Elastic Light Tunable Tissue Adhesive Dendrimers

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2016

Feng, G., Djordjevic, I., Mogal, V., O'Rourke, R., Pokholenko, O., & Steele, T. W. J. (2016). Elastic Light Tunable Tissue Adhesive Dendrimers. *Macromolecular Bioscience*, 16(7), 1072-1082.

<https://hdl.handle.net/10356/80668>

<https://doi.org/10.1002/mabi.201600033>

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DOI:

Article type: Full Paper

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Keywords: *Bioadhesion; PAMAM dendrimer; diazirine; hydrogel; UV activation.*

Abstract

Development of bioadhesive formulations for tissue fixation remains a challenge. The major drawbacks of available bioadhesives are low adhesion strength, toxic by-products and complexity of application onto affected tissues. In order to address these problems, we have developed a hydrogel bioadhesive system based on poly amido amine (PAMAM) dendrimer, grafted (conjugated) with UV-sensitive, 4-[3-(trifluoromethyl)-3H-diazirin-3-yl] benzyl bromide (PAMAM-g-diazirine). This particular diazirine molecule can be grafted to the surface amine groups of PAMAM in a one-pot synthesis. Diazirine functionalities are carbene precursors that form covalent crosslinks with hydrated tissues after low-power UV activation without necessity of free-radical initiators. The rheological properties and adhesion strength to ex vivo tissues are highly controllable depending on diazirine grafting, hydrogel concentration and UV dose intensity fitting variety types of tissues. Covalent bonds at the tissue/bioadhesive interface provide robust adhesive and mechanical strength in a highly hydrated environment. The free flowing hydrogel to elastic adhesive after UV activation allows intimate contact with

the ex-vivo swine tissue surfaces with low in vitro cytotoxicity observed, making it a promising bioadhesive formulation towards clinical applications.

1. Introduction

Technologies for production of tissue adhesives, or “bioadhesives”, present a promising strategy for non-invasive tissue mending and fixation. Bioadhesives are particularly important for minimally invasive surgery (MIS), also referred to as “laparoscopic” or “keyhole” surgery that is performed without making a major incision. MIS could potentially introduce many benefits such as: decreased bleeding, reduction in sternal wound infection, patients scar dissatisfaction, shorter times for intensive care, and reduced time to return to normal activity.^[1] Possibly the most important outcome of MIS is to find the alternative to conventional surgeries that currently require major scission and tissue reattachment with sutures and staples. For further development of effective MIS and totally endoscopic approach in procedures such as working on beating heart, lung incisions, gastrointestinal operations or vascular anastomosis there is an intense interest in using bioadhesives as sealants to replace current reliance on sutures and staples.^[2, 3]

A number of commercial bioadhesive products, based both on natural and synthetic materials, have been developed and are readily available on the market. Good examples of such products are polypeptide-based adhesives and medical grade cyanoacrylate glues. However, either type of bioadhesive suffers from serious drawbacks including weak adhesion (fibrin sealants) or toxicity to surrounding tissues (cyanoacrylate glues).^[4] For example, cyanoacrylates can achieve desirable adhesion strength through stable covalent bonds formed with tissue proteins. Rapid in situ polymerization of tissue-penetrated cyanoacrylate monomers results in mechanically interlocked polymer with contact tissue.^[5] Despite their adhesion strength, crosslinked polycyanoacrylates are brittle and do not match mechanical properties of soft

tissues. Due to the significant and well documented histotoxicity of biodegradation products generated through hydrolytic polymer breakdown, cyanoacrylate bioadhesives are approved mainly for topical use.^[5] Given the importance of tissue adhesion in surgical procedures, there is a need for new formulations that would enable rapid tissue sealing and close control over mechanical properties at the bioadhesive/tissue interface.

Of particular interest to MIS procedures are on-demand adhesive formulations that crosslink upon external activation, such as electricity or UV light.^[2, 6-9] Some promising new UV-activated, acrylate-based bioadhesive systems have been recently developed.^[2, 7] Published studies describe UV-activated polyacrylates with “Irgacure 2959” free radical photoinitiator, which has shown marginal toxicity when used in the safe UVA1 range of 340 nm – 400 nm.^[10-16] Regardless of the optimal performance with biological systems, it should be noted that the maximum UV absorbance of Irgacure 2959 falls within UVB range (280 nm) – a radiation considered to have stronger cancerogenic potential compared to UVA.^[17] Possibly the major reason for accepting Irgacure 2959 as “biocompatible” is a low concentration of free radicals formed upon radiation in UVA region that limits the crosslinking efficiency.^[18-20] The presence of free radicals, dissociated from photoinitiator molecules upon UV activation, is generally regarded as cytotoxic and even the usage of “non-toxic” free radical photoinitiators (such as Irgacure 2959) should be taken with precaution.^[19] Considering the potential toxicity of free-radicals, the optimal bioadhesive formulation should eliminate the need of harmful components. Although UVA electromagnetic wave range is regarded as biologically safe, the potential detrimental effect of high energy exposure should not be neglected and bioadhesives used for internal organs and tissues should generally require minimal UV activation energy.^[17]

Motivated by clinical potential of bioadhesives we have developed an adhesive hydrogel formulation which is activated by low energy UV light without the presence of external free

radical photoinitiators. Our novel bioadhesive is based on poly(amido amine; PAMAM) dendrimer grafted with 4-[3-(trifluoromethyl)-3H-diazirin-3-yl] benzyl bromide (referred to as “diazirine” further in text) as presented in **Figure 1**. Dendrimers in general are well documented for their biomedical application, mainly related to drug delivery and gene therapy.^[21, 22] Another reason for choosing a water-soluble dendrimer as a backbone system is the large number of pendant amine ($-NH_2$) functional groups available for reaction and formation of PAMAM-g-diazirine (**Figure 1**). Conjugated diazirine generates highly reactive carbene upon UVA (365 nm) irradiation, which further insets to most C-H bonds of extracellular tissue matrix without affecting protein conformation and activity.^[23, 24] The design and control over mechanical modulus and adhesive strength of UV-activated PAMAM-g-diazirine bioadhesive is based on following hypotheses: (i) the mechanical modulus and adhesive strength of crosslinked PAMAM-g-diazirine can be controlled by degree of grafted (conjugated) diazirine on PAMAM dendrimer periphery and the amount of PAMAM-g-diazirine dissolved in buffer; (ii) due to formation of carbene radicals capable of forming covalent bonds upon UV activation, irradiated PAMAM-g-diazirine enable rapid adhesion to tissue interfaces, even within aqueous environments.

