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1	Fixation of transparent bone pins with photocuring biocomposites
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ABSTRACT

22 Bone fractures are in need of rapid fixation methods, but current strategies are limited to 23 metal pins and screws, which necessitate secondary surgeries upon removal. New techniques 24 are sought to avoid surgical revisions, while maintaining or improving fixation speed. Herein, 25 a method of bone fixation is proposed with transparent biopolymers anchored in place via 26 light-activated, biocomposites based on expanding CaproGlu bioadhesives. The transparent 27 biopolymers serve as a UV light guide for the activation of CaproGlu biocomposites that 28 results in evolution of molecular nitrogen (from diazirine photolysis), simultaneously 29 expanding the covalently crosslinked matrix. Osseointegration additives of hydroxyapatite or 30 Bioglass 45S5 yield a biocomposite matrix with increased stiffness and pull-out strength. The 31 structure-property relationships of UV joules dose, pin diameter, and biocomposite additives 32 are assessed with respect to apparent viscosity, shear modulus, spatiotemporal pin curing, and 33 lap-shear adhesion. Finally, a model system is proposed based on *ex vivo* investigation with bone tissue for the exploration and optimization of UV-active transparent biopolymer fixation. 34 35 **KEY WORDS:** Bone implant fixation, polymer bioadhesive, bone biocomposite, 36 hydroxyapatite, Bioglass.

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43 **1. INTRODUCTION**

44 Bone fractures are rising globally with a projected 7.5 million clinical cases by 2025 in USA 45 and Europe, in part due to an ageing population and active lifestyles. Despite the advances in 46 orthopaedic surgery, the rate of surgical revision and non-union fracture is alarmingly high: 47 10 to 50% of cases end up with failures characterized by revision surgery or non-union 48 fracture.¹ One of the major reasons for unsuccessful bone tissue repair is suppression of blood 49 supply to the tissue that in most cases results in non-union of the bone due to osteonecrosis, bone resorption and ischemia.¹ Biomaterials design for bone regeneration requires 50 51 biomimetic approach from nano- to micro-scale. Properties of composite biomaterials like 52 biocompatibility, degradation rate and the type/characteristics of bioactive inclusions 53 embedded in the matrix have to be tailored to allow osseoconductivity in initial stage of healing.² Bone remodelling (i.e. healing) is a multi-phase process where biomechanical 54 properties undergo dynamic change correlated to bone mineral density³⁻⁵ as Young's modulus 55 for human granulation tissue is ~0.5 MPa and rises up to 20 GPa for mature bone.⁶ The 56 57 variation of callus mechanical moduli through the multi-phase healing process can be in the range of 20-6000 MPa.⁷ In case of implant-assisted fracture repair, the callus formation 58 59 begins at the implant surface; the tissue formation is highly responsive to interfacial / mechanical properties of the implant and the process is known as contact osteogenesis.⁸ Due 60 61 to complexity of bone tissue, the development of biomaterials that would mimic bone 62 biomechanics and structure to facilitate fracture healing still presents an unmet clinical need.⁹ 63 Bone fixation screws and pins have been employed in clinical practice for decades. Apart from standard metallic implants,¹⁰ bone fixation is also performed with biodegradable plates 64 and screws that offer less invasive approaches.¹¹⁻¹² Recently reported clinical trials indicate 65 66 that bioresorbable polymer (polycaprolactone, PCL; poly(lactic acid), PLA) and permanent implants (metallic) are equally safe and effective for non-load-bearing bone reconstruction.¹³ 67 68 Resorbable implants eliminate the need for secondary surgery which is required for metallic

implants after tissue healing is completed. The bone microenvironment repair relies on
sensitive bone / implant interface¹⁴ that is disrupted by compression (force-mediated) fixation
that causes peri-implant bone damage up to 0.9 mm in radial direction from the implant.¹⁵
This issue compromises the primary implant stability and should be addressed by noninvasive, biodegradable fixation formulations that combine principles of surgical adhesion
and tissue engineering.

75 Over the recent years we have developed a diazirine-grafted polycaprolactone polyol (named 76 CaproGlu) hydrophobic, liquid bioadhesive that can be mixed with bone mineral hydroxyapatite to yield viscous liquid biocomposite (Scheme 1A,B).¹⁶⁻¹⁷ The CaproGlu 77 platform is based on polycaprolactone triol or tetrol (PCLT) grafted with 78 trifluoromethylphenyl diazirine as a surgical adhesive.¹⁸ UV activation of diazirine generates 79 80 carbene that rapidly crosslinks with release of molecular nitrogen that causes a >200% volumetric expansion and pressures that could exceed 200 kPa (Scheme 1C).¹⁹ Carbene 81 82 covalently inserts non-specifically causing both internal and interfacial crosslinking that 83 immobilizes bone implants (Scheme 1D).¹⁷ Due to known biodegradation and 84 biocompatibility of polycaprolactone biomaterials, CaproGlu-based biocomposite bone 85 fixation formulation presents a new strategy for fixation of transparent bone pins crosslinked 86 with low energy UV light. To the best of the authors' knowledge, there has been no prior 87 research on utilizing photoactive, polycaprolactone-based biocomposite that mediates non-88 invasive fixation of light-activated bone pins.

89 In this paper for the first time, we describe the bone fixation with UV-active bone

90 biocomposite based on bioactive particles, namely hydroxyapatite (both micro- and nano-

91 particles) and glass microparticles. CaproGlu biocomposite is activated on-demand via a

92 novel fibre-optic pin (polymethyl methacrylate; PMMA) platform (Scheme 1C,D).

