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DOI:
10.1016/j.eurpolymj.2022.111502

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Document Version
Peer reviewed version

Citation for published version (Harvard):
Djordjevic, I, Wicaksono, G, Singh, J, Singh, M, Ellis, EG, Alraddadi, M, Dove, A & Steele, TWJ 2022, 'Hybrid polymer networks of carbene and thiol ene', *European Polymer Journal*, vol. 178, 111502. https://doi.org/10.1016/j.eurpolymj.2022.111502

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Hybrid polymer networks of carbene and thiol ene

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ABSTRACT

Thiol/ene-based resorbable elastomers display tough elongation but lack adhesion to soft tissues. Carbene-based bioadhesives (e.g. CaproGlu) allow soft tissue adhesion, but the covalent crosslinks limit extensibility after photoactivation. Herein thiol/ene resorbable elastomers are combined with a carbene bioadhesive into a 3-component hybrid network by exploiting tunable photoactivation of each macromolecule independently or simultaneously. Dual crosslinking was monitored by photorheometry, where 405 nm initiates formation of a thiol/ene elastomeric network, followed by 365 nm activation of diazirine-grafted polycaprolactone tetrol (CaproGlu). Dynamic shear moduli, gelation point, elongation at break, and lap shear stress of the hybrid polymer network are evaluated with respect to absorbed light energy dose. Surface-exposed unreacted CaproGlu enables adhesion of the hybrid network to various substrates, as well as intermolecular crosslinking within the transparent matrix. The network morphology and functional group conversion is evaluated through scanning electron microscopy and infrared spectroscopy, respectively. For the first time, we demonstrate hybrid thiol/ene/diazirine double sided bioadhesives with tunable dynamic moduli in the range of 10-800 kPa and 160 kPa lap-shear adhesion strength.

Keywords: *Hybrid polymer network, diazirine-grafted polycaprolactone; light curing; crosslinked elastomer; double sided adhesive.*

1. INTRODUCTION

Hybrid polymer networks consist of two or more entangled polymers which homogeneously build the polymer system by physical and/or covalent crosslinks [1]. The strategy of combining the properties of individual polymers could result in precise tuning of hybrid material for targeted applications [2]. One of the obvious examples of hybrid networks are "interpenetrated polymer network" (IPN) systems that have pushed the limits of what is capable for viscoelastic elastomers, such as elongation that exceeds 2000% and resilience that is unmatched with typical rubber networks [3]. Hybrid IPN materials can be produced both in hydrogel [4] or solvent free forms [5]. Careful selection of the type of crosslinking chemistry provides the strategy of design and control over unique material performance characteristic to hybrid polymer networks [6]. In applications such as wound management, tissue sealing and reconstruction, tissue adhesion plays a significant role. There are a number of hybrid materials reported to adhere to tissues. Recent examples include allyl-functionalized branched polymers mixed with tri-thiol crosslinking component [7], *in situ* forming multi-monomer acrylate IPN hydrogel tissue patch [8], polyacrylamide/alginate hybrid hydrogels [9] and two-component adhesive, composed by two different *p*-hydroxyphenyl-grafted polymers: chitosan and polyethylene glycol (PEG) activated by hydrogen peroxide and horseradish peroxidase [10]. Most of the bioadhesive systems (single components or hybrid network) rely on interfacial bonding realized by acrylate crosslinking and are either limited to topical use (i.e. cyanoacrylate) [11] or result in low adhesion strength (i.e. IPN hydrogels; adhesion strength ~20 kPa) [8]. Another type of tissue adhesion is by physical interaction (i.e. hydrogen or ionic bonds) where adhesion strength could be compromised by hydrolysis or changes in local pH values [12]. Current unmet clinical needs require bioadhesives that deviate from 2-part chemical curing designs. One-pot stimuli-sensitive crosslinking systems are sought with specific design parameters, such as: (i) solvent-free liquid resins; (ii) benign light activation energy that would yield rapidly gelling biomaterials; (iii) improved crosslinking chemistry to provide model systems for investigations of hybrid network adhesives. On-demand materials are sought for tissue adhesives with sufficient adhesive/cohesive strength to replace mechanical fixation methods based on sutures or staples.

Using activated esters presents another strategy for interfacial covalent bonding between hybrid adhesive and surface amines on tissue substrates [13-15]. Although some important advances have been made in the field with N-hydroxysuccinimide (NHS)-grafted

biomaterials, it should be noted that adhesion strength is dependent on concentration of surface amines that can vary for different tissues, causing modulation in adhesive performance [16]. Another potential drawback of NHS grafting technology is that requires dehydrated tissue prior to adhesive interaction, which often includes relatively complicated macromolecular designs [12, 13]. Carbene-based bioadhesives are under development to surpass current limitations of commercial tissue adhesives. Diazirine-grafted polycaprolactone polyol (CaproGlu) [17] was designed to be solvent-free liquid precursor for rapid light-induced gelation (both visible and UV light) and to be miscible with organic additives [18]. The hydrophobic nature of liquid CaproGlu allows solvent-free dissolution of other hydrophobic liquid polymers to yield hybrid network. Herein, CaproGlu's miscible nature is exploited to dissolve thiol/alkene and thiol/alkyne precursors into hybrid polymer network (**Figure 1**).

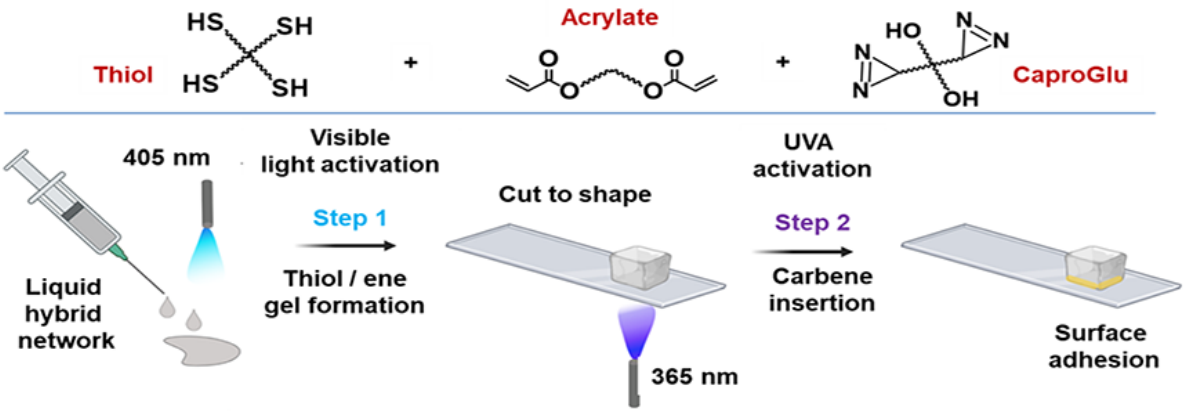

Figure 1. 3-component double sided adhesive hybrid polymer network design by solvent-free mixture of (top), 4-functional thiol, PEG-diacrylate and diazirine-grafted polycaprolactone tetrol (CaproGlu); (bottom) syringable hybrid network undergoes liquid-to-solid transition upon activation with visible light (405 nm – Step 1) followed by diazirine-to-carbene activation (UVA light at 365 nm – Step 2) that results in interfacial covalent attachment of adhesive gel with solid surface.

Polyethylene glycol-diacrylate (Ene), pentaerythritol tetrakis (3-mercaptopropionate) (SH), 2-propynoic acid, 1,1′-(1,2-ethanediyl) ester (Yne) and diazirine-grafted polycaprolactone tetrol (CaproGlu; abbreviated to "Dz" for diazirine functional groups) are mixed into hybrid networks that result in both single-step activation with polychromatic light in the range of 320-500 nm (SH/Yne/Dz) and dual-step activation by monochromatic 405 nm light followed

by UVA activation (365 nm) for SH/Ene/Dz. The general hypothesis is that thiol/ene reaction results in liquid-to-gel transition upon exposure to visible light (405 nm; Step 1) where diazirines are stable. This 3-component hybrid gel can be formed in any desirable shape/size prior to diazirine-to-carbene reaction, activated by UVA light (365 nm; Step 2;). The carbene subsequently inserts into any type of solid surface resulting in covalent adhesion of crosslinked hybrid network. The simple blending procedure of components with different molecular geometries (**Figure 2**) provide a facile preparation method of injectable synthetic biomaterials with a wide range of elastic moduli possible [19].

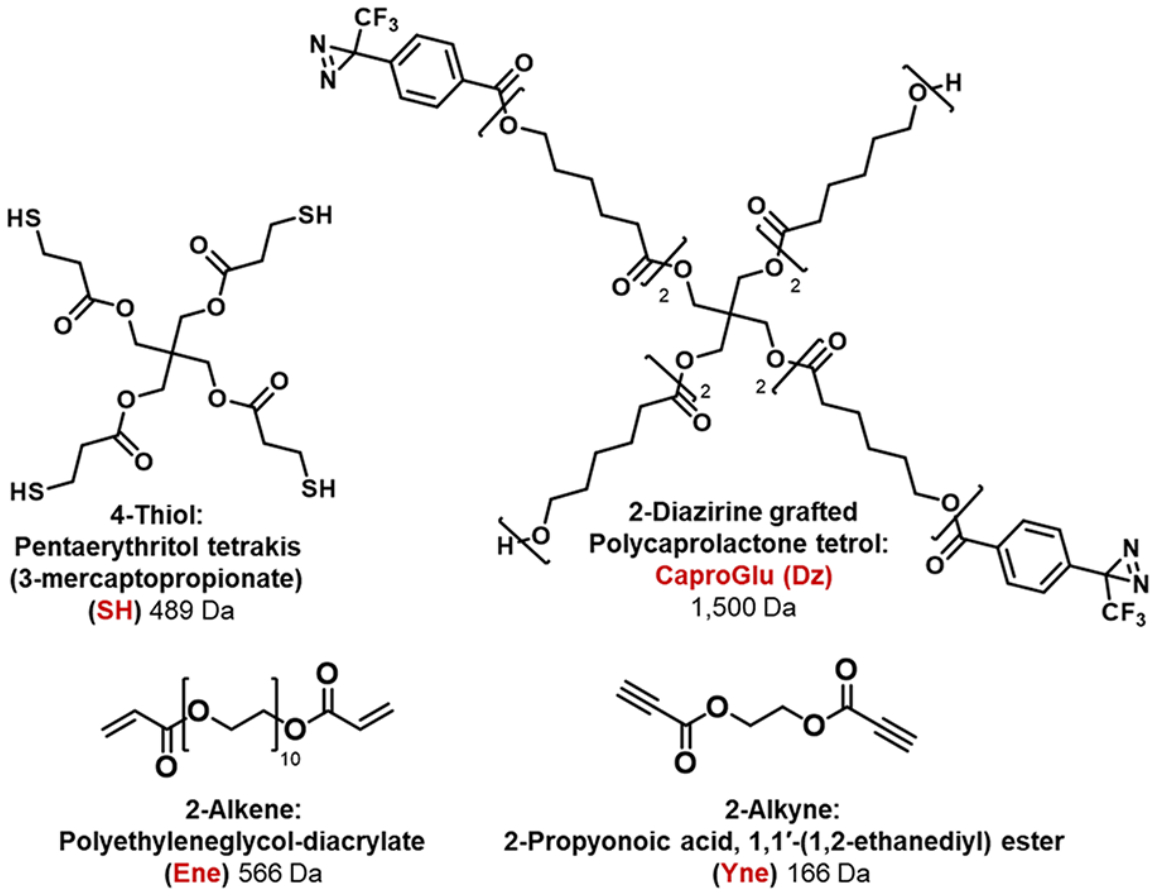

Figure 2. Chemical structures of individual components and nomenclature (in red font) used for hybrid polymer network, crosslinked by light activation with the presence of TPO initiator; CaproGlu formulation is polycaprolactone tetrol (PCLT; 1000 Da) grafted with 4-[3-(Trifluoromethyl)-3H-diazirin-3-YL] benzoic acid (Dz-COOH; 230 Da) with concentration of grafted Dz-COOH = 50% (2 diazirine functional groups per molecule).