We have previously developed two different diazirine-based adhesive systems: 1) diazirine grafted polymer films,^[25] and 2) electrocuring adhesive from diazirine grafted PAMAM dendrimer.^[6] In case of diazirine grafted films, relatively low adhesion strength was achieved (max. 4.5 kPa) with limited contact with ex vivo tissues, while the electrocuring adhesive can only be used with conductive surfaces. In order to overcome those limitations, we have investigated the potential of strong adhesive generated upon UV activation of liquid PAMAM-g-diazirine formulation. We have limited our investigation to max. 30% of diazirine conjugation (to insure water miscibility) and to a relatively low irradiation power of max. 21.3 mW cm^{-2} . The adhesion strength was characterized with ex vivo tissues and the potential

cytotoxicity of developed bioadhesive has been evaluated as well. The macromolecular system, reported in this paper, has a strong potential to provide rapid and controllable on demand bioadhesive formulation for future use in clinical practice.

2. Results and discussion

2.1 Diazirine conjugation on PAMAM dendrimer: SEC and NMR analysis

Diazirine conjugation percentage onto PAMAM dendrimer would eventually determine the performance of adhesive polymer system. In order to demonstrate the close control over conjugation reaction (max. 30% to insure water miscibility), and to correlate molecular composition to mechanical properties, it was necessary to determine the molar masses of reaction products prior to rheometry and adhesion tests. Diazirine, as the carbene precursor, was conjugated (grafted, '-g-') onto PAMAM molecules directly by nucleophilic substitution as illustrated in **Figure 1**. Grafting of diazirine increased the molar mass of dendrimer, which resulted in the shift of refractive index peak (**Figure 2**, solid lines) towards smaller elution volumes in SEC. This result is in line with the UV absorption data (350 nm, dashed lines in **Figure 2**) that show a relative increase in molar mass as a consequence of increased concentration of conjugated diazirine. The molar mass of PAMAM-g-diazirine conjugates was calculated from refractive index and multi-angle static light scattering (MALS) analysis; the established method for absolute molar mass determination from polymers in solution.^[26] The comparative data between pre-determined (theoretical) and experimental values of diazirine conjugation percentage calculated from SEC experiment are presented in Supporting Information (Table S1).

PAMAM-g-diazirine conjugates (10-30%) were analyzed with H^1 NMR spectroscopy and the results were compared to pure PAMAM and diazirine reagents (**Figure 3**). We detected 5 groups of protons and they were assigned from 'a' to 'e' according to previously reported

results for PAMAM dendrimer.^[27-28] As expected, overlapping of proton signals could not be avoided due to the complexity of dendrimer structure. Diazirine peaks 'g' and 'f' correspond to methylene and aromatic protons respectively. After reaction of diazirine with -NH_2 groups, aromatic proton signals appear as broad peaks as a results of new chemical environment in complex macromolecular system. Aromatic peaks ('f') were integrated in order to calculate diazirine conjugation percentage. The peak integration and calculation was completed by using the method from B. Huang et al. (see Supplementary Information).^[27] The conjugation percentages calculated from ^1H NMR spectra were compared to both SEC results and conjugation percentages that were calculated from initial molar ratio of reactants (Table 1). The consistency of results indicates successful synthesis of PAMAM-g-diazirine with concentration of diazirine functionalities that is closely controlled by our method.

In principle, -NH_2 groups can react twice in nucleophilic substitution and this reaction could result in both mono- and bis- functionalization of PAMAM end-groups. We recorded C^{13} NMR spectra of PAMAM-g-diazirine for HMQC 2D spectrum analysis in order to investigate the possibility of tertiary amine formation (see Supplementary Information). The spectra recorded for PAMAM-g-diazirine 30% (**Figure 3**) show that two peaks at 3.56 ppm and 3.71 ppm correspond to tertiary and secondary amine groups. This chemical shift difference pattern is typical between mono-benzyl and di-benzyl amines.^[29-31] Those two structures could only be accurately detected for the highest conjugation percentage (30%), however, the existence of tertiary amines should not be excluded from dendrimers with lower concentrations of conjugated diazirine molecules. Regardless of the diazirine position in the conjugate (mono- or bis- functionalization), the overall conjugation percentage, or the overall concentration of grafted diazirine onto PAMAM macromolecules, is the major parameter that determines the crosslinking density upon UV activation. This parameter in turn should determine both

dynamic mechanical properties and adhesion strength of PAMAM-g-diazirine bioadhesive formulation.

2.2 Tunable dynamic mechanical properties of PAMAM-g-diazirine hydrogel

An optical light guide coupled UV light (365 nm) to the transparent base of the rheometer (**Figure 4A**) was used to measure the influence of both UV irradiation time and concentration of diazirine on dynamic mechanical properties. The irradiation intensity was kept constant at 21.3 mW cm⁻² in all experiments (as measured by Newport Power Meter 1918-R). The storage (G') and loss (G'') moduli values of 15% conjugated PAMAM-g-diazirine hydrogel, prepared as 50% (w/w) concentration in PBS, were recorded as representative and monitored during UV curing at 1 minute “ON/OFF” intervals of UV light (**Figure 4B**). There was an obvious increase of measured G' during UV irradiation of PAMAM-g-diazirine (UV power ‘ON’). Upon the OFF cycle, G' plateaued and no further increase was seen as G' remained stable until the UV light was turned ‘ON’ again. This cycle was repeated up to 5 times to observe the cycle dependent changes in G' , denoted as $\Delta G'$. $\Delta G'$ was observed to continually increase in the first four cycles from 2.73 kPa to 7.24 kPa, but decreases thereafter, likely indicating the depletion of carbene formation, resulting in fewer intermolecular crosslinks. (**Figure 4B inset**). G''/G' ratio of crosslinked PAMAM-g-diazirine varied from 10 to 0.1 after UV activation by alteration of UV irradiation time (Figure S1). The G''/G' range is larger than previously reported dendrimer-based acrylate adhesive (2–0.2).^[7] The sol/gel transition (at $G''/G' = 1$) occurred in less than 1 min which translates into UV activation energy of only 1.3 J cm⁻² measured for representative 15% diazirine conjugation percentage (Figure S1).

