non-aqueous bioadhesive based on blends of polyamidoamine (PAMAM) dendrimer, conjugated with 4-[3-(trifluoromethyl)-3H-diazirin-3-yl] benzyl bromide (PAMAM-g-diazirine) and liquid polyethylene glycol (PEG 400) has been developed. PEG 400 biocompatible solvent reduces the viscosity of PAMAM-g-diazirine dendrimer without incorporating aqueous solvents or plasticizers, allowing application by syringe or spray. Upon UV activation, diazirine-generated reactive intermediates lead to intermolecular dendrimer crosslinking. The properties of the crosslinked matrix are tissue compliant, with anisotropic material properties dependent on the PEG 400 weight %, UV dose, pressure and uncured adhesive thickness. The hygroscopic PAMAM-g-diazirine/PEG 400 blend was hypothesized to absorb water at the tissue interface, leading to high interfacial adhesion, however porous matrices led to cohesive failure. The hydrophilic nature of the polyether backbone (PEG 400) shielded cationic PAMAM dendrimers with cured bioadhesive film displaying significantly less platelet activation than neat PAMAM-g-diazirine or PLGA thin films.

91 Keywords: *PAMAM, dendrimer, PEG 400, diazirine, carbene, bioadhesive*

1. Introduction

The ability of sutures and staples[1, 2] to rapidly fix tissues prevails over their complications of inflammatory response, scar tissue formation[3] and secondary operations.[4, 5] Current innovations have focused on bioresorbable materials, mechanical architectures (e.g. barbed), surface coatings[6] and alternative methods of application.[7, 8] Modern developments in the surgical theatre are placing higher demands on less invasive methods of fixation, especially in the fields of laparoscopic and minimally invasive surgery (MIS).[9] New methods of fixation based on non-toxic bioadhesives could spur new technological innovations in MIS, which currently is noted to have few novel commercial technologies.[10]

Liquid adhesives are an attractive alternative to mechanical tissue fixation in terms of stress distribution without inducing puncture injuries. Novel methods of soft tissue adhesion require biocompatibility and bioresorbability for clinical translation. For the latter, this requires degradation kinetics having an overlap with tissue ingrowth while blood contacting applications need to demonstrate non-thrombogenic surface properties. Towards ease of application, liquid tissue adhesives require shear viscosities less than 250 mPa.s or require less than 10N (1 kg x g) force for applications by syringe or catheter, especially in laparoscopic/MIS surgery.[11-13] If the tissue adhesives are aimed at replacing suturing with high interfacial adhesion strength, then application and cure time needs to be on par with suturing rates; e.g. 40 min for caesarean sections.[14] Apart from this, modulus should closely match the strain related elasticity of the tissue, suggesting the cured tissue adhesive closely match the tissue elasticity to avoid stress concentration at the interface that could lead to adhesion failure.[15]

Fibrin glues[16] though biocompatible are mechanically weak. Cyanoacrylates have a short cure time but have tissue noncompliant rigidity[17] and limited to skin fixation due to toxic leachants. Polyurethane adhesives require long cure time (tens of minutes to hours) and two-part mixing before application.[18] Low viscosity and weak physical interaction of nanoparticle solutions based adhesive (~0.25 to 3 kPa) limits them to small, clean incisions.[19, 20] These shortcomings limit commercial adhesives to provide only auxiliary support[21, 22] for sutures for tissue regeneration[23], topical wound closure or emergency use[24] where suturing alone has a low success rate.[25] There is an unmet clinical need to develop soft tissue adhesives that have sufficient bonding strength on wet substrates, have tissue compliant mechanical properties (10-100 kPa) and be hemocompatible. A tissue adhesive with these

properties could potentially replace sutures/staples in connective surgeries (for example, blood vessel anastomosis), reduce surgical theatre time, and prevent secondary complications in the removal of sutures or staples.

Adhesives based on carbene crosslinking attempt to bridge the gap of the current surgical adhesives with attributes of rapid curing (< 60 s), tunable elasticity and adhesion to wet substrates. Thin film carbene adhesives grafted diazirine precursor directly to polyester surfaces and resulted in proof-of-concept wet adhesion to swine aortas and a tunable shear adhesion strength.[26] The polyester film employed has the advantage of photo or time adjustable drug delivery and adjustable mechanical properties[27-33] but they do not conform to irregular tissue surfaces. To overcome this limitation, a liquid formulation based on dendrimers was developed that could be spread on tissue surfaces, yet photo-crosslink into a strong bonding interfacial adhesive.[34-36] Both the thin film and liquid formulations display a correlation between light dose (UV joules), diazirine grafting, shear adhesion strength (~ 5 -20 kPa) and tunable mechanical properties within the liquid dendrimer formulations. The dendrimer adhesive consists of G5 polyamidoamine grafted diazirine (PAMAM-g-diazirine) dissolved in saline (25-75% w/w ratio of PAMAM-g-diazirine/saline). These formulations require the optimization and interplay between four main parameters: initial shear viscosity (before curing), dissolved w/w ratios, diazirine grafting (10-30% diazirine/ 1° amines) and final lap shear adhesion strength. Lap shear adhesion strengths above 10 kPa requires 50-75% w/w ratios, but 75% w/w ratios exhibit shear viscosities above 250 mPa.S, preventing syringe handling.[37] Formulations dissolved in saline will suffer from carbene non-specifically reacting with H_2O , obstructing intermolecular crosslinking (weak cohesive strength) and substrate bonding.[38]

To overcome the limitation of aqueous PAMAM-g-Diazirine bioadhesive, herein aqueous buffer is replaced with polyethylene glycol (PEG, Mn 400 Da) solvent. PEG 400 is known to be a biocompatible solvent miscible with PAMAM dendrimers.[39, 40] Unlike aqueous solvents, PEG 400 offers an electron rich $-OH$ bond and $-CH_2$ group towards bridging intermolecular bonding. Diazirine activation is accompanied with evolution of nitrogen gas (N_2). The solubility of N_2 is 3X times more in alcohol[41] compared to water and in low molecular PEG at $25^\circ C$ and 1 atm is between 4.7 to 8.9 mg/g[42] and has known pressure dependency.[41, 43] The non-aqueous bioadhesive becomes hygroscopic which is hypothesized to adsorb water on the tissue interface and improve interfacial adhesion

conformal contact. Lastly, addition of PEG may improve hemocompatibility against human blood due to ‘stealth’ properties induced by hydrophilic polyether backbone and steric repulsion.[44-47]

2. Materials & Methods

2.1 Materials

Fifth generation Polyamidoamine (Mw=28.8 kDa) was purchased from Dendritech, USA. 3-[4-(bromomethyl) phenyl]-3-(trifluoromethyl)-diazirine (diazirine) was purchased from TCI, Japan. Poly-DL-lactide-co-glycolide (PLGA 53/47, IV 1.07) was purchased from Corbion N.V, Netherlands. Methanol (MeOH) and Polyethylene glycol (PEG, Mw 400) were purchased from Sigma Aldrich, Singapore and used as received.