93 Transparent PMMA is used only as a model that simulates bone fixation by transparent,

commercially available polylactide pins (e.g. Inion CPSTM).²⁰ Described bone biocomposite 94 95 integrates tissue engineering approach with bone implant (pin) fixation where the 96 biocomposite serves as a temporary support that evenly transfers stress from the healing 97 tissue to the immobilized pin. The design of fibre-optic orthopaedic implant is directed by the 98 following key requirements: (i) Biocomposite liquid conforms to the drilled gap, where 99 activation causes volume expansion that solidifies and fills complex voids and geometries; 100 (ii) Biocomposite is produced from biodegradable materials that induce osseointegration; (iii) 101 Non-exothermic *in situ* crosslinking by exposure to non-invasive, low energy UV light with 102 adhesion properties that allow flexibility towards specific bone reconstructive surgery; and 103 (iv) Transparent fibre-optic pin made from PMMA allowing delivery of UV light that 104 crosslinks CaproGlu component of biocomposite.







106 Scheme 1. Demonstration of light activation of transparent bone pins with the aid of CaproGlu biocomposite formulation. (A) Composite is produced by mixing diazirine-grafted 107 108 polycaprolactone (CaproGlu; branched polyol with diazirine end-groups, symbolically 109 presented as triangle shapes) with solid additives: Bioglass (45S5) and hydroxyapatite. (B) 110 Representative paste-like biocomposite formulation prior to UV activation. (C) UV light (365 111 nm) transmitted through light-transparent PMMA pin activates diazirine groups and turn 112 them into carbene for subsequent crosslinking of biocomposite at PMMA-bone interface; 113 diazirine photolytic degradation produces molecular nitrogen bubbles that expand 114 biocomposite and cause locking pressure for pin fixation. (D) Ex-vivo experimental setup to 115 investigate light activation of transparent bone pins with the aid of expendable, UV-active 116 biocomposite for mechanical locking at the bone / pin interface.

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It is hypothesized that the thickness of bone-implant (pin) interface should be kept below 0.2 mm in order to ensure sufficient light transmission and UVA energy distribution and to generate sufficient interfacial crosslinking for compressive stresses that are sustained through the biocomposite matrix. The results herein present the preliminary investigations of the model system towards developing of new methods of bone fixation with non-metallic implants.

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125 2. MATERIALS AND METHODS

126 **2.1** Synthesis of CaproGlu bioadhesive and biocomposite preparation methodology

127 The detailed synthesis procedure of CaproGlu has been described in a previous publication.¹⁶

128 In brief, polycaprolactone triol (CAPA 3031, 300 Da, Perstorp, Sweden) and diazirine-

129 bromide (TCI, Japan) are mixed in PCLT/diazirine molar ratio of 1/1 to yield 50% diazirine

130 conjugation. Reactants are dissolved in dioxane and allowed to react in the presence of silver

131 oxide (Ag₂O) and molecular sieve for 72 h at room temperature under nitrogen atmosphere.

132 Filtered product is precipitated in deionized water and centrifuged; the water-dioxane 133 supernatant is discarded and the PCLT-D conjugate product (viscous pale-vellow transparent 134 liquid) is further washed 3 times with water and centrifuged. PCLT-D formulations are lyophilized for 24 h and characterized with ¹H NMR to calculate the conjugation (grafting) 135 percentage (Bruker Avance; 400 MHz). Refractive index (RI) of purified CaproGlu is 136 137 measured by Mettler Toledo portable refractometer 30GS at room temperature, and RI estimation of CaproGlu bioadhesive composites are performed using Lorentz-Lorenz 138 equation for rule of mixtures.²¹ CaproGlu bioadhesive composites are prepared by directly 139 140 mixing the additive powder into the liquid CaproGlu formulation. Hydroxyapatite 141 nanopowder (hereafter referred as HNP), <200 nm particle size are purchased from Sigma 142 Aldrich. Hydroxyapatite coarse powder (hereafter referred as HMP), ultrapure grade ($10 \pm$ 143 2.0 µm particle size) were purchased from Sigma Aldrich. Bioglass 45S5 powder, <32 nm 144 particle size (hereafter referred as BG), is synthesized by melt-quenching process followed by milling and sieving, as previously described.²² 145

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5 2.2 Photorheometry measurements

147 Rheometry measurements are conducted with Anton Paar Physica MCR 102 rheometer fitted 148 with UV transparent glass plate. The applied UV intensity (365 nm) is calibrated to 100 mW cm⁻² with an IL 1400 Radiometer through handheld UV LEDs or by Thorlabs SOLIS-365C 149 150 High Power LED. Rheology tests are performed using parallel plate geometry with probe diameter 10 mm, on 0.1, 0.2 and 0.4 mm measuring gaps. Apparent viscosity is evaluated via 151 rotational rheology with shear rate 10 s⁻¹ for 60 seconds. The storage modulus (G') and loss 152 153 modulus (G") are evaluated during dynamic oscillatory rheology with amplitude of 1% and 154 frequency of 10 Hz for 160 seconds; UV irradiation is performed between t = 30 s and t =155 130 s to achieve total UV dose of 10 J. Amplitude sweep of 1-1000% shear strain are

performed onto the cured sample to evaluate yield stress and strain.

157 **2.3 PMMA Optical Fiber and surface area evaluation**

158 Optical fiber-grade PMMA rods of diameters 1 mm, 1.5 mm, 2 mm, and 3 mm were 159 purchased from Edmund Optics Pte Ltd. The fibres are cut into 3 cm, 5 cm, or 7 cm lengths 160 and their ends are polished using 120-grit sandpaper. Cured biocomposites on the optical 161 fibers are taken for image analysis using ImageJ software. The images are split into RGB channels and thresholded to identify and count the ratio of pixels representing yellow-cured 162 163 biocomposite against the total area. For the purpose of analysis, the cured area is split into 10 164 identical lengths along the direction of UV curing and the cured pixel ratio is calculated per 165 section. The resulting % cured versus UV curing distance is fitted according Gauss 166 probability distribution.