In order to prevent the mechanical failure or tissue damage, a bioadhesive should have comparable dynamic mechanical properties to the surrounding tissue and, ideally, tunable yield stress.^[32] In the case of free radical (acrylate) polymerization the chain propagation is

unpredictable^[33] and therefore resulting mechanical properties of crosslinked adhesives could be difficult to control. As presented in **Figure 4B**, no chain propagation occurred in our formulation as the modulus of PAMAM-g-diazirine remained constant in the periods where UV was switched “OFF”. This result indicates possibility of fine tuning that can be accomplished by alteration of UV power and irradiation time. Rheometry results show $G'' > G'$ prior to UV activation for all investigated conjugation percentages and concentrations in PBS. This result is generally expected for low viscosity liquids such as PAMAM-g-diazirine formulation. In other words, adhesive formulation takes the shape of the contact surface prior to crosslinking and this particular feature is important as tissue interfaces can often have irregular shapes and sizes.

Both G'' and G' values were plotted during constant UV power for different diazirine concentrations in PBS (**Figure 4C**). Gelation point (the transfer from viscous to viscoelastic state) occurred as fast as 40 seconds, and a G' plateau was reached after 4 minutes for PAMAM-g-diazirine concentrations of 50% and 75%. Lower concentration in PBS (25%) resulted in slower increase of G' due to the reduced interaction between dendrimer molecules within hydrogel matrix. A constant value of storage modulus (plateau) is indicative of crosslinking saturation, where all available diazirine functionalities have reacted.

Photoproducts from diazirines are generated through several different processes including formation of carbenes and diazo compounds.^[34] In case of PAMAM-g-diazirine both intermediate species could cause crosslinking as diazo compounds are highly reactive towards nucleophiles such as $-NH_2$ groups. However, previous studies indicate that aromatic diazirines mostly generate carbenes;^[34] from our observation the crosslinking happens immediately upon UV activation which also suggests that the generation of carbene is the predominate crosslinking mechanism.

The wide range of G' values recorded from 3.5 kPa up to 225 kPa (**Figure 4D**), as a function of both conjugation percentage and dendrimer concentration in PBS, shows the potential application to match the elasticity or modulus of various tissue types, such as: compliant tissue (ie. liver, 25 kPa^[35]), subcutaneous adipose tissue (10 kPa^[36]) and certain types of blood vessels (ie. artery, 100-400 kPa^[37-38]). Adhesive-substrate modulus mismatch is a prime cause of adhesive failure at the interface. Whilst it is advantageous for a clinician to activate an adhesive formulation on-demand, ^[8, 9] the crosslinking must occur rapidly in order to physically keep the liquid on intended site.^[2] This is particularly important for aqueous formulations (such as our hydrogel dendrimer) at the hydrophilic tissue interface. In such cases rapid crosslinking would prevent the wash out by body fluids and would also prevent deep penetration of the bioadhesive formulation into the tissue structure. The gelation time for PAMAM-g-diazirine is less than 1 min and the mechanical strength can reach 50% of its maximum value after only 2 min of UV curing at an irradiation power limited to max. 21.3 mW cm⁻²; (**Figure 4D**). This power is less than 10% of the maximum power of the mercury lamp used in our experiments. Our results demonstrate quick crosslinking that has a potential to be used in surgical procedures where reaction time is crucial. In particular, endoscopic approach in MIS procedures such as gastrointestinal operations or vascular anastomosis require bioadhesives that could be cured rapidly with minimum UV power in order to prevent possible tissue damage by high UV energy dose.

Grafting of diazirine molecules onto polymer chains offer many new possibilities for biomaterials design and processing. Good example of such technology was reported by J. Raphael et al.^[39] Authors have demonstrated that diazirine conjugated elastin-like proteins can be produced in different forms such as micropatterned surfaces and porous scaffolds. This new approach is of great significance for fabrication of various biomaterials, however, surgical bioadhesive should be cured rapidly with minimum required UV energy. Unlike our

PAMAM-g-diazirine which can crosslink in seconds, elastin proteins grafted with diazirine required timespans ranging in hours.^[39] Furthermore, our method for diazirine conjugation onto pendant -NH_2 functional groups of macromolecular system offers a flexible platform for additional grafting – it opens new possibilities for fabrication of biomaterials incorporating multiple functionalities, i.e. cellular signaling, drug delivery, optical reporters, etc.

2.3 Ex vivo adhesion strength to blood vessel tissue

Shear adhesion strength of PAMAM-g-diazirine between excised swine artery surface and PLGA films was evaluated by testing the “sandwich structure” (**Figure 5A**) in lap-shear mode under tensile loading.^[40] This experimental set-up enabled us to examine the adhesive dendrimer system in direct contact with hydrated tissue. The irradiation time was fixed to 5 minutes to allow complete crosslinking by all available carbene interaction points, as discussed in the previous section. A typical stress/strain response of PAMAM-g-diazirine under lap-shear is presented in **Figure 5B** for different diazirine conjugation percentages. No adhesion was observed from pure PAMAM dendrimer used as control. All failures were visually determined to be cohesive (**Figure S2**)—fracture was across the bulk of the adhesive film and not at the tissue or PLGA interface. Thus adhesion is seen to occur at both synthetic and natural material interfaces—this also supports the carbene-mediated covalent crosslinking mechanism (PLGA has no amines present). The linearity of the initial stress-strain responses indicates that the PAMAM-g-diazirine forms an elastic solid after UV activation, in contrast to the viscous nature of unmodified PAMAM. The shear modulus (slope of stress/strain curve) and adhesive strength (maximum shear stress before failure) were calculated from experimental data and the values are plotted as a function of diazirine conjugation (**Figures 5C and D**). As expected, adhesion strength and storage modulus both increased with diazirine conjugation percentage and the results are consistent with rheometry measurements presented in **Figure 4**. For example, the adhesive strength increased with PAMAM-g-diazirine

concentration in PBS from 3.2 kPa to 19.9 kPa for 15% diazirine conjugation. Similarly, the adhesive strength increased with diazirine conjugation percentage from 10 kPa to 28 kPa for 50% (w/w) concentration in PBS. As expected, maximum adhesion of almost 40 kPa was obtained for 30% diazirine conjugation and 75% w/w concentration in PBS, far superior to fibrin glue which has been reported to have an adhesive strength of only 5 kPa.^[2, 41]