2.2 Synthesis of PAMAM-g-Diazirine conjugate and it's PEG blends

Diazirine was grafted onto 5th generation polyamidoamine (128 1° surface amine functional group) using previously published method.[37] Briefly, in a 500 ml round bottom flask, 1000 mg of PAMAM dendrimer and 181.75 mg of diazirine was added and diluted in 200 mL of methanol to achieve 15% conjugation degree, stoichiometrically calculated as ratio between primary amine and diazirine. Methanol was removed by rotary evaporation followed by high vacuum (< 1 Torr) to yield a thick amber paste. Required amount of solvent free PAMAM-g-Diazirine (15%) and PEG 400 were added in a 2 ml Eppendorf tube in the following weight ratios of 90:10, 70:30, 50:50, 40:60 and 30:70. A homogeneous suspension was obtained by pipetting 400 µl of MeOH and vortexing for 10 minutes. The suspension was dried under vacuum (< 5 Torr) till constant blend weight was achieved.

PAMAM-g-Diazirine (15%) and PEG 400 were analysed using NMR (Bruker Advance) at 400 MHz with D₂O as solvent at room temperature. ¹H NMR spectra were recorded using TopSpin 2.1 and analysed with TopSpin 3.5 pl 7. The residual peak of solvent was used as reference and assigned 4.85ppm for PAMAM-containing samples as it was found as typical value for PAMAM derivatives solutions in D₂O as the basic and buffer properties of PAMAM shifts it from standard 4.80 ppm value, this value was observed for PEG sample in D₂O.

2.3 Real-time dynamic mechanical properties

Uncured liquid adhesive blend (10 µL) were pipetted onto a quartz plate (P=PTD120/GL, Anton Parr, Austria) setup on rheometer (Physica MCR 501, Anton Paar, USA). The sample

was irradiated from underneath the quartz plate with 365 nm UV power of 200 mW cm⁻². Upon irradiation, the change in storage (G') and loss modulus (G'') are assessed with a 10 mm parallel plate probe fitted to the rheometer. Experimental parameter was fixed at 1% strain, 1 Hz with 100 µm gap thickness at 23°C. The experimental template was configured to record the changes in the gap size when the adhesive is being photocuring. The expansion ratio based on gap size is calculated based on equation 1;

$$\% \text{ Expansion} = ((t_f - t_i) / t_i) \times 100 \quad (1)$$

Where t_f and t_i is the final gap thickness and initial gap thickness.

2.4 SEM imaging

PAMAM-g-Diazirine (15%) : PEG 400 blend were cured between PET films at UV doses of 10, 20 and 60 J cm⁻² with intensity of 200 mW cm⁻² at 365 nm (Omnicure S1000 fitted with an external 365 nm filter). PET sheets were peeled away, exposing the cohesively fractured adhesive matrix. The samples were gold sputtered (5 Pa) and scanned. Using *Image J*[48] software a greyscale SEM was digitally enhanced for contrast with a 2-pixel median filter followed by auto threshold. To achieve, black pores with white borders, a radius count of 40 is applied followed by Otsu/median methods and watershed to achieved defined boundaries for pore surface area analysis. This digital analysis was limited till sufficient contrast was achieved. As an alternate, manual counting of pore was done by Image J software. The scale was used as a measurement reference and the longest length on the pore was recorded.

2.5 Bioadhesion strength (shear) measurements with vascular tissue

Lap shear adhesion strength measurements were based on ASTM standard F2255-05. Fresh swine aortas were procured from local abattoir within 4 hours of slaughter. The aortas were washed with 1X PBS and excess fat removed. The aorta was cut (20 × 20 mm) and was glued with cyanoacrylate adhesive onto a glass slide. PLGA squares of 20 × 20 mm were fixed onto slides using double sided tape. Adhesive (~30 mg) was pipetted onto the PLGA patch and clipped on the aorta tissues with paper clips applying 27 ± 5 kPa pressure. The lap-shear samples were cured with 365 nm UV dose of 10, 20 and 60 J cm⁻². Shear adhesive strength test was performed with a tensile tester (Chatillon Force Measurement Products, USA) at strain rate of 3 mm min⁻¹ with 50 N loading cell

2.6 Swelling and Leaching behavior of cured adhesive

Swelling behavior of cured adhesive blend (PEG 400 content 0, 10, 50 and 70 %w/w) were investigated by pipetting 5-10 mg of uncured adhesive blend between two 1 cm² PET sheets and photocured with UV dose of 10, 20 and 60 J cm⁻². Post photocuring, the PET sandwich was stripped and the fractured surface was immersed in 1X PBS at 37°C for 60 minutes. The weight of the PET sheet and cured adhesive was measured before the start of the experiments. Samples were taken out of the buffer and their weight measured after cleaning their surface with lint-free paper. Neat PAMAM-g-Diazirine was used as control. The percent swelling was calculated using the following equation

$$\% \text{ Swelling} = (W_b - W_o / W_o) \times 100 \quad (2)$$

Where W_b is the weight of the hydrated sample and W_o is the initial dry weight of the cured sample.

Similarly, to quantify the amount of material leached out of the cured matrix, adhesive blend with different PEG 400 content were photocured with UV dose of 60 J cm⁻². The cured matrix was incubated in D₂O which was spiked with 0.2% w/v pyrazine. Pyrazine has a single characteristic peak in ¹H NMR (8.734 ppm in D₂O) and was used to quantify amount of dendrimer and PEG 400 being leached out. To validate the NMR method, 2% w/v uncured adhesive was dissolved in pyrazine spiked D₂O and NMR spectra recorded to obtain the dendrimer to PEG 400 ratios.