Well-known thiol/ene and thiol/yne reactions are reported to occur by two mechanisms: thiol/ene radically mediated reactions and Michael-type additions [20, 21]. Thiol/ene free-

radical reactions are light-activated in the presence of photoinitiator with controlled crosslinking kinetics [22]. This crosslinking reaction is particularly useful for acrylate systems in coatings industry, dental and tissue engineering applications [23, 24]. *In-situ* light activation with visible light or with UVA light (i.e. absorbed light energy dose: 10-20 J) is a polymerization method of choice for synthetic implant design where the implant first takes shape/size of surgical site (Step 1) and subsequently adheres to solid substrate (Step 2; **Figure 1**). For the first time, this paper describes diazirine-based 2-step crosslinked hybrid polymer network with characteristics of double sided adhesive gel and controlled dynamic mechanical modulus.

2. EXPERIMENTAL SECTION

2.1 Materials

Polyethyleneglycol-diacrylate (Ene; **Figure 2**), diphenyl (2,4,6-trimethyl benzoyl) phosphine oxide (TPO) and other reagents and solvents (KOH, $KMnO_4$, HCl, $MgSO_4$, 1,1-carbonyldiimidazole (CDI), deuterated and pure dichloromethane (DCM), diethyl ether; Et_2O) are purchased from Sigma (Singapore). Pentaerythritol tetrakis (3-mercaptopropionate; SH; **Figure 2**), 4-[3-(Trifluoromethyl)-3H-diazirin-3-yl] benzyl alcohol (Dz-MeOH) and 2-Propynoic acid, 1,1′-(1,2-ethanediyl) ester (Yne; **Figure 2**) are purchased from TCI Chemicals (Japan). Polycaprolactone tetrol (PCLT; M_w = 1000 Da) is kindly donated by Ingevity (Capa^{TM} 4101; Lot No. HTX06P024).

2.2 Synthesis and preparation of 2-step light-activated hybrid polymer networks: thiol, acrylate and diazirine-grafted polycaprolactone tetrol

CaproGlu is diazirine-grafted PCLT (**Figure 2**) synthesized by previously published method [25]. In brief, diazirine grafting is obtained by esterification reaction between PCLT and 4-[3-(Trifluoromethyl)-3H-diazirin-3-YL] benzoic acid (Dz-COOH produced by Dz-MeOH oxidation) conducted with 1,1- carbonyl diimidazole (CDI) used as a coupling agent. The molar ratio of Dz-COOH/PCLT = 2/1 is deliberately chosen to yield ~50% diazirine conjugation. Hybrid polymer networks: Yne/Ene/SH/CaproGlu are prepared by mixing liquid components (**Figure 2**) in predetermined concentrations into glass vials; 2-component mixtures (SH/Yne and SH/Ene) and pure CaproGlu (Dz) are used as controls for crosslinking method in photorheometry experiment (**Table 1**). The solvent (DCM) is added to the mixture

(approximately up to 10% w/v polymer/solvent) and vortexed 3 times (20 sec each time). Stock solution of TPO (10% w/v; DCM) is added (maximum 0.1% w/w TPO/polymer) to the polymer solution and vortexed again 3 times (20 sec each time). The solvent is evaporated under vaccum to produce solvent-free hybrid polymer blends in the molar ratios displayed in **Table 1** (number of mmols of SH, Yne and Ene are normalized to CaproGlu set as 1 mmol).

Table 1. Molar ratios of hybrid networks with controls normalized to CaproGlu concentration (pure CaproGlu is used as Control-1 in all measurements) and light activation methods: 405 nm and 365 nm are two consequent activation steps used in dual curing photorheology experiment.

Hybrid network composition	4-Thiol (SH)	2-Alkyne (Yne)	2-Alkene (Ene)	2-Diazirine (CaproGlu)	Light activation		
					320-500 nm	405 nm	365 nm
CaproGlu (Control-1)	-	-	-	1	x	√	√
SH/Yne (Control-2)	1	1	-	-	√	x	x
SH2/Yne2/Dz	2.3	2.3	-	1	√	x	x
SH/Ene (Control-3)	1	-	2	-	x	√	√
SH2/Ene2/Dz	2.0	-	2.2	1	x	√	√
SH1/Ene2/Dz	1.1	-	2.1	1	x	√	√
SH1/Ene1.5/Dz	1.1	-	1.5	1	x	√	√

2.3 Photorheometry analysis of hybrid polymer networks

Samples are analysed with Anton Paar MCR302 rheometer (SH/Yne/Dz: PP08 / 8 mm diameter probe / 0.2 mm probe-base gap) and MCR302 rheometer (SH/Ene/Dz: PP10 / 10 mm diameter probe; 0.1 mm probe-base gap), equipped with UV-transparent glass base. The following is an example of rheometry evaluation: rotational shear at 10 Hz for 30 seconds, followed by dynamic shear for 120 seconds (1% amplitude; 10 Hz frequency) together with photoirradiation (UV light; OmniCure Series 1500 UV Spot Curing System with 320-500 nm bandpass filter (100 $mW.cm^{-2}$) for 200 seconds (SH/Yne/Dz). Dual crosslinking (SH/Ene/Dz) is performed with two different light sources: each 3-component network and controls (**Table 1**) are first subjected to rotational shear at 10 Hz for 60 seconds, followed by dynamic shear (1% amplitude; 10 Hz frequency) during which the samples are irradiated with 405 nm exposure (power: 100 $mW.cm^{-2}$; Thorlabs SOLIS-405C High-Power LED), followed by 365 nm UVA exposure (Convoy S2+ 365 nm Nichia UV Waterproof LED Flashlight; 100

184 mW.cm^{-2}). Light diodes are calibrated with IL 1400 Radiometer. Storage (G') and loss (G'')
185 moduli are recorded over time during the dynamic shear. G' and G'' are measured both as a
186 function of irradiation time and energy (J.cm^{-2}; referred to as "J" further in text).

187 **2.4 Fourier-transform Infrared Spectroscopy (FTIR) analysis**

188 FTIR spectra of 3-component hybrid networks (crosslinked by exposure to ambient light for
189 24 h) and pure CaproGlu are recorded before and after UVA activation (single components:
190 SH, Ene and CaproGlu are recorded as controls). Solid hybrid network sample is centred
191 between 2 UVA diodes (Supplementary Information; **Figure S1-2**) and activated from both
192 sides (10 J each side). Liquid samples (pure components) are placed on glass slides and
193 activated with 10 J dose of UVA light. FTIR spectra are recorded before and after UVA
194 activation in attenuated total reflection (ATR) mode at the following timepoints: before UVA
195 (neat), immediately after UVA activation and 10 min, 20 min, 30 min and 24 h post-UVA.
196 FTIR spectroscopy experiment is performed using PerkinElmer Frontier IR equipped with
197 ATR sampling accessory. Spectra are recorded over accumulation of 8 scans at resolution 4
198 cm^{-1}, at range of 4000-600 cm^{-1}. The theoretical calculation of the concentration of diazirine
199 groups, contained at 1 cm^2 surface with 1 µm thickness is performed by using the estimated
200 molecular weight of CaproGlu (1,500 Da) and the density of hybrid network determined
201 directly by weighing the samples with measured dimensions (**Table S2**).

202 **2.5 Scanning electron microscopy analysis of crosslinked hybrid polymer network:**
203 **cross-section morphology profile**

204 Crosslinked hybrid network is prepared by the same method as for FTIR spectroscopy and
205 lap shear adhesion experiments (10 J of absorbed light energy from each side of the square
206 sample (**Supplementary Video_1**) is cut in cross-sections and analysed with SEM. Samples
207 are subjected to platinum coating (90 s, chamber pressure <5 Pa at 20 mA). Images are
208 obtained by JSM 6360 SEM at an acceleration voltage of 5–20 kV and a working distance of
209 ~15 mm.

210 **2.6 Adhesion strength analysis of hybrid networks on polymer surfaces**

211 3-Component polymer networks in predetermined concentrations (**Table 1**) are casted into
212 petri dishes and irradiated with 405 nm diode for the total dose of 4 J. The samples are left to
213 crosslink for 24 h under ambient conditions to produce ~1 mm thick films for peel test
214 (**Figure S3**). Samples are placed on collagen film fixed with cyanoacrylate onto glass slide.

The sample is placed onto a collagen surface with one collagen film on the top of the sample thus forming collagen/HPN/collagen sandwich structure (**Figure S4**). Both glass slides with fixed collagen film (bottom surface) and the collagen strips (top surface) are soaked in purified water followed by removal of excess water with lint free paper. The sample is fixed with an additional glass slide (from the top) with the aid of paper clips (**Figure S4**). The sample is irradiated with UVA (365 nm) light from both sides with the total dose of 20 J (10 J from each side). After UVA activation, the paper clips and the glass slide from the top are removed. The sample is mounted onto a peel test cell and the top collagen film is pulled upwards to record the peel strength (N/m) vs displacement (mm). Each HPN composition as well as CaproGlu control are measured with Series Force Measurement System (Chatillon Force Measurement Products, USA) equipped with 100 N loading cell (n = 3). Gel-like samples (representative SH1/Ene2/Dz; **Supplementary Video_2**) are cut in square films and the weights / dimensions are recorded for each sample to estimate the density values of tested materials. Sample cuts (~1 mm thickness) are produced with a surgical blade and are placed on PMMA slide centered between 2 UVA diodes (top and bottom of the sample). A PET sheet is placed on the top of the sample, hand-pressed with the aid of glass microscope slide, UVA diodes are turned ON, simultaneously to deliver the total dose of 20 J (counted together from both sides; bottom through PMMA and top through glass + PET) to produce PMMA-hybrid network-PET sandwich structure for lap shear adhesion test. Shear adhesion strength is measured with Series Force Measurement System (Chatillon Force Measurement Products, USA) equipped with 100 N loading cell (n = 7).