167 **2.4 Shear adhesion test on ex vivo bovine femur bones**

Bovine femur cortical bone samples are prepared at length of ~4 cm. Holes are drilled 168 through the outer cortical bone with diameter of 3.4 mm; only 3 mm diameter optical fibers 169 170 are tested, and the extra 0.4 mm allows ~0.2 mm thickness of biocomposite coating. 171 Approximately 15 mg of adhesive is applied at 2.5 cm of the fiber length then inserted into 172 drilled hole, and any excess adhesive outside the bone is removed. UV is applied at intensity 100 mW cm⁻² for 5 minutes (30 J) through the fibre optic; excess dose is required to 173 compensate for irregular curing efficiencies. Load is applied to the photocured PMMA pin in 174 175 the axial direction, and the shear stress calculated with respect to surface area and 0.2 mm 176 coating thickness with the aid of a modified tensile tester (Chatillon Force Measurement Products, USA) at the strain rate of 3 mm min⁻¹ with 50 N capacity force cell ($\pm 0.25\%$ 177 178 resolution).

179 **2.5 SEM/EDX analysis**

180 CaproGlu is manually mixed with BG, HNP and HMP particles (10% w/w; solid/CaproGlu) 181 and applied in thin layer (~50 mg) between PET sheets (sandwich structure) and cured with 182 10 J of UV. PETs are separated with cured CaproGlu composite on both sheets. Composite + 183 PET is cut in 2 x 2 mm squares for SEM/EDX analysis with JEOL 5500LV electron 184 microscope. Samples are subjected to platinum coating (90 s, chamber pressure <5 Pa at 20 185 mA). Images are obtained by JSM 5510 SEM at an acceleration voltage of 5-20 kV and a working distance of 15 mm. The composition of the composites is analysed by EDX using an 186 187 Oxford Inca 200 EDX detector under low Vacuum and a measuring time of 300 s. Pore size 188 distribution analysis is performed with ImageJ software by measuring the pore sizes recorded over the 7.5 x 10^{-3} cm² area. The SEM images are thresholded to outline the porous 189 190 morphology and the resulting pore sizes are measured using the built-in particle analysis 191 function.

192 **2.6 Data analysis**

193 All data processing, plotting and curve fitting are performed using OriginPro 2020 software.

194 SEM Image analysis are performed using Fiji ImageJ 1.52. All biocomposite

195 characterizations are performed in triplicate. One-way ANOVA statistical analysis is

196 performed by Tukey's comparison and P < 0.05 was set as significant in all the tests.

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198 **3. RESULTS AND DISCUSSION**

Nine biocomposite formulations (3 additives at 3 concentrations each) are evaluated for light activated fixation of transparent plastic implants. Several inorganic additives are available for inducing osseointegration, however we have limited the structure property relationship parameters to two different types of inorganic particles: hydroxyapatite and silica-based bioactive glass²³⁻²⁵ (BG; 45S5 composition). In order to demonstrate the relationship between

204 mechanical properties and the size of inorganic solid phase, we report the investigation of 205 particles in following sizes: hydroxyapatite nanoparticles and microparticles (HNP < 200 nm 206 and HMP = $10 \pm 2.0 \,\mu\text{m}$, respectively) and bioactive glass (BG < $32 \,\mu\text{m}$). Additive loading is 207 hypothesized to improve the adhesive stiffness and shear adhesion strength, so each additive 208 formulation is evaluated from 5 - 20% w/w loading. Below 5% observed no additional 209 increase in shear modulus (vs. neat CaproGlu) and above 20% yield viscous pastes with 210 viscosity above 10 Pa.s (Fig. 1). All biocomposites are evaluated by real-time 211 photorheometry, in a multi-step protocol that yields a robust analysis of uncured liquid, joule-212 dependent viscoelasticity, gelation time, and strain-dependent shear modulus. The latter 213 correlates to lap shear adhesion assuming cohesive failure. Each photorheometry experiment 214 is done in triplicate. Three thickness profiles (0.1, 0.2, and 0.4 mm) evaluate effects of UV 215 light attenuation through the biocomposite for total of (9 biocomposites x 3 thickness profiles 216 x triplicates) 81 independent rheometer evaluations. Four diameters of UV transparent polymethacrylate (PMMA) are evaluated as light-transparent pins. Optical fiber-grade 217 218 poly(methyl methacrylate) PMMA is required for sufficient UV transparency (hobby grades 219 were UV opaque, data not shown). PMMA serves as a model bone pin material, as it is UV 220 transparent, readily available, and having an elastic modulus slightly softer than cortical bone at 3 GPa.^{26 27} In order to assess the lap shear adhesion at the bone implant interface, fresh ex 221 222 vivo bovine femur bones are drilled at 3.4 mm diameter (pin diameter + 0.4 mm) and excess 223 biocomposite is applied into a bone pin mimic, inserted into the hole. As the adhesive 224 composite requires UV activation, the optical fiber-grade PMMA serves as the model 225 transparent pin material.

226 **3.1 Real-time photorheometry of composites**

Biocomposites of liquid CaproGlu and three inorganic additives are prepared in three weight
ratios. A multistep photorheometry protocol evaluates the biocomposites at all stages of the

229 curing from liquid, UV-induced gelation, to determining the strain-dependent modulus and 230 maximum shear strain (prior to ex vivo experiment) with the following framework; i) parallel 231 plate rotational shear (η_{app} UV off, 60 s), ii) oscillatory (G"/G' for 30 s UV off + 100 s UV 232 on + 30 s UV off), iii) followed by an amplitude sweep (G''/G' from 1 - 1000%, UV off). 233 The photorheometry setup is shown in Fig. 1A, with UV source below the biocomposite 234 sample placed on a quartz surface. Fig. 1B shows pictures of the various composites tested: pure CaproGlu is translucent while CaproGlu mixed with BG, HNP, and HMP additives are 235 236 opaque from particle light scattering. Fig. 1C shows the apparent viscosity as function of 237 additive concentration, with values listed in Table 1.