The quantification by NMR was based on peak area ratios of spiked pyrazine and compound of interest. Pyrazine provided a singlet at 8.53 ppm with 4 H atoms per 80.09 Da, the compound of interest was either PEG, quantified by peaks of CH₂ groups, presented as two multiplets in 3.70-3.86ppm range, integrated together and assigned 4 H atoms per 44 Da as well as PAMAM, quantified by peak of α-methylene carbonyl groups, presented as broad singlet in 2.27-2.58ppm range with 508 H atoms per 28864 Da. (Table S3). The molar concentration of PAMAM (carbonyl group, peak @ 2.45) and PEG 400(ethylene oxide, peak @3.6-3.8) was calculated using the eq.3

$$K = (A \times W_{py}) / (I_{peak} \times M_w), K_{Pyrazine} = K_{PAMAM} = K_{EO} \quad (3)$$

A: number of protons (4,508 and 4 for pyrazine, PAMAM and EO)

W_{py} : known weight (pyrazine, 0.2% w/v in D₂O)

I_{peak} : Integration area, M_w : Molecular weight

Rearranging eq.3, the residual mass PAMAM-g-Diazirine and PEG 400 is calculated. Concurrently, the PET sheets with intact adhesive were dried in vacuum oven at 37°C for 48 hours and its weight recorded. Residual weight of the cured matrix on the PET sheet after leaching is calculated using eq.4

$$\text{Residual Weight} = 1 - (W_{\text{mass after leaching}} / W_{\text{dry weight of adhesive}}) \quad (4)$$

2.7 Human-derived platelet adhesion and activation evaluation

PAMAM-g-diazirine (15%) with increasing PEG content (10, 50 and 70% w/w, 10 μ l) was pipetted between two PLGA patches (2 X 2 cm) and cured with an UV dose of 20 J cm⁻². Before platelet incubation, the cured adhesives were sterilized for 15 min at 254 nm in a sterilized cell culture hood. The platelet adhesion test was performed by the standard procedure for evaluating blood compatibility of artificial materials.[49, 50] Briefly, freshly drawn blood from the cubital veins of healthy human volunteer was 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 separate platelet-rich plasma (PRP). The PRP layer was pipetted in 2 mL eppendorf and diluted with 1X PBS in a 1:1 (v/v) ratio. Diluted PRP (60 μ L) was dispensed onto the sterilized bioadhesives surface in a 24 well tissue culture plate and incubated at 37°C for 1 hour in a 5% CO₂ cell culture incubator after which it was 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 1X PBS thrice and subjected to serial dehydration with 10%, 25%, 50%, 75%, 90%, and 100% ethanol for 10 mins each. The bioadhesive surfaces were dried overnight under vacuum and coated with platinum for 40 seconds prior to SEM observation. The samples were imaged using a SEM (JEOL 6360, Japan) at accelerating voltage of 10 KV for platelet adhesion. The quantitative analysis was done by calculating the adhered and activated platelet per mm² of the adhesive surface. The average number was obtained by analyzing triplicate images at 5000X at three different areas of each sample image. The distribution of different shape platelets were explained in terms of resting stage on non-activated (spherical non-dendritic) and activated (deformed pseudopodial or dendritic) platelets.[50]

2.8 Statistical analysis

All the experiments were performed in triplicate. Statistical analysis is performed using one-way ANOVA * = $p < 0.05$, ** = $p < 0.00$ (Originpro software). Strong correlation (Pearson's r) exists is positive for $r > 0.7$, negative or inverse for $r > -0.7$.

3. Results

Five non-aqueous adhesive blend with varying weight ratios of PAMAM-g-Diazirine (15%) dissolved within PEG 400 are evaluated by photorheometry and lap shear adhesion. Mechanical properties are assessed as a function of PEG 400 w/w ratio, 365 nm UV dose, gel thickness and normal force set during the curing process. The bioadhesion strength on ex vivo vascular tissue (swine aorta) is assessed to gauge the dependence of wet tissue adhesion in regard to PEG content and UV dose. Platelet adhesion and activation from human platelet rich plasma (PRP) is assessed as a function of PEG content at constant UV dose.

3.1 Bioadhesives exhibit UV dose dependent moduli with J-curve stress strain response

Aqueous-free bioadhesive blend are made by mixing PAMAM-g-Diazirine (15%) and PEG 400 in varying weight ratios. Homogenous blend is prepared through a methanol medium and later evaporated, as neat PAMAM-g-diazirine (15%) is too viscous for magnetic stirrers. With the addition of PEG, the dynamic viscosity is calculated by dividing the loss modulus by angular frequency (1 Hz, 6.26 rad/s) after 60 s of shearing. PEG acts as a diluent reducing the dynamic viscosity of the uncured bioadhesive blend. As seen in **Fig. 1a**, the dynamic viscosity of the neat PAMAM-g-Diazirine is reduced by three orders of magnitude, from 56800 ± 40 to 76 ± 0.3 mPa.s as a function of PEG content.

The bioadhesive blend is irradiated with 365 nm (UV power 200 mW cm^{-2}) and the changes in the material properties i.e. storage (G') and loss modulus (G'') are recorded in real time as a function of UV energy (joules). As seen in **Fig. 1b**, the uncured bioadhesive is a viscous liquid ($G'' > G'$) and sheared for two minutes before UV irradiation. Upon UV irradiation, instantaneous change in G' is observed which is attributed to intermolecular crosslinking. The time of UV irradiation was based on the net UV dose applied; 10, 20 and 60 J cm^{-2} (UV Dose = UV power x time). The bioadhesive transitions into a viscoelastic solid ($G'' < G'$) at the defined gelation point ($G'' = G'$). The gelation point depends on the PEG content in the bioadhesive blend. As seen in **Fig. 1c**, crossover for neat PAMAM-g-Diazirine (15%) is achieved within 45 ± 4 s, which reduces to 25 ± 2 s for bioadhesive with 50% w/w PEG content and 16 ± 1 s for 70% w/w PEG content. The *effective* storage modulus (G_e') of the UV cured matrix is so named due to its foam structure that results from N_2 evolution. The dependency of the effective storage modulus is assessed as a function of PEG content and UV dose applied. Neat PAMAM-g-Diazirine (15%) is compared with PEG dispersant. UV doses 10 to 60 J cm^{-2} increases G_e' 6-fold from 40 ± 5 to 245 ± 25 kPa, as seen in **Fig. 1d**, but high dynamic

viscosity (56800 mPa.S) prevents syringe application and has difficulty in uniform spatula spreading. With 10% PEG, G_e' changes from 38 ± 12 to 134 ± 7 kPa (10 to 60 J cm⁻²), while at 70% PEG content G_e' changes from 34 ± 2 to 41 ± 2 kPa, which is significant. At UV dose of 10 J cm⁻², G_e' ranges between $30 - 40$ kPa with no statistical correlation vs. PEG content (Pearson's r : 0.088, data not shown). An inverse correlation is observed between G_e' and PEG content at UV dose of 20 and 60 J cm⁻².