2.7 Data processing

All the calculations and graphs are produced in OriginPro software.

3. RESULTS

3.1 Hybrid networks with diazirine-grafted polymer crosslinker: the scope

The combination of polymers (**Figure 2; Table 1**) is hypothesized to form an instantaneous hybrid gelation by: (i) single step curing with polychromatic light range of 320-500 nm (thiol/alkyne/diazirine) or (ii) dual (2-step) crosslinking with monochromatic light wavelengths at 405 nm (thiol/alkene; Step 1) and UVA (diazirine-to-carbene; Step 2; **Figure 1**). The overall design intent gives a light activated hybrid network for use as double sided

tissue adhesive patches. CaproGlu's liquid polymer nature creates the opportunity to dissolve free radical and step growth polymerization monomers to create stimuli-based biomaterials. Light irradiation at specific wavelengths can be selectively chosen to activate the monomers. The following thiol/ene and thiol/yne monomers are common hydrogel precursors: 4-functional thiol (SH), 2-functional alkene (Ene), 2-functional alkyne (Yne) and 2-functional diazirine grafted polycaprolactone tetrol (CaproGlu; Dz). The exact mol ratios and respective nomenclature are shown in **Table 1**; for example, the network "SH2/Ene2/Dz" is a 3-component liquid mixture in the following molar ratio: PTHT/PEGDA/CaproGlu = 2/2.2/1 (structures are shown in **Figure 2**). Neat CaproGlu and 2-component hybrids (SH/Yne and SH/Ene) are used as controls. Thiol/ene and thiol/yne crosslinked resins are selected for their miscibility in CaproGlu and allow simultaneous or independent activation when doped with visible light photoinitiators. Simultaneous activation is first evaluated with polychromatic light in the range of 320-500 nm (2SH/2Yne/Dz; **Table 1**). Independent activation is evaluated for the SH/Ene/CaproGlu hybrid elastomer by visible light (405 nm: thiol/ene polymerization) [26], followed by CaproGlu activation with UVA light (365 nm: carbene-based crosslinking).

The specific monomers (SH, Yne and Ene; **Figure 2**) are chosen as they are capable of bioresorption via ester hydrolysis similar to polycaprolactone-based materials. Surface-distributed diazirines are hypothesised to enable double sided adhesion by carbene insertion upon UVA light activation. To investigate crosslinking kinetics, a custom photorheometer is used to evaluate the real-time dynamic mechanical properties. One of the objectives is to find compositions with low yield stress, also known as Bingham plastics (**Table 1**) that allow substrate conformation for double sided adhesion. Structure-property relationships are determined with respect to light energy exposure ($J.cm^{-2}$; referred to as "J" further in text), and tertiary ratios of the hybrid components. Qualitative analysis of surface chemistry is evaluated by ATR-FTIR spectroscopy to observe chemical reactions of surface-exposed functional groups before and after light activation. All hybrid networks are tested for their peel strength when UVA-crosslinked between two hydrated collagen surfaces. Scanning electron microscopy (SEM) evaluates the depth of CaproGlu activation through porous morphologies and fundamental mechanical properties (lap shear adhesion strength and toughness) are evaluated in regard to the cross-sectional depth of diazirine activation.

3.2 Dynamic mechanical properties and crosslinking kinetics profiles are tuned by the rule of mixtures between individual polymers

Thiol/yne/Caproglu hybrid (SH2/Yne2/Dz; the exact mol ratios are shown in **Table 1**) is activated with polychromatic light in UVA/visible range (320-500 nm; mercury lamp) for simultaneous activation of CaproGlu (UVA) and the thiol/yne network (visible light). Storage (G') and loss (G'') moduli are recorded as a function of time and irradiation dose (**Figure 3A**). Gelation (G' = G'') of SH2/Yne2/Dz hybrid network is reached at the dose of 4.2 J (absorbed per cm^{-2} in all photorheometry experiments) which is almost three times higher than gelation dose measured for pure CaproGlu (1.6 J; control-1) activated with mercury lamp (320-500 nm; Supporting Information, **Figure S5**). The 2-component mixture SH/Yne (control-2; **Table 1**) required a higher gelation dose of 6 J (**Figure 3B**).

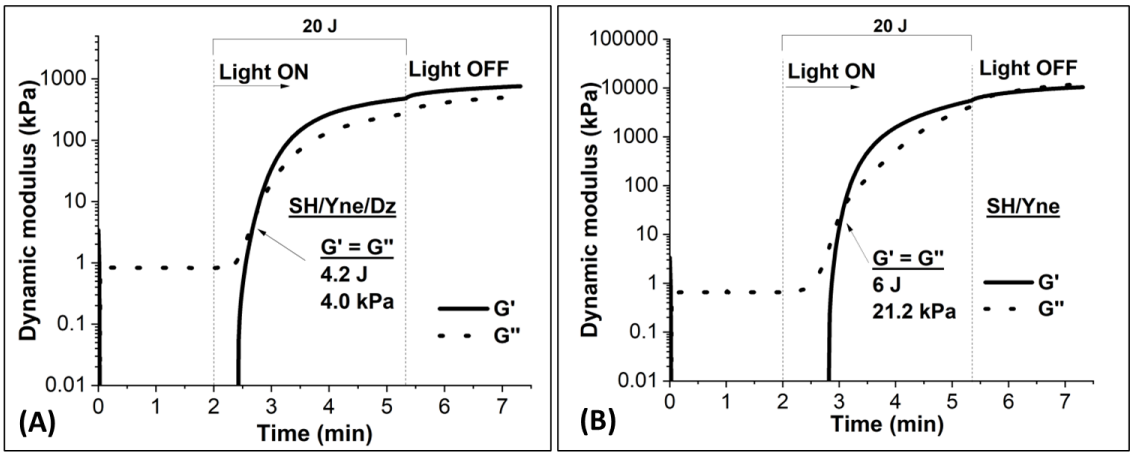

Figure 3. Light activation of SH2/Yne2/Dz hybrid samples. Liquid-to-solid transition of hybrid networks analysed with photorheometry – dynamic change of storage (G') and loss (G'') moduli with indicated gelation points (G' = G") upon light activation (320-500 nm; 100 $mW.cm^{-2}$) over measured time (bottom) and energy dose ($J.cm^{-2}$; top): (A) 3-component thiol/alkyne/CaproGlu hybrid: SH2/Yne2/Dz; (B) 2-component thyol/alkyne hybrid: SH/Yne (control-2).

This result demonstrates that introduction of CaproGlu into the SH/Yne/Dz mixture results in lowering the gelation point in simultaneous crosslinking process of thiol/yne and covalent carbene insertion. Upon absorption of light at the total dose of 20 J, the hybrid 3-component polymer network reaches dynamic modulus (G') value of 500 kPa (**Figure 3A**) that is an order of magnitude lower than value reached for 2-component control-2 (G' > 5 MPa; **Figure**

3B). Lower G' value likely results from emission of nitrogen upon diazirine photolysis and formation of foam like structure [17, 18, 25]. Introduction of diazirine-grafted polymer into thiol/yne reaction system results in 1-step reaction within seconds activated by polychromatic mercury light (320-500 nm). This result expands the application of thiol/yne reaction that is relevant in both bioorganic chemistry and polymer synthesis [27]. It should be noted that the relatively high energy dose (20 J) is necessary to reach G' plateau for both SH2/Yne2/Dz (**Figure 3**) and pure CaproGlu (**Figure S5**). This is a result of polychromatic light emitted from mercury lamp (320-500 nm) where only the portion of the total absorbed light (320-390 nm) activates diazirine groups [17].

Thiol/ene precursors are selected for independent light activation investigations. These precursors are available with similar molar mass (489 Da and 566 Da; **Figure 2**) to closely align polymer and functional group molar ratios. Independent 2-step light activation of three hybrid networks (**Table 1**) is evaluated by visible, followed by UVA activation. All 3-hybrid network samples in **Table 1** with the two-step light activation are first sheared for 1 min followed by irradiation with 405 nm light (Step-1). After 2 min no further changes in modulus are observed and the sample is irradiated using a UVA diode (365 nm) with a 6 J dose (Step-2; **Figure 4**). Light activation intervals are marked with dashed lines in **Figure 4** and **Figure S6**. The representative evolution of G' for SH1/Ene2/Dz hybrid is compared to pure CaproGlu (control-1) and 2-component diazirine-free SH/Ene mixture (control-3) as shown in **Figure 4A**. Both SH/Ene (control-3) and SH1/Ene2/Dz hybrid show an increase in G' values upon activation at 405 nm, indicating free-radical (thiol/ene) polymerization in the presence of CaproGlu that remains unreactive until the UVA light is turned on.

According to rheometry data in **Figure 4A**, the rapid free-radical polymerization appears to be faster in comparison to carbene-induced CaproGlu crosslinking (judging from the G' vs irradiation time required to reach G' plateau). The 2-component control-3 (SH/Ene; no CaproGlu) shows no change in G' upon UVA activation. Neat CaproGlu indicates liquid-to-biorubber transition when activated either by polychromatic light (**Figure S5**) or monochromatic UVA (365 nm) diode (**Figure 4B**). However, 3-component hybrid networks (**Table 1**) undergo 2-step crosslinking without indication of gelation point (**Figure 4C; Figure S6**). Note that pure CaproGlu takes 6 J UVA dose to reach G' value of ~170 kPa (**Figure 4B**) consistent with previously published work on CaproGlu bioadhesive [17, 25].