Figure 1. Photorheometry experimental setup: (A) Schematics presentation of rheometer
fitted with light-transparent base with outlined dimension parameters. (B) Close-up pictures,
from left to right: pure CaproGlu, CaproGlu + 20% BG, CaproGlu + 20% HNP, CaproGlu +
20% HMP. (C) Summary of viscosity values measured for biocomposites as a function of
additive concentration in comparison to pure CaproGlu (control; 0%).

Table 1. Apparent viscosity (Pa.s) of composites: shear ^{rate} 10 s⁻¹; base-probe thickness 0.2
mm.

Additive concentration	Bioglass 45S5 (BG)	Hydroxyapatite nanopowder (HNP)	Hydroxyapatite coarse powder (HMP)
0% (control)	5.55 ± 0.37		

5%	7.60 ± 0.36	7.10 ± 0.54	6.56 ± 0.75
10%	8.29 ± 0.70	8.54 ± 0.51	9.22 ± 1.42
20%	11.1 ± 0.40	11.3 ± 0.54	26.1 ± 3.21

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248 CaproGlu by itself (no additives) has average viscosity of 5 Pa.s. Inclusion of both BG and 249 HNP additives up to 10% still results in viscosity lower than 10 Pa.s, and subsequent addition 250 of solid particles increase the viscosity significantly. In particular, addition of 20% HMP 251 displays considerable increases, likely surpassing the contact percolation threshold. Most of the uncured formulations display aspects of a Bingham plastic and are able to coat surfaces 252 253 with thickness greater than 0.2 mm under the force of gravity. 254 Photorheometry is performed using 365 nm wavelength (defined here as UV light) at 255 intensity of 100 mW.cm⁻² for 100 seconds, for a total dose of 10 J.cm⁻². Before UV curing, the sample is pre-sheared for 30 seconds under oscillatory rheometry, which disrupts any 256 257 structures, placing the biocomposite in viscous liquid state where G'' > G'. During UV 258 exposure, CaproGlu crosslinks, evidenced by an increase in G' (storage modulus). The 259 sample turns from viscous liquid to viscoelastic solid, represented by gelation point G' = G''260 (see Fig. 2A): an irreversible transition from liquid to elastomeric material consistency. After curing, the biocomposites are crosslinked and G' >> G". Fig. 2A shows a representative plot 261 262 of G" and G' versus curing time, comparing the properties of pure CaproGlu vs CaproGlu 263 with 20% BG additive, at 0.1 mm thickness. Fig. 2B displays a comparison of all three 264 additives at 20% loading, 0.2 mm thickness. An increase of G' values with BG microparticles

after curing as a function of loading is presented in **Fig. 2C** and a plot of G' vs. thickness for

BG, HNP and HMP is shown in **Fig. 2D**. **Table 2** lists complete values of G' after 10 J of UV

267 curing. In addition, the process of crosslinking CaproGlu generates the maximum force of

268 expansion which can be detected by the rheometer probe (Table S1). The values are

dependent on the base-probe distance and the maximum recorded force is 52 ± 6 kPa for 0.1 mm distance. The expansion force drops for an order of magnitude with increase of distance to 0.4 mm (**Table S1**). Even at the maximum value, the expansion force caused by CaproGlu crosslinking reaction is significantly lower than rupture stress measured for adult cranial human bone (100 MPa order of magnitude).²⁸



Figure 2. Photorheological properties of CaproGlu biocomposite formulations: (**A**) Plot of biocomposite photocuring showing the evolution of G' and G'' versus UV curing time, representative for pure CaproGlu vs Caproglu + 20% BG. (**B**) Comparison of G' after curing as function of additive type, representative for 20% (w/w) loading and 0.2 mm probe-base gap. (**C**) Comparison of G' after curing as function of additive loading, representative for BG and 0.2 mm thickness. (**D**) Comparison of G' after curing as a function of base-probe thickness, representative for BG at 20% (w/w) loading.

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Measurement thickness	Additive concentration (w/w)	Bioglass 45S5 (BG)	Hydroxyapatite nanopowder (HNP)	Hydroxyapatite coarse powder (HMP)
	0 %		155 ± 1.75	
0.1 mm	5 %	171 ± 35.0	172 ± 1.95	138 ± 24.9
0.1 1111	10 %	241 ± 35.9	191 ± 8.0	176 ± 27.6
	20 %	247 ± 12.9	250 ± 17.1	467 ± 22.1
	0 %		64.4 ± 0.93	I
0.2 mm	5 %	99.6 ± 3.89	167 ± 14.6	66.3 ± 32.5
0.2 mm	10 %	113 ± 24	199 ± 12.6	112 ± 13.8
	20 %	142 ± 8.29	252 ± 12.2	325 ± 32
	0 %		49.5 ± 3.65	
0.4 mm	5 %	31.4 ± 1.58	54.1 ± 0.22	48.6 ± 6.67
0.4 11111	10 %	74.6 ± 3.56	61.2 ± 4.51	37.6 ± 2.45
	20 %	75.0 ± 8.89	67.3 ± 5.26	16.5 ± 5.02

Table 2. Values of G' (storage modulus; kPa) after photocuring at total dose of 10 J.cm⁻².