For the cured bioadhesive blend to be tissue compliant, its deformation profile (stress vs. strain) should mimic the J-curve present in natural materials, hybrid composites, and soft tissues.[51-53] This is assessed by an amplitude sweep under physiological strains. The deformation profile of the neat and cured bioadhesives is displayed in **Fig. 1e** for a strain range of 0.1 to 100% . Upon applied shear stress, photocured adhesives mimic tissue properties that result in J shaped stress-strain curve with anisotropic moduli. Tissue response is further discussed in **sec 3.4**. In comparison to neat PAMAM-g-Diazirine (15%), addition of PEG creates softer adhesive matrix with lower modulus; as a function of PEG content and UV dose, per given strain. Increase in elongation when PEG ratios are greater than 50% is also reported (**Fig. 1e**). The failure strain of the cured bioadhesive is between $70-90\%$ for low PEG content (10 and 30% w/w) and increases up to 150% strain at higher PEG content ($\geq 50\%$).

Figure 1: a) Dynamic viscosity of bioadhesive blends (SD < 1%). b) Storage modulus (G') and loss modulus (G'') of PAMAM-g-Diazirine (15%) with varying PEG content cured with UVA dose: $0 - 60$ J cm⁻². c) Gelation time/UV dose for bioadhesive blend. d) Summary of effective storage modulus (G_e') for cured bioadhesive as a function of PEG content and UV dose. e) Shear stress-strain response of cured bioadhesive blends (UV dose: 60 J cm⁻²) and f) Linear fit of effective storage modulus (G_e') against PEG content in the bioadhesive blends.

3.2 Pore count in cohesively fracture films is inversely correlated to PEG content.

After photocuring, the bioadhesives blend are observed to cohesively fail, with fracture occurring within the matrix and not at the substrate. SEM images of the cohesively fractured bioadhesive blend are displayed in **Fig. 2a-2f**, with pore size distribution seen in **Fig. 2g** and **2h**. Neat PAMAM-g-Diazirine has a porous structure with pore sizes of 50 μ m or less. The interconnected pores could not be summed for a comparable size distribution. The average pore length of PAMAM-g-Diazirine: PEG is 170 ± 53 and 280 ± 43 μ m at 10 and 30%

PEG 400 (w/w). The pore size distribution becomes broader as PEG content increases, but no correlation is seen with average pore length. The number of pores per cm^2 is inversely dependent on the PEG content, as expected (Pearson's r : -0.948). Based on qualitative (visual) assessment, pore wall thickness increases as the PEG content increases.

Figure 2: Representative SEM images of cohesive fracture surface (a-f) of UV cured PAMAM-g-Diazirine (15%) with increasing PEG 400 (10, 30, 50, 60 and 70% w/w). h) Pore size distribution of the cured bioadhesive as function of PEG content (10, 30, 50, 60 and 70% w/w). g) Dependency of the average pore size and density as a function of PEG 400 content. Scale bar 200 μm .

3.3 Low PEG content has a strong correlation between G_e' and applied load.

The major factors governing G_e' comprise N_2 gas nucleation (foaming) and intermolecular dendrimer crosslinking—limiting the former is predicted to enhance the latter. To assess this hypothesis, gap thickness (100 μm initial minimum) is varied to maintain a constant static load when the expanding bioadhesive blend is photocured. The normal force and gap thickness is continuously monitored by the rheometer software to maintain the normal load desired with a minimum gap thickness. The bioadhesive blend is subjected to three static loads of 0.1, 1.0 and 10 N (1.3, 13 and 130 kPa) with a minimum gap thickness of 100 μm and subsequently photocured with UV dose: 60 J cm^{-2} . G_e' displays a strong correlation at low PEG 400 content (10%), with a decreasing dependence as the PEG 400 content (**Fig. 3a**). The change in the gap size is due to evolution of nitrogen gas (N_2) when diazirine converts to carbene. At higher diazirine concentration, higher amount of N_2 is evolved and the adhesive expands which can be restricted by applying opposing static loads. These loads act as counter to the pressure generated due to N_2 generation. For every adhesive blend, higher % expansion is observed at lower static load; % expansion @1.3kPa > % expansion @13kPa > % expansion @130kPa, as seen in **Fig. 3b**. Lower %expansion is expected to yield denser pores making photocured matrix stiffer as observed by increasing G_e' as the static load increases as seen in **Fig. 3a**. The magnitude of this changes i.e. G_e' and %expansion reduces as the PEG content in the adhesive blend increases. Representative gap thickness vs joules/time is given in **Fig. 3c**, which demonstrates the rheometer feedback with respect to gap thickness and applied load. An initial delay (ca. 15 s) in expansion is observed at all 3 loads, where the static load is less than set due to the 100 μm gap precondition—rheometer is programmed not to adjust below this gap minimum.

Figure 3: Influence of static load on the material properties of photocured bioadhesive blend: a) Effective storage modulus (G'_e) as a function of static loads (0.1, 1 and 10 N ~ 1.3, 13 and 130 kPa) pressure applied during photocuring. b) Expansion ratio before and after photocuring. c) Thickness as function of UV dose and pressure applied for 50% PEG 400 as a representative formulation.

3.4 Initial viscosity controls G'_e dependence on constant thickness curing

Opposite to the above, gap thickness is held constant and the normal load is varied to maintain it. G'_e and normal load is measured in real-time as a function of gap thickness and UV dose. **Fig. 4a** summarizes the results of G'_e vs. gap thickness. Bioadhesive blend with 10% PEG 400 (and highest dyn. viscosity, **Fig. 1c**) displays no correlation between modulus and thickness, 135-145 kPa G'_e @ 60J is constant across all thicknesses. Initial thickness displays a strong inverse correlation on G'_e as shown in **Fig. 4b** from adhesive blends with 50% PEG content or more. A maximum normal force of 10 N is observed at 100 μ m which falls precipitately to 0.1 N at 200 μ m gap thickness. Once established (< 1 min), the normal force remains constant, but decreases slowly (ca. 0.5 N min^{-1}) after UV irradiation is off. For 50 % PEG 400 content, stress-strain curves exhibit an anisotropic curve with dependence on gap thickness, which is representative of the high PEG 400 content formulations. As shown in **Fig 4.c**, the stress strain response of the photocured bioadhesive mimic J shaped stress-curve observed when soft tissues are subjected to stresses.[54]

