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1 **Fixation of transparent bone pins with photocuring biocomposites**

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ABSTRACT

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Bone fractures are in need of rapid fixation methods, but current strategies are limited to metal pins and screws, which necessitate secondary surgeries upon removal. New techniques are sought to avoid surgical revisions, while maintaining or improving fixation speed. Herein, a method of bone fixation is proposed with transparent biopolymers anchored in place via light-activated, biocomposites based on expanding CaproGlu bioadhesives. The transparent biopolymers serve as a UV light guide for the activation of CaproGlu biocomposites that results in evolution of molecular nitrogen (from diazine photolysis), simultaneously expanding the covalently crosslinked matrix. Osseointegration additives of hydroxyapatite or Bioglass 45S5 yield a biocomposite matrix with increased stiffness and pull-out strength. The structure-property relationships of UV joules dose, pin diameter, and biocomposite additives are assessed with respect to apparent viscosity, shear modulus, spatiotemporal pin curing, and 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.

KEY WORDS: *Bone implant fixation, polymer bioadhesive, bone biocomposite, hydroxyapatite, Bioglass.*

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,
50 bone resorption and ischemia.¹ Biomaterials design for bone regeneration requires
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 osseointegration in initial stage of
54 healing.² Bone remodelling (i.e. healing) is a multi-phase process where biomechanical
55 properties undergo dynamic change correlated to bone mineral density³⁻⁵ as Young's modulus
56 for human granulation tissue is ~0.5 MPa and rises up to 20 GPa for mature bone.⁶ The
57 variation of callus mechanical moduli through the multi-phase healing process can be in the
58 range of 20-6000 MPa.⁷ In case of implant-assisted fracture repair, the callus formation
59 begins at the implant surface; the tissue formation is highly responsive to interfacial /
60 mechanical properties of the implant and the process is known as contact osteogenesis.⁸ Due
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

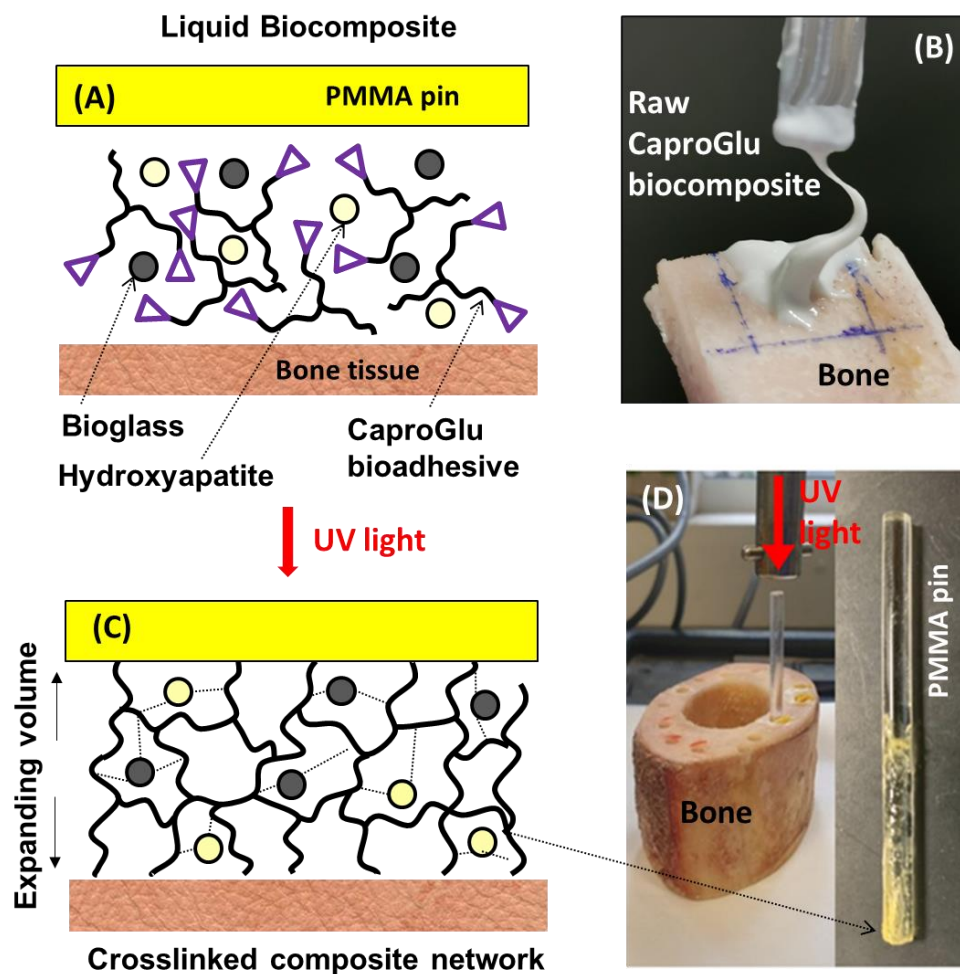
63 Bone fixation screws and pins have been employed in clinical practice for decades. Apart
64 from standard metallic implants,¹⁰ bone fixation is also performed with biodegradable plates
65 and screws that offer less invasive approaches.¹¹⁻¹² Recently reported clinical trials indicate
66 that bioresorbable polymer (polycaprolactone, PCL; poly(lactic acid), PLA) and permanent
67 implants (metallic) are equally safe and effective for non-load-bearing bone reconstruction.¹³
68 Resorbable implants eliminate the need for secondary surgery which is required for metallic