2.4 Morphology of tissue-adhesive interface

The surface morphology of bioadhesive/tissue interface was evaluated with SEM. Cross-sectional micrographs of the PAMAM-g-diazirine /endothelium interface is shown in **Figure 6**. For clarity reasons the two phases, tissue and gel, are displayed in different colors (the original SEM results are presented in Figure S3). The collagen surface layer of the tissue appears to be infiltrated by adhesive gel, which is expected due to the formation of covalent bonds at the interface. One product of UV-induced free radical formation from conjugated diazirine is N₂, which forms bubbles during UV irradiation.^[6] As the reaction occurs very rapidly even under relatively low UV power, these bubbles remained trapped after solidification of gel layer. This “foaming” of diazirine could present a certain advantage of bioadhesive system: as the foam expands, it increases the specific surface area in direct contact with tissues. On the other hand, the porous bulk structure might result in lower mechanical modulus. The amount of foaming can be adjusted through the intensity of UV activation – visual observations have shown that low intensities (<1 mW cm⁻¹) produce little to no foaming – presumably N₂ dissolves into the surrounding medium (data now shown). The porous bulk of the material and the adhesive-tissue interface could be observed at higher magnification (**Figure 6**, inset) suggesting both covalent and mechanical interlocking adhesion mechanisms are responsible for the cohesive bulk failure (Figure S2). We also investigated the presence of PAMAM-g-diazirine residue on ex vivo tissues after adhesion testing. Tested tissue samples were soaked in water for a prolonged period of time and the

residual water was analyzed by SEC. No polymer was detected, indicating that PAMAM-g-diazirine forms an insoluble 3D network structure almost immediately after UV activation (Figure S4).

2.5 3T3 fibroblasts cytotoxicity test: influence of diazirine conjugation and UV-activated crosslinking

The potential cytotoxicity of PAMAM-g-diazirine was evaluated by measuring the reductive power of living 3T3 fibroblast cells cultured with PAMAM-g-diazirine, both with and without UV irradiation. The half maximal effective concentration (EC50) refers to the concentration of PAMAM-g-diazirine that reduces 50% of cell metabolic activity after 24 hours (**Figure 7A**). Results show that EC50 concentration of PAMAM-g-diazirine is up to four times higher than pure PAMAM dendrimer. In other words, PAMAM-g-diazirine is significantly less cytotoxic than unmodified PAMAM. This reduction in toxicity is thought to arise from modification of the terminal -NH_2 groups to non-cationic amides.^[42] PAMAM dendrimers have the ability to penetrate cell membranes which is desirable in certain drug delivery applications.^[21] In case of tissue regeneration (or bridging of broken tissues) this particular feature of PAMAM makes it device specific towards antibacterial applications, as the dendrimer-induced cytotoxicity increases with dendrimer generation.^[21] Our results indicate that diazirine conjugation acts in favor of lowering PAMAM cytotoxicity by reducing the concentration of -NH_2 groups that are exposed to cell contact. It is important to note that UV irradiation has shown no significant effect to 3T3 fibroblast viability in comparison to non-irradiated samples (**Figure 7A**). We also quantified fibroblast activity in the environment of crosslinked PAMAM-g-diazirine films (**Figure 7B**). There was no significant cytotoxicity detected after 3 days for PAMAM-g-diazirine 30% in comparison to control (tissue culture plate) in aqueous medium, indicating intact films with little to no leaching of potentially toxic monomers or other unknown components. However, lower conjugation percentage (15%)

showed significant toxicity (* $P < 0.05$; **Figure 7B**) when compared to cells in culture plate. Possible reason for this behavior is cell exposure to higher concentration of $-NH_2$ surface groups (from 15% conjugated dendrimer). Despite the potential adverse effects, dendrimer surface groups ($-NH_2$) could be used in future to in antibacterial bioadhesives or in grafting additional bioactive compounds that would facilitate tissue regeneration, for example protein sequences (such as RGD).^[3, 43-44] We speculate that the presence of additionally grafted bioactive molecules allows a unique flexibility towards multifunctional bioadhesives that could initiate tissue regeneration simultaneously with tissue adhesion, assuming that the bioactivity remains after crosslinking.^[45] Apart from bioactive molecules, PAMAM dendrimers offer many possibilities for chemical modification such as grafting of PEG chains for drug delivery applications^[46], covalent attachment of fluorescent dyes and complexes for magnetic resonance imaging.^[47] PAMAM-g-diazirine, described in this paper, opens new possibilities for development of multifunctional bioadhesives, which could take advantage of dendrimers in various biomedical applications.

3. Conclusion

We have developed a bioadhesive hydrogel formulation based on diazirine-conjugated PAMAM dendrimers crosslinked by low-power UV activation. Our results demonstrate that mechanical modulus and adhesion strength of crosslinked dendrimer can be tuned by following parameters: 1) concentration of diazirine conjugated on dendrimer surface; 2) concentration of PAMAM-g-diazirine conjugate in aqueous medium; and 3) UV activation energy. The adhesion of PAMAM-g-diazirine to ex vivo swine aorta was investigated and we conclude that the storage modulus and shear adhesive strength can be tuned by the above parameters for application to different tissue types. Our novel strategy in development of UV-activated bioadhesives requires no additional components (free-radical UV initiators) in order to reach the outstanding shear adhesion strength of 40 kPa. In vitro tests with fibroblast cells

showed that the diazirine binding mechanism does not increase the cytotoxicity; in fact, the toxicity of PAMAM dendrimer was reduced by diazirine modification. Our novel formulation has potential for further development with grafting of proteins or protein sequences via the un-reacted amine groups – an exciting prospect for future tissue adhesives.