Figure 4: Influence of initial uncured bioadhesive thickness (gap size) on the material properties of the photocured bioadhesive: a) Normal force as a function of UV dose and thickness for PAMAM-g-Diazirine (15%) : PEG 400 (50:50). N_2 evolution during photocuring expands the bioadhesive, generating the normal force. b) Effective storage modulus (G'_e) as function of PEG 400 and thickness at 13.0 kPa of pressure. c) Stress-strain curve of bioadhesive cured with different thickness with overlay of known stress-strain response of carotid, thoracic and transverse artery.[54]

3.5 High ratios of PEG 400 reduce swelling, but is rapidly extracted across all ratios

The photocured bioadhesive blend is immersed in 1X PBS and mass is measured at 5,15 and 60 minute time interval. As seen in **Fig. 5a**, the bioadhesive blend with higher dendrimer

content displays higher % swelling. % swelling decreases as function of PEG 400 in the bioadhesive blend. Neat PAMAM-g-Diazirine (15%) has no UV dose dependency with % swelling (~300%).

PEG 400 and PAMAM-g-Diazirine are completely miscible in water and tested as a leachate in photocured bioadhesives. Neat PAMAM-g-Diazirine and three bioadhesives with varying PEG 400 content are photocured with UV dose of 60 J cm^{-2} . The amount of material leached is quantified via NMR and gravimetric mass balance. PAMAM-g-Diazirine, PEG 400, and presence of residual methanol was assessed using NMR. Uncured bioadhesive is dissolved in D_2O with pyrazine (0.2 %w/v) as internal standard and NMR spectra recorded (supplementary information **Fig. S2**). Based on eq.2, mass ratio between uncured PAMAM-g-Diazirine (15%) and PEG 400 is similar to the adhesive weight ratio (%w/w) (supplementary information, Table S3). The absence of methanol peak (δ : 3.34ppm in D_2O) indicates the bioadhesive were solvent free. The water peak (δ : 4.85 ppm in D_2O) is due to the water present in D_2O and differs from expected 4.74 because of basic properties of PAMAM. **Fig. 5b** summarizes residual mass of cured matrix on PET sheets. The residual mass of neat PAMAM-g-Diazirine via NMR is $96.0 \pm 1.5\%$ and gravimetrically is $86.0 \pm 2.1\%$. Photocured bioadhesive with higher PEG 400 content show higher mass loss with strong inverse correlation (Pearson's r : -0.999).

Figure 5: a) %Swelling of cured bioadhesive as function of UV dose, PEG 400 content, and incubation time. b) Residual weight of bioadhesive with varying PEG 400 content photocured with 60 J cm^{-2} after incubation in 0.2% w/v pyrazine spiked D_2O for 60 minutes assessed by NMR and gravimetric mass balance.

3.6 Consistent lap shear bioadhesion strength is achieved at higher PEG 400 content.

Addition of PEG 400 is expected to make the dendrimer bioadhesive hygroscopic, leading improved interfacial conformal contact by surface water absorption. This is indirectly assessed with lap shear bioadhesion strength, each at three UV doses (10, 20 and 60 J cm^{-2}). The bioadhesive is photocured against endothelial (intima layer) of hydrated swine aorta and subjected to lap shear. The sample is strained until failure. Representative load displacement curve for bioadhesive cured with effective UV dose of 60 J cm^{-2} is shown in **Fig. 6a**. The average lap shear adhesion strength of the bioadhesive as function of PEG content and UV dose is summarized in **Fig. 6b**. Bioadhesive with less than 50% PEG 400 has adhesion strength

between 2 - 4 kPa with no observed dependency on UV dose. Bioadhesive with 50% or higher amount of PEG 400 have lap shear adhesion strength between 5 -15 kPa. Bioadhesive with 60% PEG 400 has consistent adhesion strength between 9-11.5 kPa with maximum adhesion strength of 11 ± 3 kPa when photo-cured with UV dose of 20 J cm^{-2} . No statistical difference is noted between the 3 UV doses however.

Figure 6: Lap shear assessment against ex vivo swine aorta: a) Lap shear load vs. elongation of bioadhesive cured with UV dose 60 J cm^{-2} (inset: fractured bioadhesive – ex vivo tissue). b) Lap shear adhesion strength at failure as a function of PEG 400 content and UV Dose.

3.7 PEG shields cationic PAMAM and prevents activation of human platelet rich plasma.

Platelets present in human blood undergo thrombosis[55] when in contact with foreign materials. Surface modification and use of non-thrombogenic additive are employed to minimize the response.[56-58] PAMAM bioadhesives utility in blood environment is evaluated through human derived platelet activation and adhesion.[49, 59, 60] Thrombus formation is the result of non-specific protein (fibrinogen) adsorption, platelet adhesion, activation and subsequent aggregation.[60, 61] **Fig. 7a** displays the SEM images of the adhered platelets over the surface of cured PAMAM-g-Diazirine (15%): PEG 400 samples. Neat PLGA and cured PAMAM-g-Diazirine (15%) serve as controls (**Fig S3**). As seen in **Fig. 7b**, addition of PEG 400 shifts the surface concentration and ratio of platelet activation and adhesion. The surface distribution over neat PAMAM bioadhesive displays nearly complete activation of all adhered human platelets (**Fig. S3**). Cellular deformation of pseudopodia or filopodial outgrowth and subsequent degranulation and spreading are the characteristics of activated platelets[50, 62]. While in presence of PEG 400, majority of platelets had a non-dendritic, spherical morphology when imaged on three-dimensional porous architecture. **Fig. 7b** summarizes the quantitative data of platelet adhesion and activation as a function of PEG 400 content. PEG 400 formulations observed a statistically lower surface density than neat bioadhesive, but was higher than PLGA. The quantitative data demonstrated that all the PEG formulations showed significantly lower activation than neat bioadhesive or PLGA.

Figure 7: (a) SEM photomicrograph of adhered platelets over the surface of cured PAMAM-g-Diazirine (15%) with varying PEG content (0, 10, 50 and 70 %w/w). (b) Quantitative data of platelet adhesion and activation as a function of PEG 400 content.