69 implants after tissue healing is completed. The bone microenvironment repair relies on
70 sensitive bone / implant interface¹⁴ that is disrupted by compression (force-mediated) fixation
71 that causes peri-implant bone damage up to 0.9 mm in radial direction from the implant.¹⁵
72 This issue compromises the primary implant stability and should be addressed by non-
73 invasive, biodegradable fixation formulations that combine principles of surgical adhesion
74 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
77 hydroxyapatite to yield viscous liquid biocomposite (**Scheme 1A,B**).¹⁶⁻¹⁷ The CaproGlu
78 platform is based on polycaprolactone triol or tetrol (PCLT) grafted with
79 trifluoromethylphenyl diazirine as a surgical adhesive.¹⁸ UV activation of diazirine generates
80 carbene that rapidly crosslinks with release of molecular nitrogen that causes a >200%
81 volumetric expansion and pressures that could exceed 200 kPa (**Scheme 1C**).¹⁹ Carbene
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,

94 commercially available polylactide pins (e.g. Inion CPS™).²⁰ Described bone biocomposite
 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.



105

106 **Scheme 1.** Demonstration of light activation of transparent bone pins with the aid of
107 CaproGlu biocomposite formulation. (A) Composite is produced by mixing diazirine-grafted
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.

117

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

124

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-yellow transparent
134 liquid) is further washed 3 times with water and centrifuged. PCLT-D formulations are
135 lyophilized for 24 h and characterized with ^1H NMR to calculate the conjugation (grafting)
136 percentage (Bruker Avance; 400 MHz). Refractive index (RI) of purified CaproGlu is
137 measured by Mettler Toledo portable refractometer 30GS at room temperature, and RI
138 estimation of CaproGlu bioadhesive composites are performed using Lorentz-Lorenz
139 equation for rule of mixtures.²¹ CaproGlu bioadhesive composites are prepared by directly
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 $2.0 \mu\text{m}$ 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
145 milling and sieving, as previously described.²²

146 **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
149 cm^{-2} with an IL 1400 Radiometer through handheld UV LEDs or by Thorlabs SOLIS-365C
150 High Power LED. Rheology tests are performed using parallel plate geometry with probe
151 diameter 10 mm, on 0.1, 0.2 and 0.4 mm measuring gaps. Apparent viscosity is evaluated via
152 rotational rheology with shear rate 10 s^{-1} for 60 seconds. The storage modulus (G') and loss
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 $t = 30 \text{ s}$ and $t =$
155 130 s to achieve total UV dose of 10 J. Amplitude sweep of 1-1000% shear strain are
156 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
162 channels and thresholded to identify and count the ratio of pixels representing yellow-cured
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**

168 Bovine femur cortical bone samples are prepared at length of ~4 cm. Holes are drilled
169 through the outer cortical bone with diameter of 3.4 mm; only 3 mm diameter optical fibers
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
173 100 mW cm^{-2} for 5 minutes (30 J) through the fibre optic; excess dose is required to
174 compensate for irregular curing efficiencies. Load is applied to the photocured PMMA pin in
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
177 Products, USA) at the strain rate of 3 mm min^{-1} with 50 N capacity force cell ($\pm 0.25\%$
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
186 working distance of 15 mm. The composition of the composites is analysed by EDX using an
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
189 over the $7.5 \times 10^{-3} \text{ cm}^2$ area. The SEM images are thresholded to outline the porous
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**