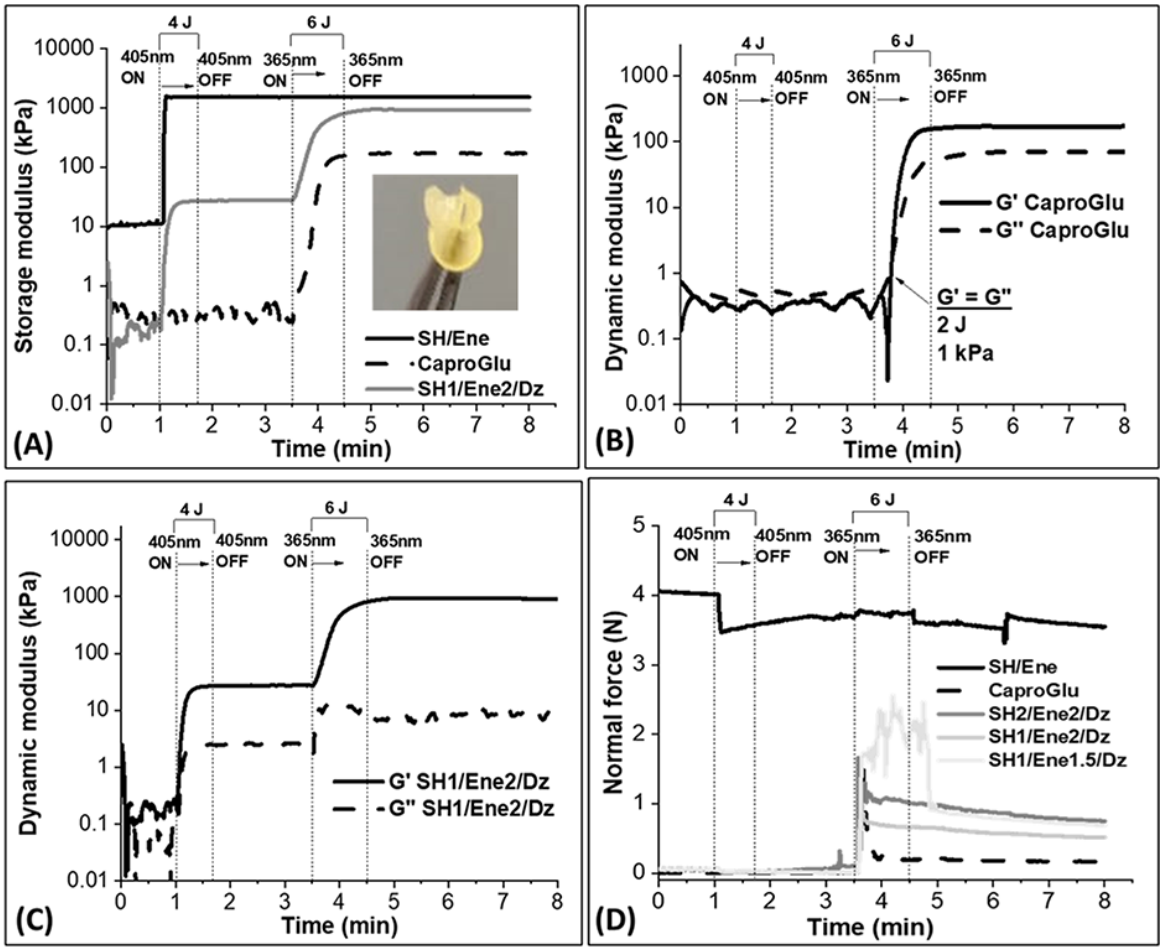

Figure 4. 2-step energy directed crosslinking of liquid-to-solid hybrid network (3-component compositions are specified in **Table 1**) analysed with photorheometry by using different wavelengths: (A) representative dynamic change of storage moduli (G') of 3-component thiol/ene/CaproGlu hybrid network (SH1/Ene2/Dz) upon crosslinking at 405 nm (Step 1) followed by activation at 365 nm (Step 2) in comparison to 2-component thiol/ene (SH/Ene; control-3) and pure CaproGlu (control-1); the power of both diodes is adjusted to the total of 10 J.cm^{-2} dose (diode power = 100 mW.cm^{-2}); (inset) SH1/Ene2/Dz transitioned into a flexible film after dual irradiation; (B) dynamic change of G' and loss modulus (G'') upon light irradiation recorded for pure CaproGlu with indicated gelation point (G' = G''); (C) G' and G'' change with irradiation energy recorded for SH1/Ene2/Dz; (D) normal force caused by the volume expansion of polymer networks upon light-activated crosslinking recorded for 3-component hybrid networks compared to 2-component (SH/Ene; control-3) and pure CaproGlu (control-1). All data points are measured over time (bottom) and energy dose (J.cm^{-2}; top)

Unlike controls that show no indication of crosslinking by either visible light (405 nm; pure CaproGlu) or by UVA light (365 nm; 2-component SH/Ene), 3-component hybrid network results in 2-step crosslinking process that is first activated by visible light (thiol/ene) followed by UVA activation of diazirine to reach the maximum at 920 kPa recorded for SH1/Ene2/Dz hybrid network (**Figure 4A and C**). Hybrid elastomeric films could be peeled off the rheometer probe after a dual curing experiment as shown in **Fig. 4A-inset** (the rheometry results are summarised in **Table S1**).

The photorheometer simultaneously assesses volumetric shrinkage by applying normal force to maintain a predetermined sample thickness. The normal force to the rheometer probe is monitored for both hybrid networks and controls 1-2 (pure CaproGlu and 2-component, diazirine-free SH/Ene mixture, respectively) and results are shown in **Figure 4D**. Control-2 demonstrates a decrease of normal force with exposure to 100 $mW.cm^{-2}$ power 405 nm diode within seconds. The SH/Ene molar ratio in control-2 (**Table 1**) is 1/2 for the highest density of crosslinking between 4-functional thiol and 2-functional acrylate. A drop in normal force is evidence for shrinkage and an increase signifies matrix expansion. Volume shrinking is observed under visible light activation due to Michael addition and free radical crosslinking. 3-Component hybrid networks (**Table 1**) all resulted in sample volume shrinkage upon 405 nm activation (Step 1) as evident from drop in normal force (see **Figure S7**). CaproGlu shows no change in normal force during Step 1 light activation (405 nm; 4 J; **Figure S7**) suggesting the inert nature of diazirine groups towards visible light (405 nm). Under UVA light exposure, diazirine photolysis releases molecular nitrogen as a byproduct that in turn causes foaming [17, 25]. Volume expansion is evident in both pure CaproGlu (control-1) and 3-component hybrid samples (**Figure 4D**). This further supports the hypothesis of hybrid network crosslinking with independent light wavelengths.

The hybrid networks are observed to spontaneously solidify over 24 h (no UVA activation) when exposed to laboratory ambient environment, which may be due to visible light activation (**Figure S2**). Note that SH1/E1.5/Dz hybrid network (refer to **Table 1** for exact mol ratio) results in viscoelastic solids (i.e. Bingham plastic) material (**Figure S1**). The elastomeric hybrid composition can be cut into solid, double sided adhesive polymer (network SH1Ene2/Dz; **Table 1**; **Supplementary Video_1**) and forms a solid material after 24 h exposure to ambient light; this formulation has a ~1/1 ratio thiol/ene (**Table 1**). The absence of gelation points in hybrid networks (**Figure 4C**; **Figure S6**) is possibly a consequence of immediate reaction upon mixing under ambient light condition as the

photorheometry analysis is performed within 30 min after sample preparation. The absence of gelation points (recorded 30 min after mixing) are consistent with the macroscopic appearance of hybrid networks that form either solid gels or Bingham plastic materials even without direct irradiation by 405 nm light diode (**Figure S1**). Note that stoichiometric SH/Ene ratio in SH1/Ene2/Dz (4-arm thiol and 2-arm acrylate; **Figure 2**) results in highest G' = 28 kPa upon 405 nm activation in comparison to SH2/Ene2/Dz (16 kPa) and SH1/Ene1.5/Dz (7 kPa) where unreacted thiol acts as plasticizer (**Figure 4**; **Figure S6; Table S1**). Although the highest relative concentration of diazirines is in SH1/Ene1.5/Dz, the stoichiometric thiol/acrylate ratio (SH1/Ene2/Dz) results in the highest recorded G' after second activation step with UVA (920 kPa) compared to 410 kPa and 630 kPa recorded for SH2/Ene2/Dz and SH1/Ene1.5/Dz respectively (**Figure 4**; **Figure S6**). Carbene reacts both at the surface and within the bulk of materials, depth limited by macromolecule scattering. The unreacted thiol within the hybrid networks might act as carbene scavenger thus reducing the extent of surface reaction with both base and the probe of photorheometer, that in turn results in relatively low modulus.

3.3 FTIR spectroscopy identifies covalent crosslinks within hybrid networks

FTIR spectroscopic analysis is performed to qualitatively observe the depletion of reactive functional groups upon photoreactions (**Figure 5**), namely: out of plane –C=C– stretch vibrations at 810 cm^{-1} (2-Ene) [21, 28], S-H absorption peaks at 2570 cm^{-1} (4-SH) [29], diazirine ring at 1634 cm^{-1} [25, 30] and diazoalkane intermediate peaks at 2092 cm^{-1} [25, 31]. The network SH1/Ene2/Dz (the exact molar ratio is shown in **Table 1**) is chosen for this experiment to prove the hypothesis that under visible light only thiol reacts to completion with acrylate while diazirine groups remain unreacted. **Figure 5A** shows full spectra of both hybrid SH1/Ene2/Dz and neat components (no UVA activation) while **Figure 5B** is a magnified region used in this analysis. Note that the peaks at 1640-1610 cm^{-1} of Ene (assigned to –C=C– from acrylate) overlap with diazirine (–N=N–; 1630 cm^{-1}) and –C–N (1610 cm^{-1}) [25] peaks from CaproGlu and therefore could not be used to observe reaction of acrylates [32]. For that reason, the peak at 810 cm^{-1} is selected to analyse Ene (acrylate) reaction within hybrid matrix (**Figure 5B-inset**). Both thiol (2570 cm^{-1}) and acrylate (810 cm^{-1}) disappear in SH1/Ene2/Dz sample after photocuring by ambient light with estimated degree of Ene conversion of ~80% even before activation with UVA light (**Figure S8**).