4. Discussion

Minimally invasive surgeries account for 37% of surgeries in the USA and rising.[63]. Concurrent development in the wound closure/sealing technology is lacking, with bioadhesive formulations relying on redevelopments of conventional glues (acrylates, urethanes, two-part adhesives). Development of minimally invasive suturing devices[64] aid in wound closure, but it is accompanied with additional ergonomic and instrumentation training which adds on to the complexities associated with MIS suturing.[65] Hence, there exists an unmet clinical need to develop adhesives and sealants that replace suturing while being amenable to catheter delivery. This disqualifies moisture activation (cyanoacrylates), two-part mixing (fibrin, polyurethanes), and exothermic reactions (acrylates). In this regard, this paper presents an alternative technology: non-aqueous carbene crosslinking bioadhesives. Carbene precursor bioadhesives were plasticized with liquid PEG 400 to allow a free flowable, one-pot tissue bioadhesive that cures within minutes. Aqueous-free formulations provide a longer shelf-life and allow incorporation of hydrophobic and particle-based therapeutics. Diazirine (carbene precursor) upon 365 nm UV irradiation forms highly reactive carbene and diazo intermediates. Carbene nonselectively reacts with the primary amines on the PAMAM dendrimer as well as undergo O-H and C-H insertion in the PEG 400 backbone. However, the leachate results in **Fig. 4** indicate carbene-amine is the dominant intermolecular cross-linking, as PEG 400 is found in stoichiometric ratios. This suggests a diazo-dominant pathway[66], which is known to decay to carbene with sufficient UV intensity.[67] Regardless, crosslinking is accompanied by N₂ gas, which is visually observed as foaming. This imparts a degree of porosity at the cost of weaker mechanical properties. This could be offset through binary or tertiary ratios of PEG molar mass ratios, but complicates the structure activity relationships and will be part of our future work. Use of PEG 400 imparts the following advantages: 1) PEG 400 acts as a water-free plasticizer, lowers dynamic viscosity and improves surface wetting; 2) improves N₂ solubility towards less foaming; 3) imparts less platelet activation; 4) prevents hydrolysis of active compounds; and 5) allows in situ drug depots and volatile dispersant formulations.[68, 69]

Bioadhesives with low viscosity and fast gelation times are critical selection parameters in the clinical theatre[70]. Addition of PEG 400 reduces the dynamic viscosity of neat PAMAM dendrimers by 3 orders of magnitude. At 60% or higher PEG 400, Cilurzo et.al's viscosity criteria are met for bioadhesive to be syringed with 21G needles[13]. Upon photocuring, the

bioadhesive transitions from liquid like to elastic like material due to carbene induced intermolecular covalent crosslinking. The gelation time ($G' = G''$) is dependent on UV dose, where lower doses are favourable for faster curing and optical engineering. Neat PAMAM-g-diazirine required 9 J cm^{-2} for gelation, while only $3\text{-}5 \text{ J cm}^{-2}$ is required for the PEG 400 formulations (**Fig. 1c**). With irradiation power of 200 mW cm^{-2} , the time required to achieve gelation required is 15 - 45 seconds. The material properties of the bioadhesive blend can be tuned by varying the UV dose or diazirine molal concentration. While neat PAMAM-g-Diazirine has a wide modulus range of 40-245 kPa, it's high viscosity requires VOC components dilution (typically flammable) for practical applicability. By selecting a combination of UV dose and PEG 400 content, the properties of the cured bioadhesive can be tuned to match the specific shear modulus of human soft tissues such as lungs (10-54 kPa), stomach (8-45 kPa), ventricle heart wall (60 - 148 kPa) and liver (37 - 340 kPa).[71] Comparatively, surgical bioadhesive have shear modulus 1 to 4 order magnitude higher than soft tissues. Vakalopoulos et.al have measured the shear modulus of surgical glues under similar conditions of the photorheometry employed herein (1 Hz, 1% strain); storage modulus of Dermabond (cyanoacrylate) 40 MPa, Bio-glue (two component, BSA/glutaraldehyde) 25 MPa, Evicel (fibrin) 2 MPa and Tissucol (fibrin) 0.6 MP.[17] None of the formulations would function in a biocompatible solvent like PEG 400, which also imparts beneficial gas colligative properties.

The adhesive performance of the bioadhesive blend was assessed against hydrated swine aorta instead of standard skin surface. While commercial adhesives have shown superior performance for skin tissue, their performance is severely diminished in hydrated environment due the presence of water layer at tissue adhesive interface. To assess the performance of bioadhesive blend against such limitation, the swine aorta was selected as tissue model and adhesion strength was assessed against the endothelial side. Addition of PEG 400 overcomes the limitation of interfacial water layer owing to it's hygroscopic nature. Increasing PEG content improves the adhesion strength while reducing the reliance on diazirine concentration. At 50% or higher PEG content, the adhesion strength of the bioadhesive blend (8-12 kPa) is higher than reported value for fibrin based adhesive (5 kPa).[72]

As the bioadhesive transitions to bioelastomer, it is accompanied by N_2 release. N_2 nucleation within the matrix forms pores. Pore size and distribution observes an inverse relationship with PEG 400. Solubility of N_2 in pure PEG 400 is known to reach 4.7 mg/g, thus increasing PEG

400 content should improve the solubility of N₂. However, PEG 400 ratios lead to larger pore sizes, suggesting dynamic viscosity was the major factor (faster stress dissipation = smaller pores).[42] Compared to neat PAMAM-g-Diazirine (50 μ m, high dynamic viscosity), average pore size increases by factor of 3 (170 μ m) and at 50% PEG 400 the pore size increases by a factor of 9 (~ 450 μ m, lowest dynamic viscosity). The porosities observed meet the requirements for fibroblast ingrowth (5–15 μ m), bone osteogenesis (100–350 μ m) and angiogenesis (>500 μ m) assuming the requisite biochemical milieu is present[73-75]. Thus, the porous formulations may be exploited for tissue regeneration and soft tissue mimicry.