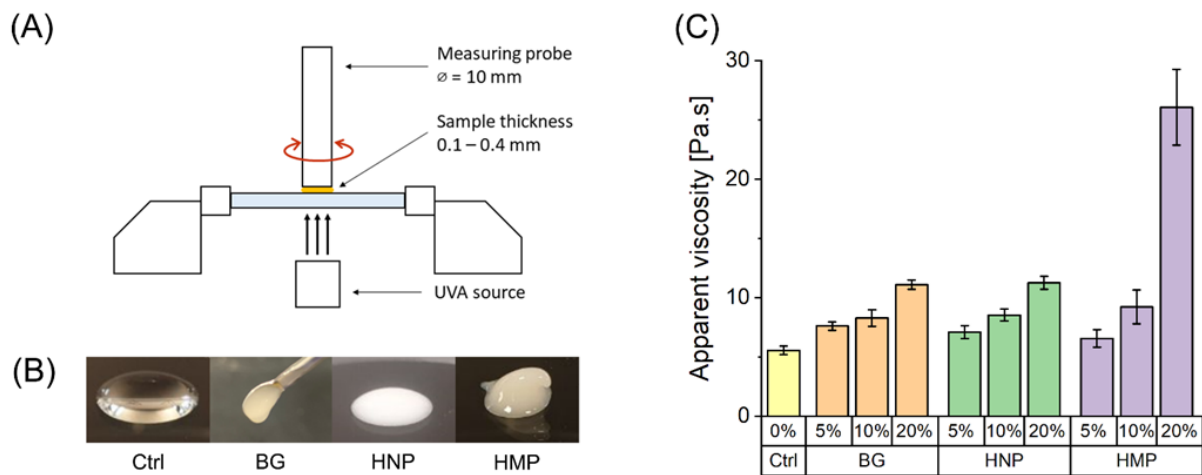
199 Nine biocomposite formulations (3 additives at 3 concentrations each) are evaluated for light
200 activated fixation of transparent plastic implants. Several inorganic additives are available for
201 inducing osseointegration, however we have limited the structure property relationship
202 parameters to two different types of inorganic particles: hydroxyapatite and silica-based
203 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
217 polymethacrylate (PMMA) are evaluated as light-transparent pins. Optical fiber-grade
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
221 at 3 GPa.^{26 27} In order to assess the lap shear adhesion at the bone implant interface, fresh *ex*
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**

227 Biocomposites of liquid CaproGlu and three inorganic additives are prepared in three weight
228 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:
 235 pure CaproGlu is translucent while CaproGlu mixed with BG, HNP, and HMP additives are
 236 opaque from particle light scattering. **Fig. 1C** shows the apparent viscosity as function of
 237 additive concentration, with values listed in **Table 1**.



238
 239 **Figure 1.** Photorheometry experimental setup: (A) Schematics presentation of rheometer
 240 fitted with light-transparent base with outlined dimension parameters. (B) Close-up pictures,
 241 from left to right: pure CaproGlu, CaproGlu + 20% BG, CaproGlu + 20% HNP, CaproGlu +
 242 20% HMP. (C) Summary of viscosity values measured for biocomposites as a function of
 243 additive concentration in comparison to pure CaproGlu (control; 0%).

244

245 **Table 1.** Apparent viscosity (Pa.s) of composites: shear ^{rate} 10 s⁻¹; base-probe thickness 0.2
 246 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

247

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
 252 the uncured formulations display aspects of a Bingham plastic and are able to coat surfaces
 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 $100 \text{ mW}\cdot\text{cm}^{-2}$ for 100 seconds, for a total dose of $10 \text{ J}\cdot\text{cm}^{-2}$. Before UV curing,
 256 the sample is pre-sheared for 30 seconds under oscillatory rheometry, which disrupts any
 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
 261 curing, the biocomposites are crosslinked and $G' \gg G''$. **Fig. 2A** shows a representative plot
 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
 265 after curing as a function of loading is presented in **Fig. 2C** and a plot of G' vs. thickness for
 266 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