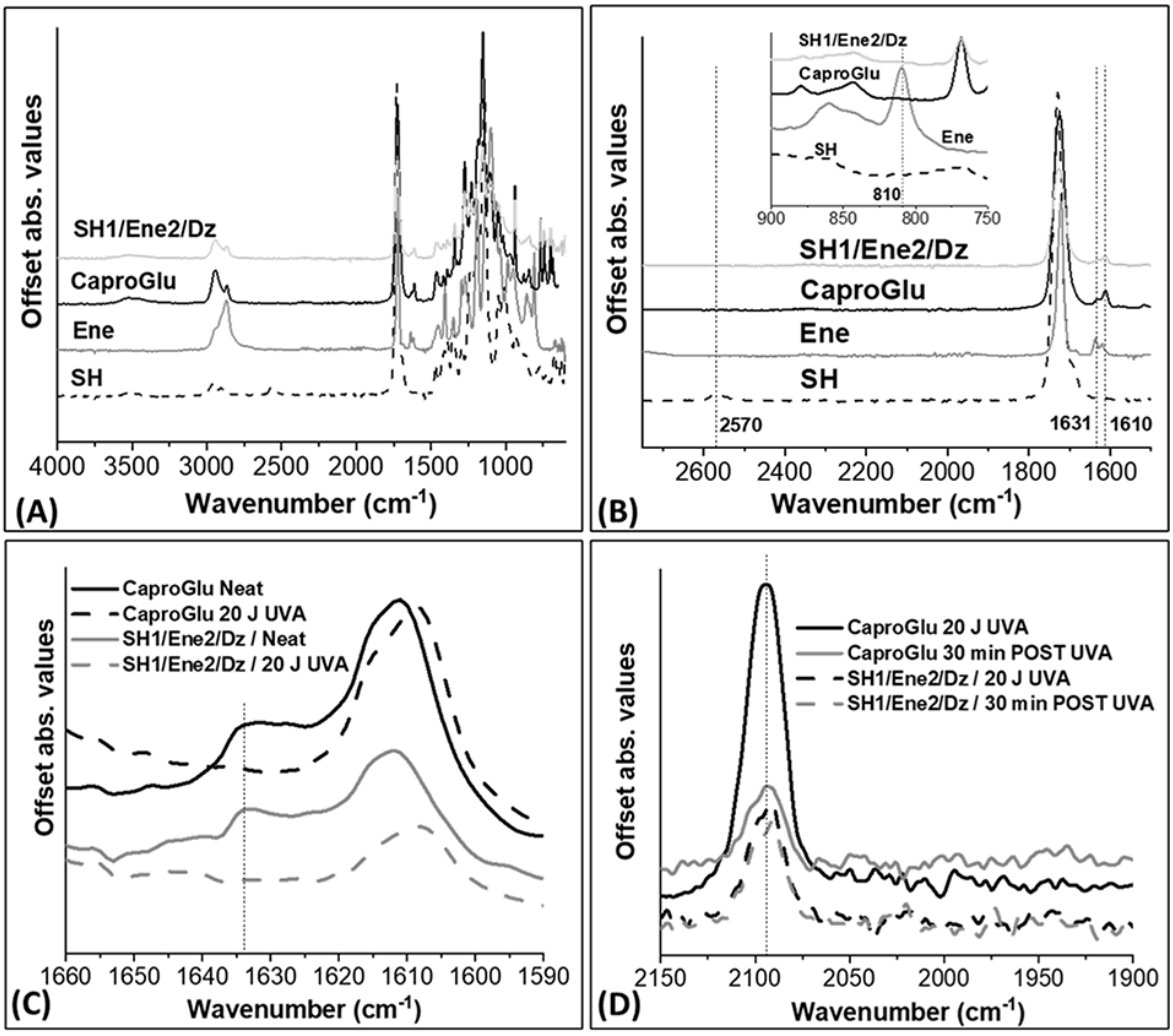

Figure 5. FTIR spectral regions recorded for representative 3-component thiol/ene/CaproGlu hybrid network (SH1/Ene2/Dz) and pure polymer components (structures and nomenclature of individual component are shown in **Figure 1**): (A) full FTIR spectral region (no UVA); (B) diazirine (1631 cm^{-1}; CaproGlu and SH1/Ene2/Dz), –C–N (1610 cm^{-1}), –S–H (2570cm^{-1}; pure 4-functional thiol: SH) peaks; (inset) –C=C– stretch vibration at 809 cm^{-1} from diacrylate groups (Ene; no UVA); (C) disappearance of diazirine peak (1634 cm^{-1}; dashed line) upon UVA activation; (D) diazoalkane (2094 cm^{-1}) persistence over the period of 24 h upon UVA activation of 3-component hybrid network (SH1/Ene2/Dz) and pure CaproGlu.

Upon UVA activation, diazirine undergoes photolysis as recorded from disappearance of –N=N– peak at 1630 cm^{-1} (**Figure 5C**). Two products are possible from diazirine photolysis: carbene and diazoalkane. The semi-stable nature of the diazoalkane is demonstrated by the presence of 2090 cm^{-1} peak absorbance of both SH1/Ene2/Dz hybrid and neat CaproGlu

(control) 30 min post-UVA activation (**Figure 5D**). Previously published FTIR results provide the evidence that the diazoalkane decay kinetics is dependent on the functional groups adjacent to diazirine [33]. The intermolecular environment (in this case SH/Ene mixture) is likely to influence the fate of diazoalkane, however this requires dedicated kinetics study in future research. It is hypothesized that the adhesion to solid surfaces of 3-component hybrid network is facilitated by the fraction of the diazirine groups that are distributed at the hybrid surface after the first crosslinking step. From theoretical estimation (according to molar ratio in **Table 1**) the SH1/Ene2/Dz network contains 70 nmol.cm^{-2} of diazirine groups for the sample thickness of 1 µm (the density of this particular network is 1.1 g.cm^{-3}; **Table S2**). Indeed, diazirine groups are detected in ATR-FTIR experiment (**Figure 5C**) with penetration depth of ATR probe to be ~100 nm [34]. With evidence that diazirine surface groups remain unreacted, double sided adhesives are possible.

3.4 UVA activation of diazirine component results in porous surface micro-morphology detected by SEM

As indicated by the double sided adhesive nature of the 3-component SH1/Ene2/Dz hybrid network (**Table 1**), UVA irradiation activates the sample surface causing covalent insertion of carbenes onto solid polymer interfaces (**Supplementary Videos 1 and 2**). SEM analysis of cross-section (**Figure 6**) is performed to examine the penetration depth of diazirine activation visually observed by the colour change (from white opaque to yellow; **Figure 4A-inset**). Arrows pointing out from **Figure 6A-inset** indicate different parts of SH1/Ene2/Dz cross-section (surface **Figure 6B** and bulk **Figure 6C**) analysed with SEM. UVA activation of CaproGlu causes evolution of molecular nitrogen that in turn results in porous crosslinked matrix [25]. From **Figure 6A-B**, the porous matrix is formed at the surface of the hybrid network with estimated penetration depth of molecular nitrogen in the range of 100-150 µm while the middle portion of the sample (**Figure 6C**) shows micro-wrinkled morphology, characteristic for elastomeric surfaces [35]. The pore size generated by molecular nitrogen (diazirine photolysis) is in the range of 5-10 µm (**Figure 6B**), 10x smaller than neat CaproGlu with pore size of 50-100 µm (**Figure 6D**).

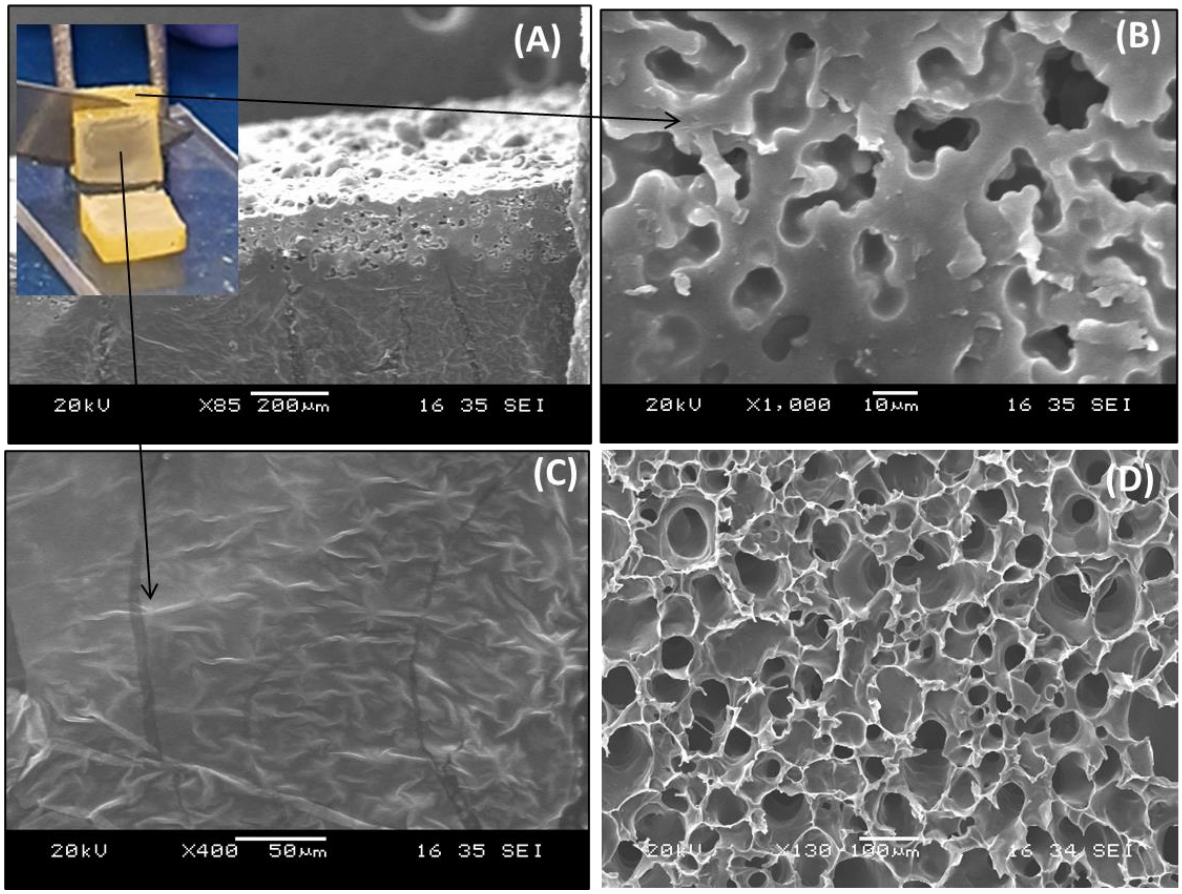

Figure 6. SEM images of cross-section of 3-component thiol/ene/CaproGlu hybrid polymer network after 10 J.cm^{-2} of UVA activation indicating the outer porous and inner homogeneous morphology of UVA activated sample - 2 parts of the sample, surface and bulk (inset photography) are indicated by arrows: (A-C) SH1/Ene2/Dz; (D) pure CaproGlu (control-1) activated with 10 J.cm^{-2} of UVA (magnification bar: 100 µm).

Decrease of pore size is a direct consequence of material properties – unlike the crosslinked hybrid network that results in a semi-solid material, liquid (neat) CaproGlu results in lower stress that inhibits nitrogen bubble expansion. Knowing the penetration depth of CaproGlu crosslinking within the SH1/Ene2/Dz hybrid network (100-150 µm; **Figure 6A**) attention should be given to the rheometry base-gap probe that is set for 100 µm, and therefore dynamic moduli (G') in **Figure 4** and **Figure S6** are representative of complete diazirine (CaproGlu) crosslinking within the total volume of analysed hybrid network sample (**Figure 4A-inset**) as confirmed by SEM. Elastomeric wrinkled morphology [36] in the bulk of SH1/Ene2/Dz hybrid is in line with visual observation of the material (**Supplementary Video_1**).

Crosslinked SH1/Ene2/Dz (Step 1; 405 nm) does not allow pore expansion deeper and larger than measured by SEM, however, future work could demonstrate that the pore size might be controlled by the following parameters: (1) concentration of grafted diazirine and molecular weight of polycaprolactone polyol; (2) CaproGlu concentration within composite (hybrid) network; and (3) crosslinking density activated with visible light, prior to UVA activation. Furthermore, diazirine can be activated with longer wavelength at 445 nm (with the aid of photocatalyst) [18] that will possibly allow light-activated crosslinking deeper than 150 μm.