4. Experimental Section

Diazirine grafting of PAMAM dendrimer: Poly (amido amine) dendrimer (PAMAM, 5th generation, Mw=28.8 kDa, 128 surface amine functional groups) was purchased from Dendritech, USA. 3-[4-(bromomethyl)phenyl]-3-(trifluoromethyl)-diazirine (Br-diazirine) was purchased from TCI, Tokyo. Methyl alcohol (MeOH) and glacial acetic acid (AcOH) were obtained from TEDIA, Singapore. Poly-DL-lactide-co-glycolide (PLGA 53/47) from Sigma (Singapore) was used as received. Phosphate buffer saline (PBS; Gibco, Singapore) was used in all experiments. PAMAM-g-diazirine was synthesized by previously developed method.^[6] In brief, PAMAM solution in MeOH (0.5% w/v) was prepared and different amounts of diazirine were added directly into 5 mL of PAMAM solution (according to theoretical calculations) in order to obtain different diazirine conjugate percentages (**Table S1**; Supporting Information). Examined conjugation percentages in all experiments were in the range of 5% - 30%. The conjugation reaction was set for 36 hours with stirring under room temperature. MeOH was removed by vacuum and PAMAM-g-diazirine was collected as a pale yellow viscous liquid.

Size exclusion chromatography – multi-angle laser light scattering - ultraviolet (SEC-MALS-UV) analysis of PAMAM-g-diazirine conjugate: Agilent 1100 series high-performance liquid chromatography (HPLC) pump complete with degasser and PLGel aqueous 50 column in a 35° C oven with refractive index (RI) detector (Agilent Technologies, Santa Clara, CA, USA) was incorporated with a Wyatt MiniDawn 3-angle light scattering (LS) detector (Wyatt

Technology Corporation, US) in line. A solution of 1% w/v AcOH (166.7 mmol) with 0.2% (w/v) NaN_3 (30.8 mM) was prepared as the eluent buffer. Pure PAMAM and PAMAM-g-diazirine conjugate samples were dissolved in eluent buffer and the concentration was set for 2 mg mL^{-1} . RI, LS and UV absorption (350 nm) were recorded and processed by Wyatt ASTRA software (version 5.19.1), Wyatt Technology, Santa Barbara, CA, USA). RI increment, dn/dc of 0.185 was used for PAMAM with the UV extinction coefficient of 1009.5 $\text{mL cm}^{-1} \text{g}^{-1}$ @ 350 nm. The UV extinction coefficient of Br-diazirine in aqueous medium was measured as 1009.5 $\text{mL cm}^{-1} \text{g}^{-1}$ @ 350 nm which was used to calculate the experimental conjugation percentage.^[48] PAMAM-g-diazirine (15% modification degree; 75% concentration in PBS) was tested for residual polymers after UV-activated crosslinking for 5 min at 21.3 mW cm^{-2} . Crosslinked PAMAM-g-diazirine was soaked in DI water for 24 h and the suspension liquid was analyzed with SEC.

Nuclear Magnetic Resonance (NMR) spectroscopy analysis: Both reactants (PAMAM dendrimer and diazirine) and PAMAM-g-diazirine conjugates were analyzed with NMR (Bruker Avance) at 400 MHz with DMSO-d_6 used as solvent. ^1H and ^{13}C depth 135 HMQC HMBC NMR spectra were collected and analyzed. The peak assignment, 2D spectrum analysis and peak integration (^1H NMR) were performed with SpinWorks 4.2 software.

Dynamic mechanical analysis (DMA) of PAMAM-g-diazirine under UV irradiation: PAMAM-g-diazirine with each conjugate percentage (5%, 10%, 15%, 20% and 30%) were prepared into 3 different concentrations (25%, 50% and 75% w/w in PBS balanced at pH = 7.2) and each formulation was pipetted onto a light transparent rheology testing base (P-PTD120/GL, Anton Paar, Austria). The UV light was generated and transferred by a S1000-1B-3188 UV initiator (OmniCure, Canada) with 365 nm UV filter (019-01036). The power intensity was maintained as 10% intensity setting, which was determined to be 21.3 mW cm^{-2} (1918-R Power Meter, Newport, Singapore). Dynamic properties were assessed in real-time

with a quartz parallel plate Physica MCR 501 Rheometer (Anton PAAR, US) at 1% amplitude @ 1 Hz with 8 mm diameter parallel stainless steel probe (PP08/150, Anton PAAR, US) maintained at 25 °C with a 100 μ m gap.

Shear adhesive strength measurements with ex vivo cardiovascular tissue: Shear adhesive strength measurements were based on ASTM standard F2255-05. Fresh swine artery, obtained from a local abattoir, was cut into 40 $\times$ 20 mm slides with a thickness around 1 mm (no animal subjects were used for the purpose of experiment). The fat was removed and the tissue with endothelial (intima) side up was glued with cyanoacrylate adhesive onto a glass slide (25.4 $\times$ 76.2 mm, 1 – 1.2 mm thick, CLP, China). PLGA thin films were processed by a previously reported method.^[25] PLGA samples were cut into a 40 $\times$ 20 mm squares and glued onto glass slides with cyanoacrylate adhesive. PAMAM-g-diazirine in PBS (0.3 mL) with different concentrations and conjugation percentages were pipetted in between the swine artery surface and PLGA film. Joined glass substrates were immediately exposed to UV light (365 nm) through the glass. The UV intensity at the surface of this sandwich structure was kept constant at 21.3 mW cm⁻². An average force of 1.37 ± 0.25 N cm⁻² was applied on the sandwich system during the UV curing by two paper binder clips (SQ-0107, 19 mm width, SureMark, Singapore). The exact area of the gel (~20 $\times$ 20 mm PLGA/tissue overlap) was calculated by analyzing the photograph in Adobe Photoshop CS2. Shear adhesive strength test was performed by Series Force Measurement System (Chatillon Force Measurement Products, USA) at the controlled linear speed of 3 mm min⁻¹ with 10 N loading cell (n = 5).

Scanning electron microscopy (SEM) analysis of ex vivo tissues: The cross-sections of hydrated artery slides after adhesion test were examined by SEM. The samples were immersed into 4% (w/w) glutaraldehyde solution in PBS for 1 hour and lyophilized. Cross-section slides were coated with gold for 70 s under a chamber pressure of less than 5 Pa at 20 mA (JEOL JFC-1600, Auto Fine Coater, Japan). Images were obtained by JSM-6340F field

emission scanning electron microscope (FESEM, JEOL, Japan) at acceleration voltage of 3 kV, emission current of 12 μ A and working distance of 8 mm.