For adhesives that attempt to mimic tissue compliance, tuneable anisotropic moduli (j-curve, non-linear stress-strain) are desired. Photocured bioadhesive herein demonstrate anisotropic moduli when stressed, mimicking elastin and collagen dominated deformation at low and high strains. Such response can be attributed to synergetic effect of dendrimer crosslinking, PEG 400 plasticizer, and the porous voids. When initially stressed the matrix is strained due to shear deformation through pores. As the matrix is continuously strained, crosslinked dendrimers align and pores collapse, increasing the relative modulus of the matrix, referred as strain hardening. The load-displacement curve displays outsized strains at failure (assuming adhesive cured thickness of 100 μ m, 1000-2500% strain at break is calculated) which is a magnitude higher than the oscillatory failure strain. The outsized strain is due to combination of shear deformation of crosslinked adhesive and mm-thick ex vivo tissues. Under physiologically relevant stain (10% strain), the relative modulus of the sandwich substrate is less than 0.5 kPa (obtained by linear fitting load-displacement curve from **Fig .6a**) and is similar to tissue's Young's modulus at 10% tensile strain for common carotid artery (~0.2 kPa).[76] Adhesive that mimic soft tissue mechanical properties may serve as a mechano-transducer to signal and influence cell biology and growth.[77, 78] This is known to reduce scar tissue formation, a known complication associated with sutures and staples.[79]

PAMAM[80] and PEG 400 are known to hydrophilic, hence the photocured bioadhesive displays considerable swelling. The properties may serve to address unmet clinical needs in terms of sealants (e.g. pneumothorax) or towards more advanced drug depots (e.g. microparticle, labile proteins, or inorganic encapsulation).[81, 82] Optimizing UV dose will vary the rate of therapeutic release through modulus gradient, which is unattainable by cyanoacrylate or two-part adhesives. The presence of water layer at the tissues interface limits

the ability of most adhesives to crosslink with the tissue substrate directly. Carbene is known to react with water thereby reducing the crosslink density with the tissue surface ultimately leading to weaker adhesive strength. This was addressed by hygroscopic nature of PEG 400, which upon application absorbs the water from the tissue surface leading to better wetting. This hypothesis is supported by comparing the shear modulus with lap shear adhesion. Higher molal diazirine did not correlate with lap shear strength (**Table S3**). Bioadhesive with 50% or more PEG 400 content observed lap shear adhesion higher than neat PAMAM-g-diazirine or fibrin adhesives (5 kPa)[83]. The average lap adhesion strength increases with the amount of PEG 400 (better surface wetting) and is independent of UV dose. The swelling ratios suggest the cationic PAMAM dendrimers are hypertonic, drawing water and anions (e.g. Cl^- , HPO_4^{2-}) from the tissue substrates, impeding surface bonding. PEG 400 may alleviate or slow this osmotic imbalance, which is demonstrated in significantly lower swelling. The load-displacement curve display outsized strains at failure (assuming bioadhesive cured thickness of 100 μm , 1000-2500% strain at break is calculated) which is a magnitude higher than the oscillatory failure strain. The outsized strain is due to combination of shear deformation of crosslinked bioadhesive and mm-thick ex vivo tissues. The pressure applied by lap shear paper clips is 13.5 kPa (5.4 N normal force/4 cm^2 tissue area), which is similar to the bioadhesive normal force expansion (**Fig. 3b**, 13.3 kPa). At these curing pressures, the relative modulus of the sandwich substrate is less than 0.5 kPa, which is similar to tissue's young's modulus at 10% tensile strain for common carotid artery (~ 0.2 kPa).[76] Thus, under these activation parameters, soft tissue adhesion of vascular conduits is feasible, but one must consider formulations for bioinert blood interactions—a formidable hurdle for any tissue adhesive or vascular implant.[35, 36, 84]

Interactions with non-specific plasma proteins is a discerning parameter for tissue closure devices—steering application towards hemostatic agents or hemocompatible coatings. Previously, we have demonstrated that aqueous-based, photocured bioadhesive reduces platelet adhesion and activation with heparin and alginate additives.[36] But the bioadhesive developed is dissolved in a saline-based solvent. Without additives, nearly 80% of adhered platelets are activated. PEG 400 composite bioadhesive significantly reduces the platelet adhesion with limited or no activation, requiring no additional additives. The higher surface density of PEG 400 formulations over PLGA is likely related to increase in porous surface area relative to the SEM image. Thrombus formation can be accelerated or arrested through platelet adhesion and activation. Platelet adherence[85] is first step of thrombosis process when subjected to tissue damage. The adhered platelets release tissue factors[60, 86] which enhances

platelet-platelet interaction leading to aggregation and blood clotting. Cancerous tumors are known produce procoagulant materials thereby activating the blood clotting cascade[87] leading to thromboembolism.[88] Management of thrombosis in a cancer patient is a challenge as they are subjected to secondary factors such as surgery and chemo which further increases the risk of thromboembolism. Photocuring the adhesive within tumor vasculature would arrest the blood supply without cascading thrombosis events. PEG 400 blended PAMAM bioadhesive may have utility in development of targeted tumor therapies. The bioadhesive blend with soft tissue mimicking mechanical properties, porous structure and ability to adhere in wet environment provides a platform for further development of medical implants where non-specific carbene reactivity and PEG 400 serve to immobilize and locally deliver hydrophobic and particulate therapeutics. Future work will address therapeutic incorporation and UV dose tunable release kinetics.

5. Conclusion

There exists an unmet need to develop injectable bioadhesive formulation which are tissue compliant, have adhesion to soft tissues, water-free, and cures in minutes. Herein aqueous-free bioadhesive is developed to meet these parameters; PAMAM-g-Diazirine (15%) dissolved in PEG 400. The structure activity relationships of the bioadhesive was studied as a function of PEG 400 content, UV dose (joules), applied pressure, and thickness. PEG 400 acts a diluent with 3 orders of magnitude viscosity reduction (55,000 to 75 mPa.S) allowing injectable viscosity ranges (>50% %w/w) or further development in hydrophobic dispersants. Bioadhesive blends displayed tunable mechanical modulus with variable porous structures. The synergetic effect of these attributes yields anisotropic stress-strain curves, mimicking the mechanical properties of vascular tissues. Reducing the dendrimer concentration increases wet adhesion values with corresponding decreases in platelet adhesion and reducing platelet activation. PEG shields cationic charge of the dendrimer reducing platelet activation would find utility for some vascular applications, such as anastomosis and anti-tumor treatments. In addition, the PEG 400 based PAMAM bioadhesives could be used as porous scaffolds for vascular tissue engineering applications owing to their excellent porosity, pore interconnectivity, and platelet inertness.

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Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

a)

b)

Figure 7

SUPPLEMENTARY INFORMATION

Non-aqueous, tissue compliant carbene-crosslinking bioadhesives

Ankur Harish Shah^a, Oleksander Pokholenko^a, Himansu Sekhar Nanda^b, Terry W.J. Steele^{a,}*

^aSchool of Materials Science and Engineering, Division of Materials Technology, Nanyang Technological University, Singapore 639798.