3.5 Double sided adhesive hybrid network properties

Hybrid network SH1/Ene2/Dz (**Table 1**) has demonstrated light sensitivity in both visible light (405 nm; G' = 28 kPa at 4 J dose) and UVA (365 nm; G' = 920 kPa at 6 J dose) resulting in the highest dynamic moduli in comparison to other hybrid networks (**Figure 4; Table S1**). Predetermined SH/Ene ratio (1/2) results in complete thiol/ene reaction (as evident from FTIR spectroscopic analysis; **Figure 5B**) forming a gel polymer network after activation by visible light at low energy dose (4 J; **Figure 4A**). Rheometry results demonstrate that CaproGlu was unaffected by visible light and could be activated independently – visible light activation of SH1/Ene2/Dz hybrid allows mm thick specimens to be setup even in the presence of CaproGlu (**Supplementary Videos 1 and 2**). Diazirines are known to convert into carbene upon UVA activation that in turn results in unselective crosslinking, both polymer bulk (intermolecular) and at any proximate surface (i.e. polymer, tissue proteins) [17]. Peel strength experiment is performed for hybrid networks where the samples (**Figure S3**) are placed between two hydrated collagen surfaces (**Figure S4**) and subsequently activated with UVA light from both sides of the "sandwich" structure with the total dose of 20 J (10 J delivered through each collagen film). The obtained results are compared to the following controls: 1) neat CaproGlu before and after UVA activation; 2) two-component blend SH/Ene (without CaproGlu) previously crosslinked under ambient light (**Figure S3**) and subsequently irradiated with 20 J of UVA light through collagen sheets; 3) hybrid polymer networks fixed between two collagen films without UVA activation (**Figure 7**).

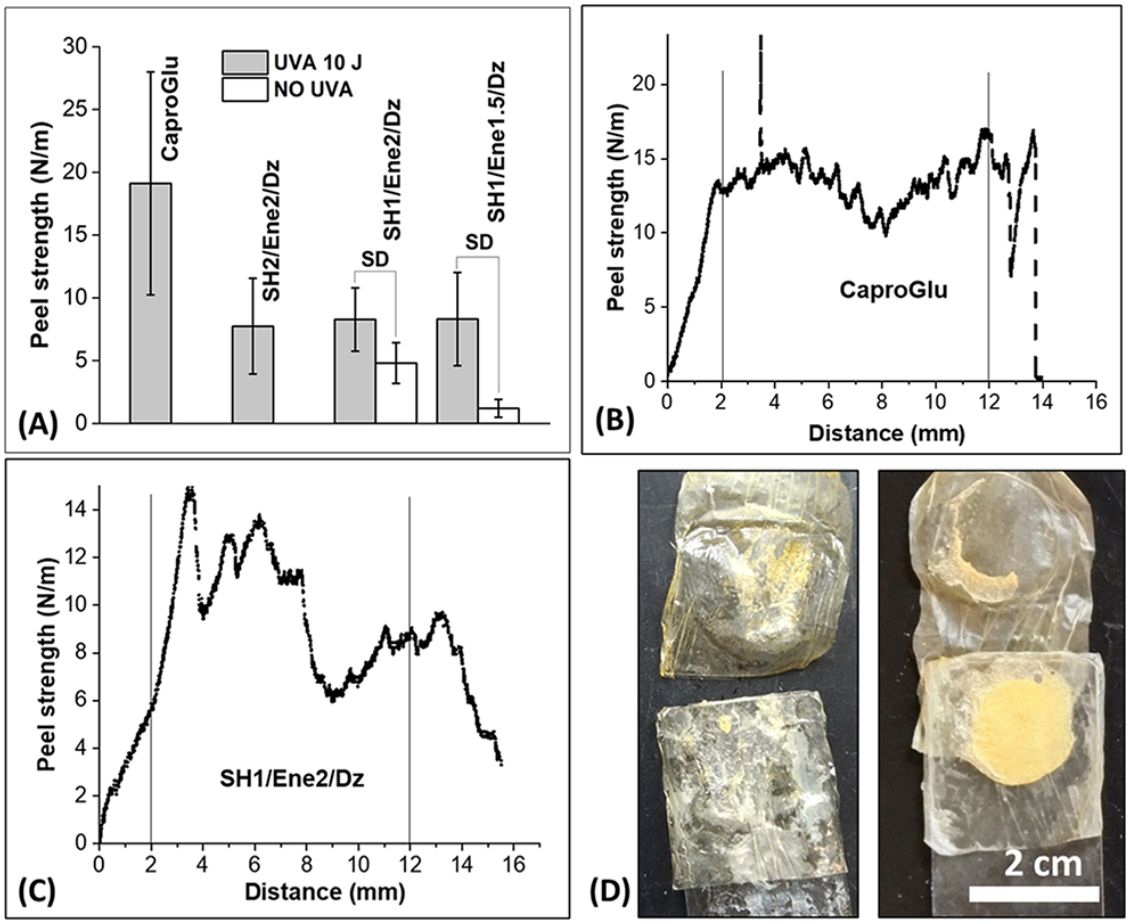

Figure 7. Peel adhesion strength measured for HPN samples (and pure CaproGlu used as control) activated through hydrated collagen surfaces with 2 UVA diodes – each side of all samples absorbed the UVA energy = 10 J.cm^{-2} (total absorbed dose = 20 J.cm^{-2}): (A) peel strength measured for HPN samples compared to CaproGlu control (n = 3; ANOVA: $p<0.05$; SD = statistically different); (B and C) representative peel strength profiles collected for pure CaproGlu and SH1/Ene2/Dz respectively (data between 2 vertical lines is used to calculate average peel strength for each sample – 2 mm after the beginning of the test and 2 mm before adhesion failure); (D) representative photographs of collagen strips with crosslinked CaproGlu (left) and SH1/Ene2/Dz (right) samples after peel strength experiment.

All tested hybrid networks resulted in peel adhesion strength (after UVA activation) of 8 ± 3 N/m with a relatively large standard deviation and without significant difference between samples (**Figure 7A**). All the raw data used to calculate values in **Figure 7A** are shown in **Figures S9-11**. This result corresponds with photorheological properties where no change in dynamic modulus could be recorded upon UVA activation without the presence of diazirine

component (**Figure 4**). Hybrid polymer networks SH1/Ene2/Dz and SH1/Ene1.5/Dz show adhesion when pressed between hydrated collagen sheets with the aid of paper clips (**Figures S4 and S11**). However, the values of 5 ± 2 N/m and 1.2 ± 0.7 N/m (SH1/Ene2/Dz and SH1/Ene1.5/Dz respectively) are significantly lower than peel adhesion strength recorded after UVA activation (**Figure 7A**). Both neat CaproGlu and two-component network (SH2/Ene2) controls do not show any adhesion that could be recorded without UVA irradiation. Together with FTIR analysis (**Figure 5**), this result supports the hypothesis that the diazirine groups are present at the hybrid network surface and result in adhesion when activated with UVA light at the hydrated biopolymer interface (collagen). Neat CaproGlu results in significantly higher peel strength after UVA activation (19 ± 9 kPa) when compared to tested hybrid networks (**Figure 7A**). This result is expected because diazirine groups in gel networks (**Figure S3**) are diluted and thus cause lower peel strength than crosslinked CaproGlu. Furthermore, during the peel experiment, the fracture of crosslinked polymer propagates evenly for CaproGlu as the fracture strength is distributed over the entire sample volume (**Figure 7B; Figure S9**). Representative peel adhesion data for hybrid polymer network (SH1/Ene2/Dz; **Figure 7C**) shows peak and trough indicating larger strain fracture toughness. CaproGlu fails cohesively during peel test [17, 18], unlike in crosslinked hybrid networks that demonstrate adhesive failure due to diazirine dilution (representative photographs are shown in **Figure 7D**). Increased toughness by formation of a hybrid network matrix correlates with rheology results (**Figure 4**) that indicate crosslinked matrix upon thiol/ene reaction activated by visible light (Step-1; **Figure1**).

Cohesive nature of CaproGlu adhesive is consistent with previously published results on pure CaproGlu used in artery anastomosis aided with polycaprolactone (PCL) mesh tape [17]. The biorubber nature of hybrid network (**Supplementary Video_2**) allows compliance with soft tissues and potentially could prevent implant failure and injuries due to biomechanical mismatch. The elastic nature of crosslinked hybrid network is further investigated in lap shear adhesion test to polymer surfaces that resulted in ultimate adhesion strength of 160 ± 50 kPa (representative SH1/Ene2/Dz; **Figure S12A-B**). The elastic behaviour is also evident from modulus vs strain diagram (**Figure S12C**) calculated as the first derivative of stress vs strain function. The modulus value increases from 140 kPa to the maximum value of 197 kPa at 0.42 (mm/mm) strain and sustains strain > 0.8 (marked with dashed arrow in **Figure S12C**). The strain energy density (toughness) of the hybrid network is measured to be 80 ± 40 $kJ.m^{-3}$ (**Figure S12D**) for the samples of ~2 mm thickness (**Figure S12B-inset**; **Table S2**). The

toughness of the hybrid network is an order of magnitude lower that the toughness value reported for thin CaproGlu film (~20 µm) activated at biological surfaces (~20 $MJ.m^{-3}$ collagen/CaproGlu/porcine skin) [18]. This result indicates that UVA activation is spatially limited by UVA penetration depth through hybrid polymer network since all measured samples failed cohesively (**Figure S13C**). The measured lap shear adhesion strength of hybrid network is comparable to pure CaproGlu (170 ± 10 kPa; **Figure S13**). Unlike the peel test performed with irregular, hydrated collagen films that results in adhesive failure (**Figure 7**), the hybrid network failed cohesively when crosslinked on flat, fully transparent polymer surfaces (PET and PMMA; **Figures S12** and **S13**). The diazirine adhesion is stronger than thiol/ene crosslinked network as evident by repetitive cohesive failure in lap shear adhesion tests. Due to the cohesive nature of the double sided adhesive hybrid network, the diazirine adhesion strength remains unknown. It is evident that the diazirine surface adhesion is stronger than thiol/ene cohesive strength that always fails first.