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Note that the HMP microparticles appear to have the highest light attenuation as judged by G' from 0.1 to 0.4 thickness. The rheometer probe evaluates the biocomposite surface with the least amount of light exposure. Taken together, the results suggest that thickness should be kept at 0.2 mm or smaller in order to limit gradients. Gelation point is reached within first 10 seconds of UV curing for sample thickness of 0.1 mm, up to 34 s for 0.2 mm, and 82 s for 0.4 mm (*Supplementary information:* **Fig. S1-S3**). It is shown that osseointegration additives can improve modulus and yield stress of CaproGlu without compromising gelation time/ gelationdose, therefore granting user control on the application of the adhesive.

289 Performing amplitude sweep on the UV-cured composites allows to plot a dynamic stress vs 290 strain plot as shown in Fig. 3A, representative for pure CaproGlu vs CaproGlu with 20% BG 291 additive, at 20% loading. Fig. 3B displays the comparison for additives at 20% loading, 0.2 292 mm thickness. Addition of BG up to 20% by weight greatly increases the yield stress, from 293 16 kPa to 58 kPa, while addition of HMP increases it up to 95 kPa. Additives loading 294 improves stress at break, representative for BG at 0.2 mm thickness (Fig. 3C). The stress at 295 yield point (break) decreases with sample thickness, as shown in Fig. 3D for all additives 296 used in experiments. The complete values of stress at break are listed in Table 3. This points 297 to evidence of decreasing the effectiveness of UV curing with increasing thickness.



- 299 Figure 3. Rheological amplitude sweep profile of CaproGlu biocomposites: (A) Plot of
- 300 dynamic stress vs strain of photocured biocomposite, representative for pure CaproGlu
- 301 (control) vs Caproglu + 20% BG. (B) Comparison of stress at break as function of additive
- 302 type, representative for 20% (w/w) loading and 0.2 mm thickness. (C) Comparison of stress
- 303 at break as function of additive loading, representative for BG and 0.2 mm probe-base
- 304 thickness. (**D**) Comparison of stress at break as function of thickness, representative for BG at
- 305 20% (w/w) loading.

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Measurement thickness	Additive concentration (w/w)	Bioglass 45S5 (BG)	Hydroxyapatite nanopowder (HNP)	Hydroxyapatite coarse powder (HMP)
	0 %		36.4 ± 0.33	
0.1 mm	5 %	56.9 ± 6.97	112 ± 2.60	71.7 ± 16.6
0.1 1111	10 %	78.3 ± 8.65	113 ± 4.79	84.4 ± 13.1
	20 %	100 ± 9.38	127 ± 9.51	155 ± 2.93
	0 %		16.5 ± 2.11	
0.2 mm	5 %	31.2 ± 4.02	88.7 ± 3.79	40.7 ± 20.0
0.2 1111	10 %	42.0 ± 12.4	85.6 ± 5.12	56.4 ± 21.5
	20 %	57.6 ± 3.1	74.8 ± 5.14	95.4 ± 11.4
	0 %		12.1 ± 1.52	
0.4 mm	5 %	9.10 ± 0.75	20.5 ± 0.66	18.8 ± 2.18
	10 %	31.3 ± 0.56	21.8 ± 2.02	12.2 ± 1.65
	20 %	19.4 ± 2.62	21.8 ± 1.71	1.78 ± 0.75

307 **Table 3.** Shear stress (kPa) of photocured composites at yield point.

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309 3.2 Light transmission properties of PMMA optical fiber

Optical fiber-grade PMMA of different diameters 1 mm, 1.5 mm, 2 mm, and 3 mm are cut into different lengths 3 cm, 5 cm, and 7 cm. The UV LED is fitted to a custom 3D-printed adapter to direct the light onto the 3mm diameter PMMA pin. Axial and lateral intensity measurements are performed to assess pin transparency (intensity loss) and length dependent attenuation. **Fig. 4A** shows the schematics of intensity measurement setup; for measurement 315 on axial direction, the PMMA optical fiber (pin) is placed directly between the UV torch and 316 the radiometer sensor. The distance from UV source to sensor equals to the optical fiber 317 length. For lateral direction, spectrometer is placed on the side of the PMMA optical fiber. 318 The result of this axial intensity measurement is plotted as a function of optical fiber length 319 and diameter (Fig. 4B). The control values used are intensity reading through air but at different distance, and the highest intensity achieved is 20 mW.cm⁻² at 3 cm. With increasing 320 distance, the intensity reading reduces slightly. For lateral intensity measurement performed 321 322 using a spectrometer, the results are plotted as a normalized relative light unit (Fig. 4C).





Figure 4. Optical properties of PMMA pins: (A) Schematic presentation of UV intensity measurement from axial and lateral directions. (B) Results of intensity measurement over the axial direction of PMMA optical fibers (pins) as a function of distance and optical fiber diameter; control values are measurements through air (no optical fiber). (C) Results of intensity measurement over the lateral direction of PMMA optical fibers as function of

distance and optical fiber diameter. (**D**) Plot of absorbance of CaproGlu + BG at
representative wavelengths of 365 nm and 400 nm, showing light attenuation as function of
loading concentration.

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333 The results demonstrate that the longer the distance is, the difference between intensity readings are getting closer as dispersion starts taking effect. In both directions, the larger 334 335 diameter of the optical fibers used, the more effective the light transmission becomes, and 336 that in itself depends on the travel distance. Fig. 4D displays the absorbance plot of 337 biocomposite with BG, tested at 365 nm and 400 nm, showing the light attenuation as 338 function of loading concentration. Following the results above, for subsequent experiment 339 results, the PMMA optical fibers with 3 mm diameter is used. Fibre length chosen is 5 cm to 340 allow better handling of experiments.