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

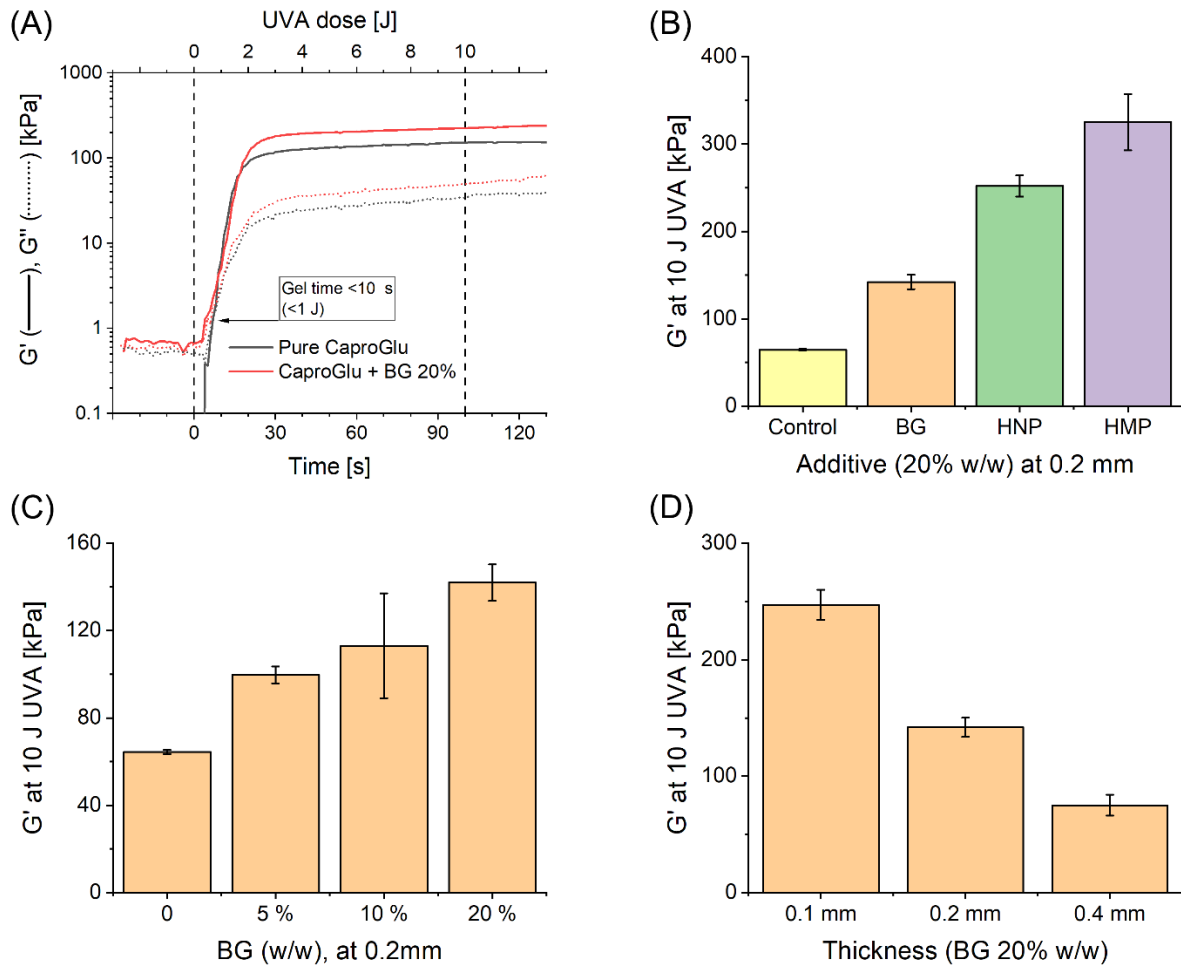


Figure 2. Photoreological properties of CaproGlu biocomposite formulations: **(A)** Plot of biocomposite photocuring showing the evolution of G' and G'' versus UV curing time, representative for pure CaproGlu vs Caproglu + 20% BG. **(B)** Comparison of G' after curing as function of additive type, representative 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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279 **Table 2.** Values of G' (storage modulus; kPa) after photocuring at total dose of 10 J.cm⁻².