4. DISCUSSION

Hybrid polymer blends that incorporate independent light activated crosslinking mechanisms have been explored for the first time. Hybrid composites of thiol/ene and carbene-based biomaterials serve as a model system to explore how viscous formulations can be rapidly photocured into tough bioelastomers. The design requires liquid precursors that are miscible yet remain relatively inactive. Liquid mixtures of CaproGlu, ester alkyne, PEG-based alkene, and multi-arm thiol were miscible and chemically inert (**Figure 2**). Both alkenes and alkynes react with thiols via radical polymerization [37] and require a visible light-activated initiator – for independent activation between the two polymer networks [26]. CaproGlu is inert to free-radical or visible light exposure, a considerable advantage for gamma sterilization and ambient shelf stability [17]. Upon photoactivation, CaproGlu emits nitrogen, resulting in a porous biorubber in both neat CaproGlu and hybrid polymer networks presented in this work. The surface porosity of implanted biomaterials is known to accelerate tissue resorption while the tunable elastic modulus of hybrid polymer network is possible in the range of 400-950 kPa in broad wavelength light activation (320-500 nm) and focused activation by visible light (405 nm) followed by crosslinking and surface adhesion activated by UVA (365 nm) light. Diacrylate/tetrathiol system has also shown crosslinking under ambient light. However, the 2-

step crosslinking might be possible with other multifunctional alkenes to produce systems with higher stability and more controlled photocuring mechanism [38].

The solvent-free hybrid network material allows for a flexible liquid-to-elastomer transition through various optical stimulation profiles. Thiol/ene crosslinking proceeds in the presence of inert and transparent CaproGlu, resulting in dynamic modulus transition from liquid to ~30 kPa (modulus) gel upon activation with visible light (405 nm). Subsequent exposure to UVA (365 nm), activates non-specific carbene insertion. The hybrid networks display a relatively broad range of shear modulus from 10-800 kPa, which can be easily tuned through both optical exposure and precursor molar ratio. The modulus near the surface (100 µm depth) of the hybrid network may allow modulus gradients to obtain matching elasticity profiles [39]. Apart from the potential control over depth of CaproGlu crosslinking, the porous surface of hybrid network (pore size: ~10 µm) is beneficial for tissue engineering strategies where neovascularization/cell migration is facilitated through interfacial porous structure of implanted scaffolds [40].

Due to the homogeneous mixing of all three components, diazirine groups are present at the surface of the hybrid network. Hybrid polymer networks result in adhesion to both dry substrates and wet collagen surfaces. Surface diazirine groups (from CaproGlu component) resulted in carbene covalent insertion onto solid polymers (PMMA and PET). This covalent insertion adheres hybrid network to polymers by reaching ~160 kPa of adhesion strength, limited by cohesive failure after applied lap shear adhesion stress. The penetration depth of diazirine photolysis was found to be in the range of 100-150 µm while the diazirines embedded within the network presumably remain unreacted. Diazirines are known to degrade into ketones, alcohols, ethers or other chemical groups [41]. Both kinetics of diazirine degradation (photolysis) and the nature of degradation products are dependent on the chemical environment [33, 41]. However, the results in this paper indicate possibility of double adhesive tape with 100 µm thickness where all diazirine groups are reacted, both within the bulk of material and at the substrate interface.

Low molar mass diazomethane precursors require precautions due to their explosive nature [42]. However, trifluoromethyl diazirine-based compounds (that lead to carbene and diazoalkane intermediates) are known for their stability with reported crosslinking activation that initiates at 110 °C [43]. CaproGlu synthesis reaction is stable (no detectable exothermic effect) at 40 °C with exceptional shelf stability even after 25 kGy gamma sterilization, ergo

the aryl-diazirine is inert to free-radical exposure and most nucleophilic functional groups—a claim few crosslinking groups hold [17, 25]. Apart from the stability of the diazirine used for polymer grafting, polycaprolactone tetrol (PCLT) precursors are available in food-grade quality and therefore present few risks towards medical devices. PCLT belongs to the platform of PCL-based biodegradable materials that are known to undergo ester hydrolysis and physiological elimination of degradation products goes through well-defined metabolic reactions such as citric acid and fatty acid pathways [69]. Polymerization of thiol/ene/CaproGlu hybrid networks, initiated by exposure to gamma irradiation, may be exploited for selective activation or depletion of acrylates without need of photoinitiators. CaproGlu would remain intact and to provide the same on-demand crosslinking / adhesion characteristics. In addition, CaproGlu can be activated with visible light (445 nm) when mixed with photocatalysts, which opens up possibilities for dual crosslinking activated by two distinct visible wavelengths [18]. Unlike NHS-grafted bioadhesives [13], grafting of carbene-generating diazirine onto liquid polycaprolactone polyols resulted in bioadhesive with non-discriminated covalent insertion to both hydrated biologically-derived surfaces and solid synthetic polymers [17]. CaproGlu is one example of the emerging carbene-based bioadhesive platform. The liquid polymer requires no refrigeration or rehydration and can be processed into ready-to-use implantable medical devices that are stable to gamma sterilization, a key attribute for industrial scale-up. CaproGlu has displayed little to no inflammation tested *in vivo* [17] and low-risk skin sensitization *in vitro* (OECD-regulated genotoxicity and sensitization tests) [25].

Thiol/ene crosslinking is available for many different polymer systems, including acrylate-grafted polysaccharides and PEG macromolecules with a wide range of molecular weights and geometries [44]. In particular, PEG diacrylate is known commercial photopolymer precursor, available in a wide range of molecular weights [45]. PEG-based polymer networks are known for their application in hybrid bioprinting technology [46] and the ester bonds aid miscibility within CaproGlu, allowing solvent-free mixing. In addition, polycaprolactone polyols (triols and tetrols) are readily available with molecular weights between 300 and 2000 Da, allowing a library of materials with various viscoelastic properties [47]. Hybrid systems mentioned herein could be extended beyond hydrophobic PCLTs. Amphiphilic formulations could be designed with dendrimers [48-50]. This extends diazirine activation method to applied voltage (Voltaglue) providing that the crosslinkers are dispersed in conductive medium. The choice of initiator would determine the wavelength of light used for free-radical

activation. For example, UV-active Igracure 2929 can be replaced with Eosin Y activated with visible light (405 nm) to crosslink acrylate-thiol systems [51]. However, it is also known that photoinitiators pose risks as toxic leachates [52]. Hybrid networks based on thiol/ene/carbene may eliminate photoinitiators through gamma initiation. Future work will explore this process to form sterile double sided adhesives.

5. CONCLUSION

Diazirine-grafted polycaprolactone (CaproGlu) can be mixed with acrylates, thiols and alkynes to form hybrid polymer networks with a high degree of control over material properties. The dual curing macromolecular systems are independently activated by visible light followed by mild UVA activation. When activated by visible light, polymer hybrid only partially crosslinks into gel-like material and remains reactive for subsequent adhesion onto solid surfaces by on-demand UVA activation of surface diazirine groups. Unselective crosslinking of diazirine-generated carbene enables chemical anchoring of double sided adhesive gels to any types of solid substrates without surface pre-treatment that is normally required for formation of interfacial heterogenous chemical bonding. These attributes of CaproGlu crosslinking formulation demonstrate multifunctional nature, both intermolecular and interfacial crosslinking, that would lead towards biomedical applications with careful selection of network components without need of solvents or photoinitiators.

CRediT authorship contribution statement

Ivan Djordjevic: Investigation, Formal analysis, Data curation, Writing – original draft. Gautama Wicaksono: Data curation, Methodology. Manisha Singh: Data curation, Methodology. Elizabeth G. Ellis: Data curation, Methodology. Maher A. Alraddadi: Data curation, Methodology. Andrew P. Dove: Conceptualization, Supervision, Writing – review & editing. Terry W.J. Steele: Conceptualization, Formal analysis, Supervision, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

T.W.J. Steele and I. Djordjevic are co-inventors of the following IP: Hygroscopic, Crosslinking Coatings and Bioadhesives; PCT/SG2018/050452. Authors declare no competing interests. CaproGlu is an abbreviation for this technology and is not trade marked.

Acknowledgements

This work is funded by Institute of Advanced Studies Birmingham Visiting Fellowship, 'Strong Elastomeric Bioadhesives Towards Tendon Repair'; Ministry of Education Tier 1 Grant RT07/20: Fiber-optic orthopaedic implants for bone-implant adhesion, Ministry of Education Tier 2 Grant (MOE2018-T2-2-114): CaproGlu, Double sided wet-tissue adhesives, NTUitive POC (Gap) Fund NGF/2018/05: Aesthetic Applications of CaproGlu Bioadhesives, and A*STAR IAF PP Grant (H19/01/a0/0II9): CathoGlu Bioadhesives-preventing catheter extravasation and skin infections.

References:

[1] S. Czarnecki, T. Rossow, S. Seiffert, Hybrid Polymer-Network Hydrogels with Tunable Mechanical Response, Polymers 8(3) (2016) 82.
[2] M.A. Haque, T. Kurokawa, J.P. Gong, Super tough double network hydrogels and their application as biomaterials, Polymer 53(9) (2012) 1805-1822.
[3] J.P. Gong, Why are double network hydrogels so tough?, Soft Matter 6(12) (2010) 2583-2590.
[4] H. Yuk, T. Zhang, S. Lin, G.A. Parada, X. Zhao, Tough bonding of hydrogels to diverse non-porous surfaces, Nature materials 15(2) (2016) 190-196.
[5] J. Deng, Z. Dai, J. Yan, M. Sandru, E. Sandru, R.J. Spontak, L. Deng, Facile and solvent-free fabrication of PEG-based membranes with interpenetrating networks for CO2 separation, Journal of Membrane Science 570-571 (2019) 455-463.
[6] M. Sangermano, W. Carbonaro, G. Malucelli, A. Priola, UV-Cured Interpenetrating Acrylic-Epoxy Polymer Networks: Preparation and Characterization, Macromolecular Materials and Engineering 293(6) (2008) 515-520.
[7] V. Granskog, O.C.J. Andrén, Y. Cai, M. González-Granillo, L. Felländer-Tsai, H. von Holst, L.-A. Haldosen, M. Malkoch, Linear Dendritic Block Copolymers as Promising Biomaterials for the Manufacturing of Soft Tissue Adhesive Patches Using Visible Light Initiated Thiol–Ene Coupling Chemistry, Advanced Functional Materials 25(42) (2015) 6596-6605.
[8] A.H.C. Anthis, X. Hu, M.T. Matter, A.L. Neuer, K. Wei, A.A. Schlegel, F.H.L. Starsich, I.K. Herrmann, Chemically Stable, Strongly Adhesive Sealant Patch for Intestinal Anastomotic Leakage Prevention, Advanced Functional Materials 31(16) (2021) 2007099.
[9] L. Zeng, J. He, Y. Cao, J. Wang, Z. Qiao, X. Jiang, L. Hou, J. Zhang, Tissue-adhesive and highly mechanical double-network hydrogel for cryopreservation and sustained release of anti-cancer drugs, Smart Materials in Medicine 2 (2021) 229-236.
[10] H.Y. Jung, P. Le Thi, K.-H. HwangBo, J.W. Bae, K.D. Park, Tunable and high tissue adhesive properties of injectable chitosan based hydrogels through polymer architecture modulation, Carbohydrate Polymers 261 (2021) 117810.
[11] S. Nam, D. Mooney, Polymeric Tissue Adhesives, Chemical Reviews (2021).
[12] X. Chen, H. Yuk, J. Wu, C.S. Nabzdyk, X. Zhao, Instant tough bioadhesive with triggerable benign detachment, Proceedings of the National Academy of Sciences 117(27) (2020) 15497-15503.