Cytotoxicity test: Half maximal effective concentration (EC50) test was performed for PAMAM-g-diazirine samples of 5%, 15% and 30% diazirine conjugation, using unmodified PAMAM as control. 3T3 fibroblasts cells (ATCC, Singapore) were used for EC50 test. Cells were cultured at 37 °C with 5% CO₂ in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS; both purchased from Gibco, Singapore). Cells suspended by trypsinization were counted and seeded on to 24 well plates (Thermo Scientific, China). Seeding density was 10,000 cells/well (1 mL of medium) and the cells were pipetted into 24 well plates (n = 4 for each concentration of test sample). The cells were incubated for 24 hours in order to allow cell attachment on the bottom of the wells and the medium was replenished prior to addition of dendrimer. The dendrimer concentrations were as follows: 200, 1, 0.1, 0.01 and 0.001 μ g mL⁻¹, measured for each conjugation percentage (15% and 30%). Two series of samples were prepared: one set of samples was irradiated with UV (365 nm) at 21.3 mW cm⁻² for 5 min while the other set was examined without UV irradiation. Fibroblast viability was quantified with alamarBlue assay (Thermo Fisher Scientific Inc, USA) and the fluorescence reading was performed by Microplate reader with 560EX nm/590EM nm filter settings (Infinite M200, TECAN, Switzerland). The effect of possible extracts from crosslinked gel on cell viability was also examined with alamarBlue test. PAMAM-g-diazirine was first spread uniformly on polyethylene terephthalate (PET) slides (20 × 20 mm with ~20 mg cm⁻² of gel) and then fully crosslinked under 365 nm UV irradiation (21.3 mW cm⁻²) for 5 min. Cured samples were cut into 4-mm-diameter disks by BIOPSY punch (4 mm, Mitex, Inc. York, USA) and washed with sterilized PBS buffer. All the samples were cultured in the medium with fibroblasts cells and the metabolic activities were examined after 1 and 3 days (n = 4). Statistical analysis was performed using a Student *t*

-test by comparing the means of each PAMAM-g-diazirine conjugation percentage. A *P* value < 0.05 was considered to be of significant difference.

Acknowledgements

Authors acknowledge support from the following research grants: Ministry of Education Tier 1 Grant: Rapid Development of biocompatible surfaces through acrylate combinatorial libraries (RG 46/ 11); Ministry of Education Tier 2 Grant: Tailored soft-tissue bioadhesives for site-specific therapy (MOE2012-T2-2-046); NTU-Northwestern Institute for Nanomedicine Grant: 3D-Printing of Electro-Curing Nanocomposite Living Electrodes for Cardiac Tissue Regeneration.

Received:

Revised:

Published online:

References

- [1] D. C. H. Cheng, J. Martin, A. Lal, A. Diegeler, T. A. Folliguet, L. W. Nifong, P. Perier, E. Raanani, J. M. Smith, J. Seeburger, V. Falk, *Innovations*. **2011**, 6, 84.
- [2] N. Lang, M. J. Pereira, Y. Lee, I. Friehs, N. V. Vasilyev, E. N. Feins, K. Ablasser, E. D. O'Cearbhaill, C. Xu, A. Fabozzo, R. Padera, S. Wasserman, F. Freudenthal, L. S. Ferreira, R. Langer, J. M. Karp, P. J. del Nido, *Sci. Transl. Med.* **2014**, 6, 218, 6.
- [3] M. Ryou, C. C. Thompson, *Tech. Gastrointest. Endosc.* **2006**, 8, 33.
- [4] M. Mehdizadeh, J. Yang, *Macromol. Biosci.* **2013**, 13, 271.
- [5] P. J. M. Bouten, M. Zonjee, J. Bender, S. T. K. Yauw, H. van Goor, J. C. M. van Hest, R. Hoogenboom, *Prog. Polym. Sci.* **2014**, 39, 1375.
- [6] J. Ping, F. Gao, J. L. Chen, R. D. Webster, T. W. J. Steele, *Nat Commun* **2015**, 6.
- [7] H. Zhang, T. Zhao, P. Duffy, Y. Dong, A. N. Annaidh, E. O'Cearbhaill, W. Wang, *Adv. Healthc. Mater.* **2015**, 2260.
- [8] R. H. Schmedlen, K. S. Masters, J. L. West, *Biomaterials* **2002**, 23, 4325.
- [9] J. Elisseeff, W. McIntosh, K. Anseth, S. Riley, P. Ragan, R. Langer, *J. Biomed. Mater. Res. A* **2000**, 51, 164.
- [10] M. A. Carnahan, C. Middleton, J. Kim, T. Kim, M. W. Grinstaff, *J. Amer. Chem. Soc.* **2002**, 124, 5291.
- [11] C. G. Williams, A. N. Malik, T. K. Kim, P. N. Manson, J. H. Elisseeff, *Biomaterials* **2005**, 26, 1211.
- [12] K. Ono, Y. Saito, H. Yura, K. Ishikawa, A. Kurita, T. Akaike, M. Ishihara, *J. Biomed. Mater. Res. A* **2000**, 49, 289.
- [13] O. Jeon, J. E. Samorezov, E. Alsberg, *Acta Biomater.* **2014**, 10, 47.