341 **3.3** Lap shear testing on bovine bones and refractive index of CaproGlu biocomposites

342 Bovine femur cortical bones are prepared with holes of 3.4 ± 0.1 mm diameter drilled into the 343 bone. Excess biocomposites (~15 mg) are applied to 2.5 cm of the length and inserted into the bone. UV activation is performed by exposing the PMMA optical fibers with UV for 5 344 345 minutes (Fig. 5A, left). Subsequently, the cured adhesive is subjected to shear test by pushing 346 the PMMA optical fiber using a tensile tester (Fig. 5A, middle). Once the PMMA optical 347 fiber is removed, it is shown that the biocomposites are only partially cured down the length 348 of the PMMA rod, with uncured region in the middle (Fig. 5A, right). An image analysis 349 estimates the amount of surface curing through the clear to yellow biocomposite colour 350 change (Fig. 5B,C), where the yellow tint is caused by diazoalkane formation.²⁹ At the air/PMMA interface, UV light is internally reflected (42° critical angle, refractive index of 351 1.49; Fig. 5D, i). Internal reflection no longer occurs at the CaproGlu interface because 352 polycaprolactone (major constituent of CaproGlu) has refractive index of 1.46, similar to 353

354 PMMA. Diffracted UV light is therefore absorbed by the biocomposite that caused 355 crosslinking (Fig. 5D, ii), but the light flux decreases along the length, creating a gradient of 356 crosslinking as function of distance from UV source. Non-uniform crosslinking caused by 357 this effect will be addressed in future by applying more sophisticated optics than simple UVA 358 diode used as a proof of concept in this paper (Fig. 5A). Regardless of recorded non-uniform 359 light energy distribution (Fig. 5C,D) the reflection of UV on the opposite PMMA surface creates a second virtual light source (Fig. 5D, iii), which is responsible for curing from the 360 361 opposite end of PMMA fiber. This explains the Gaussian distribution of biocomposite curing 362 between real and virtual light source as seen in Fig. 5C.



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Figure 5. *Ex vivo* investigation of PMMA fixation by UV-activated CaproGlu biocomposite
formulations: (A) *Left:* UV-curing setup of composites on PMMA optical fiber surface,
inserted into holes drilled onto bovine femur bone. *Middle:* setup of shear test on bovine
femur bone; the fiber optic (pin) is pushed downwards, and the shear adhesion strength is

368 measured (the force direction is indicated with arrow). Right: the composites are cured 369 partially inside the bone. (B) Analysis of cured area using image editing software ImageJ by 370 dividing cured area into 10 segments for evaluation by ratio. (C) Cured area ratio is fitted to Gaussian distribution with R^2 value of 0.87591. (**D**) Schematic presentation for proposed 371 372 mechanism of UV curing through the PMMA fiber: (i) total internal reflection through air / 373 PMMA medium, (ii) UV is absorbed by the biocomposite, (iii) reflection from original UV 374 source cured the biocomposite from the opposite end of the pin. (E) Representative load vs 375 distance curve of the shear test; increasing load represents the shear force experienced by 376 cured biocomposite (insert: measured shear force interface). (F) Maximum force values from 377 each sample is normalized against cured biocomposite area to determine lap shear strength of 378 each biocomposite.

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380 Figure 5E shows a representative result of this experiment on a pure CaproGlu as shear force reading at the pin-bone interface contributed by cured CaproGlu versus PMMA pin 381 382 displacement, in the axial direction. As the optical fibers are sheared, load reading is 383 increased until a maximum yield point. This value is normalized towards the cured area of 384 adhesive, and the resulting value is defined as lap shear strength, listed in Fig. 5F. This ultimate shear stress value represents the adhesion (shear) strength of cured CaproGlu 385 386 composite at the pin-bone interface. Curing surface area appears to be inversely dependent on 387 the additive concentration. From 0-20% BG loading, over half the surface area is cured. 388 There is ~10% surface curing for 30% loading and no observed curing for 40-50% loading, and therefore no lap shear adhesion results are available. As BG has high refractive index of 389 390 1.55, it is hypothesized that the biocomposite resumes total internal reflection for >30%391 loading,³⁰ explaining the lack of curing. The standard deviation remains high due to the 392 irregular nature of the adhesive's photocuring behaviour between bone and pin surface. This work was inspired by previous investigations of polymer waveguides that elucidated the 393 394 structure activity relationships of deep tissue light delivery, transparent biopolymers, and

photochemical tissue bonding.³¹ With 900 J of visible irradiation, they demonstrated a 395 396 significant bonding of 2 kPa, a 5x increase over control. PMMA herein serves as the model 397 UV-transparent biopolymer-it is available in medical grades but is not considered 398 resorbable. The differential refractive index at the PMMA / air interface allows total internal 399 reflection, but this immediately changes to diffraction at the PMMA/ biocomposite interface. 400 Diffraction allows photocuring / tissue bonding of CaproGlu (up to 40 kPa), but the light flux 401 decreases along the length of the PMMA rod, thus causing insufficient crosslinking in the 402 center of the implant. Reflection of UV light on the opposite PMMA surface creates a virtual 403 light source which is responsible for curing from the opposite end of PMMA pin. It is 404 important to note that we did not observe curing with particle loading exceeding 30% BG in 405 the biocomposite. This shows that for the current design of photocuring with transparent 406 biopolymers, the differential refractive index between the PMMA pin (RI = 1.49) and the 407 biocomposite (Table 4) was sufficient to prevent diffraction – little to no light flux prevented 408 CaproGlu photocuring as evident from the lack of shear adhesion forces. This partial curing 409 causes less effective biocomposite crosslinking in the middle part of the pin; as such, the 410 current application limits to short pins where light flux can be maintained through the length of the pin.³² Ultimately, the lower crosslinking density is likely to cause faster resorption of 411 412 polycaprolactone component.³³

Additive concentration (w/w)	Bioglass 45S5 (BG), RI = 1.55	Hydroxyapatite (HNP & HMP) RI = 1.64	
CaproGlu	1.485 ± 0.005		
5 %	1.49	1.49	
10 %	1.49	1.50	
20 %	1.50	1.52	
30 %	1.50	1.53	

413 **Table 4**. Refractive Index (RI) estimation^{*} of CaproGlu Biocomposites.