^bDepartment of Mechanical Engineering, PDPM-Indian Institute of Information Technology, Design and Manufacturing (IIITDM)-Jabalpur, Dumna Airport Road, Jabalpur-482005, MP, India

Table S1: Loss modulus of uncured and cured PAMAM-g-Diazirine (15%) : PEG 400 adhesive

Loss modulus (kPa)				
Formulation	UV Dose (J cm ⁻²)			
	0	10	20	60
100:0	2.24 ± 0.23	28.3 ± 1.5	23.9 ± 1.5	24.8 ± 3.0
90:10	0.19 ± 0.02	8.7 ± 1.5	17.0 ± 3.5	21.2 ± 4.5
70:30	0.06 ± 0.08	7.7 ± 0.6	13.2 ± 0.2	15.7 ± 0.6
50:50	0.02 ± 0.0007	3.9 ± 0.1	6.6 ± 0.4	7.5 ± 0.4
40:60	0.005 ± 0.0002	2.4 ± 0.1	3.1 ± 0.1	3.5 ± 0.4
30:70	0.003 ± 0.0002	1.2 ± 0.03	1.3 ± 0.1	1.6 ± 0.05

Table S2: Damping coefficient of uncured and cured PAMAM-g-Diazirine : PEG 400 adhesive

Formulation	Tan δ			
	UV Dose (J cm ⁻²)			
	0	10	20	60
100:0	9.1 $\pm$ 0.2	0.72 $\pm$ 0.4	0.17 $\pm$ 0.002	0.01 $\pm$ 0.003
90:10	20.8 $\pm$ 5.8	0.21 $\pm$ 0.02	0.16 $\pm$ 0.023	0.17 $\pm$ 0.014
70:30	9.3 $\pm$ 3.7	0.20 $\pm$ 0.03	0.14 $\pm$ 0.006	0.15 $\pm$ 0.005
50:50	3.1 $\pm$ 0.2	0.15 $\pm$ 0.004	0.104 $\pm$ 0.0016	0.01 $\pm$ 0.005
40:60	0.7 $\pm$ 0.1	0.06 $\pm$ 0.001	0.058 $\pm$ 0.0005	0.06 $\pm$ 0.002
30:70	0.4 $\pm$ 0.03	0.04 $\pm$ 0.001	0.04 $\pm$ 0.004	0.04 $\pm$ 0.001

Table S3: Molal ratios Diazirine, PEG 400 and hydroxyl group of PEG chain per gram of PAMAM-g-diazirine

Molar ratios per gram of PAMAM-g-Diazirine 15% ($\mu\text{moles/g}$)				
Formulation	$m_{\text{pamam-g-Diazirine 15\%}}$	m_{Dz}	$m_{\text{peg 400}}$	m_{OH}
100:0	0.31	4.68	-	-
90:10	0.29	4.33	250	500
70:30	0.22	3.27	750	1500
50:50	0.15	2.34	1250	2500
40:60	0.12	1.87	1500	3000
30:70	0.09	1.35	1750	3500

NMR Characterisation of adhesives blend

For PEG 400 the average total amount of EO residues per molecule is 9.1 thus average amount of internal EO residues is 8.1 (peak at 3.8 ppm), they contain in average 32.4 H atoms. Two terminal CH₂-OH groups give a peak at 3.7 ppm (multiplet) and the ratio of intensities of these two peaks (from internal and “external” CH₂ groups) is equal to the number of internal EO residues, or number of all EO residues -1. Based on **Fig. S1**, for PEG 400 this ratio should be 8.1 and we observe value of 7.44.

Ratio of intensities of peaks of PEG to -CH₂-C(=O)-N < group in PAMAM will reflect ratio of these components in the mixture. Thus intensity of 252 for the peak in PAMAM will correspond to 1 mole of this component. The intensity of signals of all -CH₂- groups in PEG was 2059. As one molecule of PEG in average contains 18.2 -CH₂- groups, their peak intensity corresponds to $2059/18.2 = 113$ moles. Thus PAMAM-diazirine to PEG molar ratio is 1:113.

The set of three overlapping broad peaks at 6.7-7.4 belongs to aromatic C-H groups of diazirines attached. The ratio of intensities of those two signals indicates that 16 to 17 benzyl diazirine groups are attached to each PAMAM molecule (16.4 in average). Of 128 available amino groups it makes $16.4/128 = 0.128$ or 12.8% conjugation rate. This is close to conjugation rate degree calculated for this PAMAM-diazirine conjugate from NMR analysis of the pure sample in DMSO d₆.

Figure S1: Representative ^1H -NMR spectra with peak assignment pyrazine spiked D2O, uncured PAMAM-g-Diazirine and blend of PAMAM-g-Diazirine : PEG 400 (50:50 %w/w)

Figure S2: Representative ^1H -NMR spectra of leachant after one hour of incubation of PAMAM-g-Diazirine (15%) : PEG 400 (50:50 %w/w) photocured with UV dose of 60 J cm^{-2} in pyrazine spiked D_2O at 37°C .

Table S4: Integration peak from NMR and corresponding PAMAM to PEG ratios

PEG content	Integration Peaks			pyrazine K 10 ⁻⁵	Wt of PAMAM	Wt of PEG 400	%PEG NMR	%PEG Measured
%w/w	CO (PAMAM)	EO (PEG)	H ₂ O		mg	mg		
0	798.1	0	25024	2.733	1.39	0	0	0
10	807.4	561	25745.97	2.733	1.41	0.17	10%	11%
50	1060.27	6072.17	28060.32	2.733	1.84	1.83	49%	51%
70	859.72	11666.77	27012	2.733	1.49	3.51	70%	70%

Table S5: PAMAM-g-Diazirine (15%) and PEG 400 content in the leachant from photocured adhesive (UV dose 60 J cm⁻²).

PEG content %w/w	PAMAM-g-Diazirine (15%) : PEG ratio (%w/w) in leachant	
	PAMAM-g-Diazirine (15%)	PEG 400
	%	%
0	100 ± 0	-
10	26.1 ± 14.8	73.8 ± 14.8
50	4.1 ± 5.8	95.9 ± 5.7
70	4.2 ± 5.3	95.8 ± 5.4

Figure S3: SEM photomicrographs of the adhered and activated platelets over the adhesive surface of UV-cured (20J) PAMAM-g-diazirine (15%) (a) and PLGA (b). Red arrows indicate resting non-dendritic platelets and yellow arrows indicate activated dendritic platelets. For neat PAMAM-g-Diazirine (15%) (a), all activated platelets show pseudopodia/filopodial dendritic structure.