- 458 [14] Y. Onuki, M. Hoshi, H. Okabe, M. Fujikawa, M. Morishita, K. Takayama, J.
459 Controlled Release. **2005**, 108, 331.
- 460 [15] T. Wang, X. Mu, H. Li, W. Wu, J. Nie, D. Yang, Carbohydr. Polym. **2013**, 92, 1423.
- 461 [16] T. Wang, J. Nie, D. Yang, Carbohydr. Polym. **2012**, 90, 1428.
- 462 [17] A. de Laat, J. C. van der Leun, F. R. de Gruijl, Carcinogenesis **1997**, 18, 1013.
- 463 [18] B. D. Fairbanks, M. P. Schwartz, C. N. Bowman, K. S. Anseth, Biomaterials **2009**, 30,
464 6702.
- 465 [19] N. E. Fedorovich, M. H. Oudshoorn, D. van Geemen, W. E. Hennink, J. Alblas, W. J.
466 A. Dhert, Biomaterials **2009**, 30, 344.
- 467 [20] I. Mironi-Harpaz, D. Y. Wang, S. Venkatraman, D. Seliktar, Acta Biomaterialia **2012**,
468 8, 1838.
- 469 [21] M. Goldberg, R. Langer, X. Jia, J. Biomater. Sci. Polym. Ed. **2007**, 18, 241.
- 470 [22] C. C. Lee, J. A. MacKay, J. M. J. Frechet, F. C. Szoka, Nat Biotech **2005**, 23, 1517.
- 471 [23] G.-L. Chee, J. C. Yalowich, A. Bodner, X. Wu, B. B. Hasinoff, Bioorg. Med. Chem.
472 **2010**, 18, 830.
- 473 [24] L. Dubinsky, B. P. Krom, M. M. Meijler, Bioorg. Med. Chem. **2012**, 20, 554.
- 474 [25] V. Mogal, V. Papper, A. Chaurasia, G. Feng, R. Marks, T. Steele, Macromol. Biosci.
475 **2014**, 14, 478.
- 476 [26] E. J. Folta-Stogniew, in *eLS*, John Wiley & Sons, Ltd, **2001**
- 477 [27] B. Huang, S. Tang, A. Desai, K.-H. Lee, P. R. Leroueil, J. R. Baker Jr, Polymer **2011**,
478 52, 5975
- 479 [28] S. K. Choi, P. Leroueil, M.-H. Li, A. Desai, H. Zong, A. F. L. Van Der Spek, J. R.
480 Baker, Macromolecules **2011**, 44, 4026.
- 481 [29] A. R. Katritzky, J. Jiang, L. Urogdi, Synthesis **1990**, 7, 565
- 482 [30] S. Hanada, T. Ishida, Y. Motoyama, H. Nagashima, J. Org. Chem. **2007**, 72, 7551.
- 483 [31] C. M. Micklitsch, Q. Yu, J. P. Schneider, Tetrahedron Lett. **2006**, 47, 6277
- 484 [32] S. Ebnesajjad, *Adhesives Technology Handbook*, William Andrew Pub, Norwich **2008**.
- 485 [33] K. Satoh, M. Kamigaito, Chem. Rev. **2009**, 109, 5120.
- 486 [34] J. Das, Chem. Rev. **2011**, 111, 4405.
- 487 [35] C. Basdogan, in *Soft Tissue Biomechanical Modeling for Computer Assisted Surgery*,
488 Vol. 11 (Ed: Y. Payan), Springer Berlin Heidelberg, **2012**, 229.
- 489 [36] G. W. M. P. Marion Geerligs, Paul A.J. Ackermans, Cees W.J. Oomens, Frank P.T.
490 Baaijens, Biorheology **2008**, 45, 677.
- 491 [37] B. S. H. Gow, Clive D., Circ. Res. **1979**, 45, 588.
- 492 [38] D. Mavrilas, T. Tsapikouni, D. Mikroulis, G. Bitzikas, V. Didilis, K. Tsakiridis, F.
493 Konstantinou, G. Bougioukas, J. Mech. Med. Biol. **2002**, 02, 329.
- 494 [39] J. Raphel, A. Parisi-Amon, S. C. Heilshorn, J. Mater. Chem. **2012**, 22, 19429.
- 495 [40] A. International, in *Standard Test Method for Strength Properties of Tissue Adhesives*
496 *in Lap-Shear by Tension Loading*, ASTM International, PA, West Conshohocken
497 **2005**.
- 498 [41] I. Strehin, Z. Nahas, K. Arora, T. Nguyen, J. Elisseeff, Biomaterials **2010**, 31, 2788.
- 499 [42] A. Janaszewska, M. Studzian, J. F. Petersen, M. Ficker, J. B. Christensen, B. Klajnert-
500 Maculewicz, Nanomedicine **2015**, 11, 409.
- 501 [43] K. Uzun, E. Çevik, M. Şenel, H. Sözeri, A. Baykal, M. F. Abasıyanık, M. S. Toprak, J.
502 Nanopart. Res. **2010**, 12, 3057.
- 503 [44] J. L. Santos, D. Pandita, J. Rodrigues, A. P. Pêgo, P. L. Granja, G. Balian, H. Tomás,
504 Mol. Pharm. **2010**, 7, 763.
- 505 [45] B. D. Ratner, Sci. Transl. Med. **2015**, 7, 272fs4.
- 506 [46] D. Luo, K. Haverstick, N. Belcheva, E. Han, W. M. Saltzman, Macromolecules **2002**,
507 9, 3456.

- 508 [47] V. S. Talanov, C. A. S. Regino, H. Kobayashi, M. Bernardo, P. L. Choyke, M. W.
509 Brechbiel, Nano Lett. **2006**, 6, 1459.
- 510 [48] A. T. Yordanov, A. L. Lodder, E. K. Woller, M. J. Cloninger, N. Patronas, D. Milenic,
511 M. W. Brechbiel, Nano Lett. **2002**, 2, 595.
- 512
- 513

Figure 1. Schematic illustration of the diazirine grafting route on PAMAM dendrimer and reaction pattern of UV-activated crosslinking at the interface between transparent PLGA patch and ex-vivo tissue (3rd generation PAMAM scheme is presented for clarity reasons).

Figure 2. SEC-MALS-UV signals measured for PAMAM (control) and PAMAMA-g-diazirine with increasing conjugation percentages (solid and dashed lines correspond to RI and UV-ABS respectively).

Figure 3. ^1H NMR spectra with peak assignment (a-f) of PMMA-g-diazirine conjugates in comparison to pure PAMAM and diazirine; the chemical structure presents one branch of dendrimer after reaction between surface $-\text{NH}_2$ group and one molecule of diazirine.

Figure 4. Rheometry analysis: A) schematic illustration of the setup for rheometry measurements with UV activation; B) storage modulus (G') and loss modulus (G'') dynamics with UV activation of PAMAM-g-diazirine (50 wt% PBS, 15% conjugation); C) G' and G'' dynamics upon UV activation with 15% conjugation for different concentrations (25 wt%, 50 wt% and 75 wt%) in PBS, and; D) summary of G' values for different diazirine conjugation percentages, concentrations and exposure times.

Figure 5. Ex vivo shear adhesion tests: A) experimental setup of shear adhesion strength analysis; B) representative stress/strain curves recorded for different adhesion parameters of PAMAM-g-diazirine and un-modified PAMAM (control); C) maximum shear moduli before failure; D) summary of maximum shear strength values before failure as a function of conjugation percentage and concentration in PBS.