414 * *RI* estimation calculated by Lorentz-Lorenz equation for rule of mixtures.

While shear stresses are evaluated, we speculate the broad standard deviation results from irregular photocuring and therefore no statistical significance can be gained with respect to additive comparison. Given that the hardest part of the bone is near the surface (cortical bone), bone adhesion may not be warranted within the bone marrow and optical flux may instead be minimized within the bone marrow. Part of our future work will continue to refine the optical setup to achieve precise control over light flux to reach conclusive shear adhesion test results for UV-activated transparent bone implants.

422 **3.4 Scanning electron microscopy**

423 Figure 6 shows representative scanning electron micrographs of UV-cured pure CaproGlu 424 and composites with all 3 different additives (10%, w/w). The porous structure of all 425 composites are the result of molecular nitrogen generation as byproduct of activation of 426 diazirine from UV exposure. This is consistent with our previously reported results that demonstrate the same porous morphology of pure CaproGlu bioadhesive formulation.¹⁷ In 427 428 Fig. 6B, 6C, and 6D, the solid particles are shown embedded on the matrix as pointed on red 429 arrows. EDX analysis confirms the composition of these particles belonging to that of BG, 430 HNP and HMP (see **Table S2**). Image analysis shows the pore size distribution of each 431 composite (Fig. S4) with measured pore sizes for CaproGlu (control), BG. HNP and HMP of: 432 $43 \pm 39 \ \mu\text{m}$, $26 \pm 19 \ \mu\text{m}$, $41 \pm 31 \ \mu\text{m}$ and $37 \pm 26 \ \mu\text{m}$, respectively, which is in line of previously reported ~ 50 μ m pore size of CaproGlu³⁴. It should be noted that nanoparticle 433 434 load (HNP) caused significantly lower pore size in comparison to both control and 435 microparticle-embedding composite (HMP and BG; Fig. S4).



436

Figure 6. Morphological analysis of crosslinked CaproGlu biocomposites (UV; 10 J) by
scanning electron microscopy (SEM; arrows indicate embedded mineral particles in polymer
matrix): (A) pure CaproGlu (control). CaproGlu composites with: (B) Bioglass 45S5 (10%);
(C) hydroxyapatite nanoparticles (10%); and (D) hydroxyapatite microparticles (10%).

441

442 CaproGlu bioadhesive is designed as a solvent-free liquid pre-polymer that allows 443 incorporation of inorganic additives, such as hydroxyapatite and Bioglass 45S5 (Fig. 1). 444 Previous evaluation of CaproGlu composites displayed adhesion strength > 800 kPa on cranium substrates.¹⁶ Generation of molecular nitrogen as byproduct of diazirine activation 445 446 allows the initially liquid-like CaproGlu adhesive to expand into porous matrix, that fills gaps 447 between surfaces during photocuring, forming a solid porous matrix (Fig. 6). Herein, the 448 bone adhesion and light-activated expansion is exploited towards fixation at the implant-bone 449 interface.

450 As hypothesized, confining the thickness of bone-implant interface below 0.2 mm in 451 conjunction to transparent cylindrical bone pin, compressive stresses have been generated 452 through the adhesive matrix - a crosslinked biocomposite layer forms in situ at the implant-453 bone interface. Such unique behaviour is deemed less traumatic than compressive stresses formed by screws or pressure-fit pins: the Young's modulus of bone changes during healing 454 in the range of 20 - 6,000 MPa⁷, and residual compressive stresses could form because of 455 456 difference in modulus. With a crosslinked biocomposite layer acting as a mediation between 457 implant and bone, this modulus mismatch between implant and bone can be minimized, therefore minimizing risk of complications.³⁴⁻³⁵ The expanding matrix may act as a porous 458 459 scaffold towards cell migration and neovascularization during remodelling stage of bone 460 fracture healing. SEM images (Fig. 6) suggests that the osseointegration additive particles of 461 Bioglass 45S5 and hydroxyapatite are embedded onto the surface of the porous matrix, which 462 is expected to promote further bone healing.

Additives to liquid polymers can plasticize the matrix^{19, 36} while solid additives improve the 463 modulus and adhesive strength of photocured CaproGlu (Fig. 2; Table 2). Inorganic 464 465 additives of Bioglass 45S5 and hydroxyapatite have enough fluidity to be applied by syringe, 466 but with sufficient viscosity to allow sub-millimetre coatings to be applied. HMP additive 467 shows the largest viscosity increase, as its µm-particle size is an order of magnitude larger 468 than the HNP. As a result, its composite at 20% (w/w) have significantly increased viscosity 469 (Fig. 1). Loading concentration of additives generally increases dynamic modulus of 470 photocured biocomposite. Different types of additives result in different curing profiles (Fig. 471 S1-S3). Photocuring itself is dependent on the penetration of UV light through the matrix, 472 which is limited by thickness of the adhesive applied. Future designs will continue to 473 optimize the curing through the matrix, which is one detractor of light activated bioadhesives.