[13] H. Yuk, C.E. Varela, C.S. Nabzdyk, X. Mao, R.F. Padera, E.T. Roche, X. Zhao, Dry double-sided tape for adhesion of wet tissues and devices, Nature 575(7781) (2019) 169-174.
[14] M.H. Turabee, T. Thambi, D.S. Lee, Development of an Injectable Tissue Adhesive Hybrid Hydrogel for Growth Factor-Free Tissue Integration in Advanced Wound Regeneration, ACS Applied Bio Materials 2(6) (2019) 2500-2510.
[15] A. Shagan, W. Zhang, M. Mehta, S. Levi, D.S. Kohane, B. Mizrahi, Hot Glue Gun Releasing Biocompatible Tissue Adhesive, Advanced Functional Materials 30(18) (2020) 1900998.
[16] N. Oliva, S. Shitreet, E. Abraham, B. Stanley, E.R. Edelman, N. Artzi, Natural Tissue Microenvironmental Conditions Modulate Adhesive Material Performance, Langmuir 28(43) (2012) 15402-15409.
[17] I. Djordjevic, O. Pokholenko, A.H. Shah, G. Wicaksono, L. Blancafort, J.V. Hanna, S.J. Page, H.S. Nanda, C.B. Ong, S.R. Chung, A.Y.H. Chin, D. McGrouther, M.M. Choudhury, F. Li, J.S. Teo, L.S. Lee, T.W.J. Steele, CaproGlu: Multifunctional tissue adhesive platform, Biomaterials 260 (2020) 120215.
[18] I. Djordjevic, G. Wicaksono, I. Šolić, J. Singh, T.S. Kaku, S. Lim, E.W.J. Ang, L. Blancafort, T.W.J. Steele, Rapid Activation of Diazirine Biomaterials with the Blue Light Photocatalyst, ACS Applied Materials & Interfaces (2021).
[19] L.J. Macdougall, V.X. Truong, A.P. Dove, Efficient In Situ Nucleophilic Thiol-yne Click Chemistry for the Synthesis of Strong Hydrogel Materials with Tunable Properties, ACS Macro Letters 6(2) (2017) 93-97.
[20] C.E. Hoyle, C.N. Bowman, Thiol–ene click chemistry, Angewandte Chemie International Edition 49(9) (2010) 1540-1573.
[21] A.E. Rydholm, C.N. Bowman, K.S. Anseth, Degradable thiol-acrylate photopolymers: polymerization and degradation behavior of an in situ forming biomaterial, Biomaterials 26(22) (2005) 4495-4506.
[22] P.M. Kharkar, M.S. Rehmann, K.M. Skeens, E. Maverakis, A.M. Kloxin, Thiol-ene click hydrogels for therapeutic delivery, ACS biomaterials science & engineering 2(2) (2016) 165-179.
[23] Y. Dong, A.O. Saeed, W. Hassan, C. Keigher, Y. Zheng, H. Tai, A. Pandit, W. Wang, "One-step" Preparation of Thiol-Ene Clickable PEG-Based Thermoresponsive Hyperbranched Copolymer for In Situ Crosslinking Hybrid Hydrogel, Macromolecular rapid communications 33(2) (2012) 120-126.
[24] K. Jin, N. Wilmot, W.H. Heath, J.M. Torkelson, Phase-Separated Thiol–Epoxy–Acrylate Hybrid Polymer Networks with Controlled Cross-Link Density Synthesized by Simultaneous Thiol–Acrylate and Thiol–Epoxy Click Reactions, Macromolecules 49(11) (2016) 4115-4123.
[25] I. Djordjevic, G. Wicaksono, I. Solic, T.W. Steele, In Vitro Biocompatibility of Diazirine-Grafted Biomaterials, Macromolecular Rapid Communications 41(21) (2020) 2000235.
[26] B. Steyrer, P. Neubauer, R. Liska, J. Stampfl, Visible light photoinitiator for 3D-printing of tough methacrylate resins, Materials 10(12) (2017) 1445.
[27] J.C. Worch, C.J. Stubbs, M.J. Price, A.P. Dove, Click Nucleophilic Conjugate Additions to Activated Alkynes: Exploring Thiol-yne, Amino-yne, and Hydroxyl-yne Reactions from (Bio)Organic to Polymer Chemistry, Chemical Reviews 121(12) (2021) 6744-6776.
[28] L. Maleki, U. Edlund, A.-C. Albertsson, Synthesis of full interpenetrating hemicellulose hydrogel networks, Carbohydrate polymers 170 (2017) 254-263.
[29] N.B. Cramer, J.P. Scott, C.N. Bowman, Photopolymerizations of thiol− ene polymers without photoinitiators, Macromolecules 35(14) (2002) 5361-5365.

[30] A. Gambi, M. Winnewisser, J.J. Christiansen, The infrared spectrum of diazirine:. Rovibrational analysis of the ν3 fundamental, Journal of Molecular Spectroscopy 98(2) (1983) 413-424.
[31] R. Cataliotti, A. Poletti, G. Paliani, A. Foffani, Infrared Spectrum and Vibrational Assignment of Diazocyclopentadiene-h4 and-d4, Zeitschrift für Naturforschung B 27(8) (1972) 875-878.
[32] M.-A. Tehfe, J. Lalevée, S. Telitel, E. Contal, F.d.r. Dumur, D. Gigmes, D. Bertin, M. Nechab, B. Graff, F. Morlet-Savary, Polyaromatic structures as organo-photoinitiator catalysts for efficient visible light induced dual radical/cationic photopolymerization and interpenetrated polymer networks synthesis, Macromolecules 45(11) (2012) 4454-4460.
[33] I. Djordjevic, G. Wicaksono, I. Solic, T.W.J. Steele, Diazoalkane decay kinetics from UVA-active protein labelling molecules: Trifluoromethyl phenyl diazirines, Results in Chemistry 2 (2020) 100066.
[34] P. Luan, G.S. Oehrlein, Characterization of Ultrathin Polymer Films Using p-Polarized ATR-FTIR and Its Comparison with XPS, Langmuir 35(12) (2019) 4270-4277.
[35] I. Djordjevic, N. Choudhury, N. Dutta, S. Kumar, Poly(octanediol-co-(citric acid)-co-(sebacic acid)) elastomers: novel bio-elastomers for tissue engineering, Polymer International 60 (2011) 333-343.
[36] I. Djordjevic, N.R. Choudhury, N.K. Dutta, S. Kumar, E.J. Szili, D.A. Steele, Polyoctanediol Citrate/Sebacate Bioelastomer Films: Surface Morphology, Chemistry and Functionality, Journal of Biomaterials Science, Polymer Edition 21(2) (2010) 237-251.
[37] O. Türünç, M.A.R. Meier, A novel polymerization approach via thiol-yne addition, Journal of Polymer Science Part A: Polymer Chemistry 50(9) (2012) 1689-1695.
[38] M. Sahin, S. Ayalur-Karunakaran, J. Manhart, M. Wolfahrt, W. Kern, S. Schlögl, Thiol-Ene versus Binary Thiol–Acrylate Chemistry: Material Properties and Network Characteristics of Photopolymers Advanced Engineering Materials 19(4) (2017) 1600620.
[39] H. Saraf, K.T. Ramesh, A.M. Lennon, A.C. Merkle, J.C. Roberts, Mechanical properties of soft human tissues under dynamic loading, Journal of Biomechanics 40(9) (2007) 1960-1967.
[40] Q.L. Loh, C. Choong, Three-Dimensional Scaffolds for Tissue Engineering Applications: Role of Porosity and Pore Size, Tissue Engineering Part B: Reviews 19(6) (2013) 485-502.
[41] A.B. Kumar, J.D. Tipton, R. Manetsch, 3-Trifluoromethyl-3-aryldiazirine photolabels with enhanced ambient light stability, Chemical Communications 52(13) (2016) 2729-2732.
[42] S.P. Green, K.M. Wheelhouse, A.D. Payne, J.P. Hallett, P.W. Miller, J.A. Bull, Thermal Stability and Explosive Hazard Assessment of Diazo Compounds and Diazo Transfer Reagents, Organic Process Research & Development 24(1) (2020) 67-84.
[43] M.L. Lepage, C. Simhadri, C. Liu, M. Takaffoli, L. Bi, B. Crawford, A.S. Milani, J.E. Wulff, A broadly applicable cross-linker for aliphatic polymers containing C–H bonds, Science 366(6467) (2019) 875-878.
[44] G.M. Cruise, D.S. Scharp, J.A. Hubbell, Characterization of permeability and network structure of interfacially photopolymerized poly(ethylene glycol) diacrylate hydrogels, Biomaterials 19(14) (1998) 1287-1294.
[45] A. Bagheri, J. Jin, Photopolymerization in 3D Printing, ACS Applied Polymer Materials 1(4) (2019) 593-611.
[46] W. Li, L.S. Mille, J.A. Robledo, T. Uribe, V. Huerta, Y.S. Zhang, Recent Advances in Formulating and Processing Biomaterial Inks for Vat Polymerization-Based 3D Printing, Advanced Healthcare Materials 9(15) (2020) 2000156.

[47] W.J. Steele, Terry ; Djordjevic, I. , Hygroscopic, Crosslinking Coatings and Bioadhesives, https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2019050479 PCT/SG2018/050452 (2019).
[48] M. Singh, C.S. Yin, S.J. Page, Y. Liu, G. Wicaksono, R. Pujar, S.K. Choudhary, G.U. Kulkarni, J. Chen, J.V. Hanna, R.D. Webster, T.W.J. Steele, Synergistic Voltaglue Adhesive Mechanisms with Alternating Electric Fields, Chemistry of Materials 32(6) (2020) 2440-2449.
[49] J. Ping, F. Gao, J.L. Chen, R.D. Webster, T.W. Steele, Adhesive curing through low-voltage activation, Nature communications 6(1) (2015) 1-9.
[50] G. Feng, I. Djordjevic, V. Mogal, R. O'Rorke, O. Pokholenko, T.W.J. Steele, Elastic Light Tunable Tissue Adhesive Dendrimers, Macromolecular Bioscience 16(7) (2016) 1072-1082.
[51] A. Fu, K. Gwon, M. Kim, G. Tae, J.A. Kornfield, Visible-light-initiated thiol–acrylate photopolymerization of heparin-based hydrogels, Biomacromolecules 16(2) (2015) 497-506.
[52] R. Taschner, P. Gauss, P. Knaack, R. Liska, Biocompatible photoinitiators based on poly-α-ketoesters, Journal of Polymer Science 58(2) (2020) 242-253.