Figure 6. SEM images of PAMAM-g-diazirine at the surface of ex vivo porcine blood vessel after UV activation; inset represents magnified interface between crosslinked bioadhesive and endothelial (intima) side of the blood vessel.

Figure 7. Results from alamarBlue assay: A) EC50 cytotoxicity test of PAMAM-g-diazirine with 3T3 fibroblast cells mixed with PAMAM-g-diazirine gel after 24 h in culture (control represents unmodified PAMAM dendrimer); B) cell viability test of 3T3 fibroblast cells cultured in the medium with immersed crosslinked PAMAM-g-diazirine films;

Table 1. Conjugation percentages of PAMAM-g-diazirine obtained from NMR and SEC-MALS-UV in comparison to expected values from diazirine/PAMAM initial molar ratio used in synthesis reaction.

Theoretical conjugation (%)	Conjugation calculated from ^1H NMR (%)	Conjugation calculated from SEC-MALS-UV (%)	Conjugation calculated from initial molar ratio (%)
10	14.6 ± 0.54	13.2 ± 0.6	12.1 ± 0.1
20	22.5 ± 0.54	19.8 ± 0.8	20.1 ± 0.2
30	31.7 ± 0.54	29.6 ± 1.1	31.4 ± 0.3

Table of Contents

This paper describes bioadhesive system aimed for reconnection of soft tissues. The bioadhesive is based on poly amido amine dendrimer, grafted with UV-sensitive, diazirine,-4-[3-(trifluoromethyl)-3H-diazirin-3-yl] benzyl bromide. The dendrimer formulation adheres to hydrated tissues upon low power UV activation. The adhesion strength and mechanical modulus are dependent on grafting concentration and the energy of UV light.

Keyword: Bioadhesive

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Supporting Information

Title: Elastic light tunable tissue adhesive dendrimers

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Supporting Information is available from the Wiley Online Library or from the author.

Table S1. SEC-MALLS-UV analysis of PAMAM-g-diazirine.

^a Theoretical and experimental conjugation refers to the percentage of reacted –NH₂ groups (PAMAM) with Br-diazirine (PAMAM-g-diazirine).

^b The mass of PAMAM was calculated according to dn/dc (0.185) and the RI signal.

^c The conjugated mass of aryl-diazirine was calculated using the UV extinction coefficient at 350 nm after subtracting from PAMAM background signals.

^d Molecules with larger molecular weight (M_w) would have lower peak elution volume. Mean error is 0.01 mL or less.

^e Weight averaged M_w calculated from laser LS and RI signals.

Theoretical conjugation (%) ^a	Mass of PAMAM (μg) ^b	Mass of diazirine (μg) ^c	Peak elution volume (mL) ^d	Molecular weight (Da) ^e	Experimental conjugation (%)
0	165 ± 12	0.33 ± 0.42	8.63	28600±400	0.16
5	206 ± 22	12.5 ± 0.6	8.56	31300±500	4.8
10	189 ± 13	17.3 ± 0.7	8.52	33400±500	9.3
15	134 ± 8	20.1 ± 0.6	8.38	35000±600	14.4
20	155 ± 10	23.7 ± 1.3	8.19	36200±1100	19.9
30	162 ± 18	32.11 ± 0.8	8.10	39700±1500	28.2

Figure S1. (A) Representative ^1H NMR spectrum of PAMAM-g-diazirine (30% conjugation) with peak intensities calculated with SpinWorks software; (B) SEC-MALS-UV results from PAMAM-g-diazirine (10%-30%) of samples prepared for NMR analysis (straight lines represent RI and dotted lines represent UV absorbance @350 nm).

Calculation of conjugation percentage from NMR proton signal intensities

The intensities of ^1H NMR proton signals 'a' and 'f' from PAMAM-g-diazirine spectra are shown in Figure S1A. No UV absorbance was observed around the solvent peaks from SEC-MALS-UV chromatogram (Figure S1B), which confirms that ^1H NMR spectra were obtained for pure conjugates without residual diazirine precursor.

Peak intensities from ^1H NMR spectra were used to calculate conjugation percentages for all bioadhesive formulations by the following method:

Number of type 'a' protons in PAMAM G5 = 508

Number of type 'f' protons in diazirine = 4

Number of surface $-\text{NH}_2$ groups in PAMAM G5 = 128

I_a – Intensity of peak 'a'

I_f – Intensity of peak 'f'

$$\text{Conjugation percentage (\%)} = \frac{\frac{I_f}{4}}{\frac{I_a}{508} \times 128} \times 100\%$$

As an example, Figure S1A shows integrated ^1H NMR spectrum of PAMAM-g-diazirine 30%. The conjugation percentage for this particular composition is calculated as follows:

$$\text{Conjugation percentage (\%)} = \frac{\frac{I_f}{4}}{\frac{I_a}{508} \times 128} \times 100\% = \frac{\frac{16.1 + 16.0}{4}}{\frac{100}{508} \times 128} \times 100\% = 31.7\%$$

Other PAMAM-g-diazirine compositions were calculated by the same method.

Figure S2. 2D HMQC NMR spectrum of PAMAM-g-diazirine 30%; cross peaks at 3.71ppm x 52ppm (Ar-CH₂-NH-PAMAM) and 3.56 ppm x 57 ppm ((Ar-CH₂)₂N-PAMAM) represent mono-benzyl and di-benzyl amine structures.

SpinWorks 4: PAMAM-diazirine conjugate 30%.

Figure S3. Ratio between loss modulus (G'') and storage modulus (G'); diazirine conjugation percentage of PAMAM-g-diazirine in this test is 15 % and the concentration in PBS ranged from 25 wt% to 75 wt%.

Figure S4. Digital photograph of ex-vivo tissue and PLGA sheet after mechanical failure of PAMAM-diazirine adhesive.

Figure S5. Cross-section SEM images of the PAMAM-g-diazirine / blood vessel interface after UV-activated crosslinking.

Figure S6. PAMAM-g-diazirine residue SEC analysis after UV activation (dash line) of 15%- conjugated dendrimer at 75 wt% concentration (data was compared to SEC results obtained before UV activation, presented as a solid line).