474 CaproGlu composite's unique material properties sets it apart from conventional implant fixation by commercial cements, such as acrylate (i.e. Cemex[®], SimplexTM) or ceramic (i.e. 475 Norian[®], HydroSet[®]) formulations.³⁷⁻³⁹ Although the clinical use of modern acrylates dates 476 back to 1943⁴⁰ the next generation of fixatives seeks to avoid acrylates-based polymerization 477 478 due to their unresorbable nature, immunological rejection, and further injury due to mechanical mismatch with native osteo-tissues.⁴¹ Free-radical polymerization can be 479 activated by light-based mechanisms or two-part mixing, but the bulk of these adhesives 480 481 requires free radical initiators and preservatives that leach into surrounding tissues. The exothermic reactions can heat up to $100^{\circ}C^{42}$ if no cooling is factored into the application. 482 Modulus can only be grossly controlled, further exacerbating tissue sensitivity.⁴¹ Bone 483 484 cements have the advantage of rapid fixation, but have known risks with regards to fixation / 485 fracture failure (through accumulation of microcracks) and toxic systemic risks (bone cement implantation syndrome) caused by initiator / monomer leachates from the shrinking acrylate 486 resins.⁴³ Calcium phosphate-based cements (CPCs) were developed to overcome acrylate 487 488 impediments with major advantages over acrylates, such as osteoconductivity, 489 osteoinductivity, bio-resorbability, and interaction with bone cells. Although CPCs are of 490 biocompatible nature, they cannot be activated on-demand, have low mechanical strength and exhibit low interfacial adhesion with hydrated tissues.⁴⁴ Thus, there is still an unmet clinical 491 492 need for bone-interface fixation formulations capable for non-invasive activation without 493 exothermic crosslinking reaction and toxic leachates: features demonstrated by CaproGlu 494 biocomposites described in this work.

The results reported in this paper present novel CaproGlu composite platform as potential
alternative to conventional bone implant fixation formulations (i.e. acrylates, CPCs). An ideal
bone implant fixation formulation should have the following properties: (1) blood and bone
tissue compatibility, (2) sufficient mechanical strength to stabilize fracture, (3) straight-

forward and simplified application on hard-to-reach areas, and (4) bone healing mediation.⁴⁵ 499 500 The combination of UV curing and tunable viscosity by changing additive concentrations 501 allows greater control of adhesive application where commercial bone fixation acrylates lack 502 (i.e. spontaneous reaction, exothermic effect, toxic leachates). Gelation time is not affected 503 by additive content, therefore the amount of UV dose can be kept to a minimum. Porous 504 structure resulting from diazirine photolysis/nitrogen generation reduces the stiffness of the 505 matrix, but can be beneficial in two ways: first, access is available for bone growth through 506 the matrix, and second, the expansion of matrix allows the adhesive to fill implant/tissue gaps 507 more efficiently. These advantages are not without drawbacks; as the effectiveness of UV 508 curing is decreasing with thickness, care should be taken when applying adhesive to avoid 509 incomplete curing. The resulting implant adhesion (shear) strength remains to be improved 510 by a factor of 10 - 100x for load bearing applications, but may meet less strenuous, non-511 loading bearing applications. Our future work will continue to improve the adhesion strength 512 of light activated bone implants while expanding the technology to the latest materials available for transparent waveguides.⁴⁶⁻⁴⁸ 513

514 In vivo investigation of CaproGlu has previously demonstrated moderate immunological response¹⁶. CaproGlu was also assessed by OECD-regulated *in vitro* tests that demonstrated 515 516 no sensitization or genotoxic effect.¹⁷ CaproGlu is polycaprolactone-based crosslinked 517 material that is biodegradable like its predecessors: the family of biodegradable polymers 518 with well-defined degradation mechanism (ester hydrolysis flushed through metabolic 519 pathways) and the range of different degradation kinetics based on crosslinking density (i.e. polymerization time, molecular weight).^{33, 49-50} In our previous *in vivo* work (rabbit model) 520 521 we have observed CaproGlu resorption within 1-3 weeks due to the porous nature of UVAactivated CaproGlu bioadhesive layer in close contact with blood vessels.¹⁶ Like all 522 523 biodegradable materials, the degradation kinetics of CaproGlu biocomposite is anticipated to

524 be dependent on several factors, including the parameters reported in this paper:

525 concentration / size / type of solid bioactive particles as well as the crosslinking density

526 dependent on CaproGlu molecular weight / diazirine grafting percentage / UVA energy dose.

527 Dedicated biodegradation study is currently conducted in our laboratory and the results will

528 be reported in future.

529

530 **5. Conclusion**

531 A unique strategy of bone fixation by UV light activation of transparent biopolymers is 532 demonstrated through the unique CaproGlu biocomposites. CaproGlu-based biocomposites 533 combination of rapid expansion and interfacial crosslinking provide a less traumatic method 534 of bone implant fixation compared to metal pins or screws. When mixed with bioactive solid 535 additives, liquid CaproGlu yields composites that have tunable mechanical properties 536 controlled by; (i) concentration of solid particles in the composite; (ii) particle size; and (iii) 537 joules light dose. The synthetic nature of CaproGlu, straight-forward production of 538 composites by simple mixing, interfacial sustainability to applied mechanical load and non-539 invasive crosslinking strategy, opens a pathway for future bone fixation devices based on 540 transparent biopolymers.

541

542 ASSOCIATED CONTENT

543 Supporting Information

544 Supporting information contains the extended photorheometry data (Fig. S1-S3; Table S1),

545 pore size distribution measured form SEM images (**Fig. S4**) and EDX results (**Table S2**).

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547

548 **AUTHOR INFORMATION**

549 **Author Contributions**

- 550 The manuscript was produced through contributions from all listed authors. The final version
- 551 of the manuscript is approved by all listed authors.

552 **DECLARATION OF CONFLICT OF INTEREST**

- 553 T.W.J. Steele and I. Djordjevic are co-inventors of patent application: Hygroscopic, Cross-
- 554 linking Coatings and Bioadhesives; PCT/SG2018/050452. Authors declare no competing
- 555 interests.

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