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

280

281 Note that the HMP microparticles appear to have the highest light attenuation as judged by G'

282 from 0.1 to 0.4 thickness. The rheometer probe evaluates the biocomposite surface with the

283 least amount of light exposure. Taken together, the results suggest that thickness should be

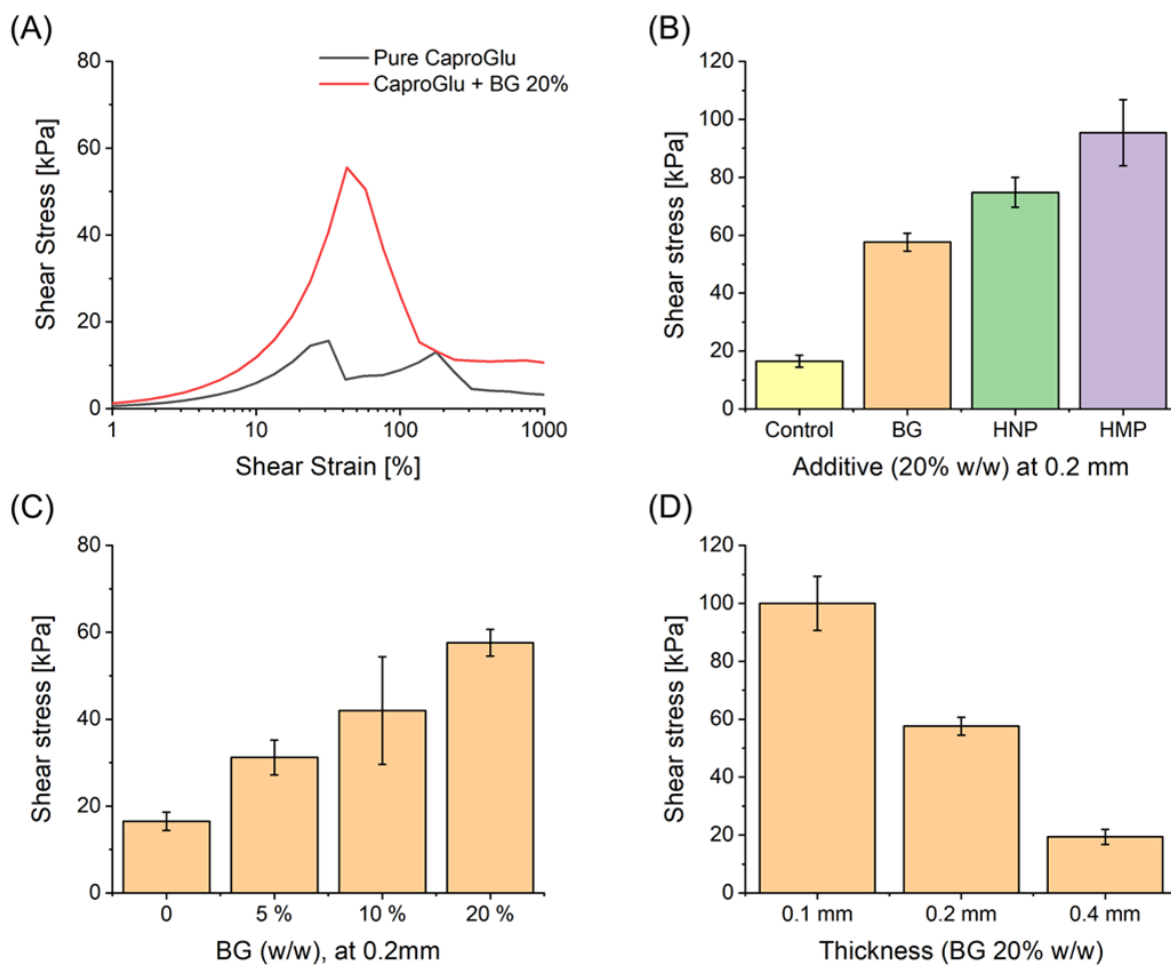
284 kept at 0.2 mm or smaller in order to limit gradients. Gelation point is reached within first 10

285 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

286 mm (*Supplementary information: Fig. S1-S3*). It is shown that osseointegration additives can

287 improve modulus and yield stress of CaproGlu without compromising gelation time/ gelation
288 dose, 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.



298

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.

306

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

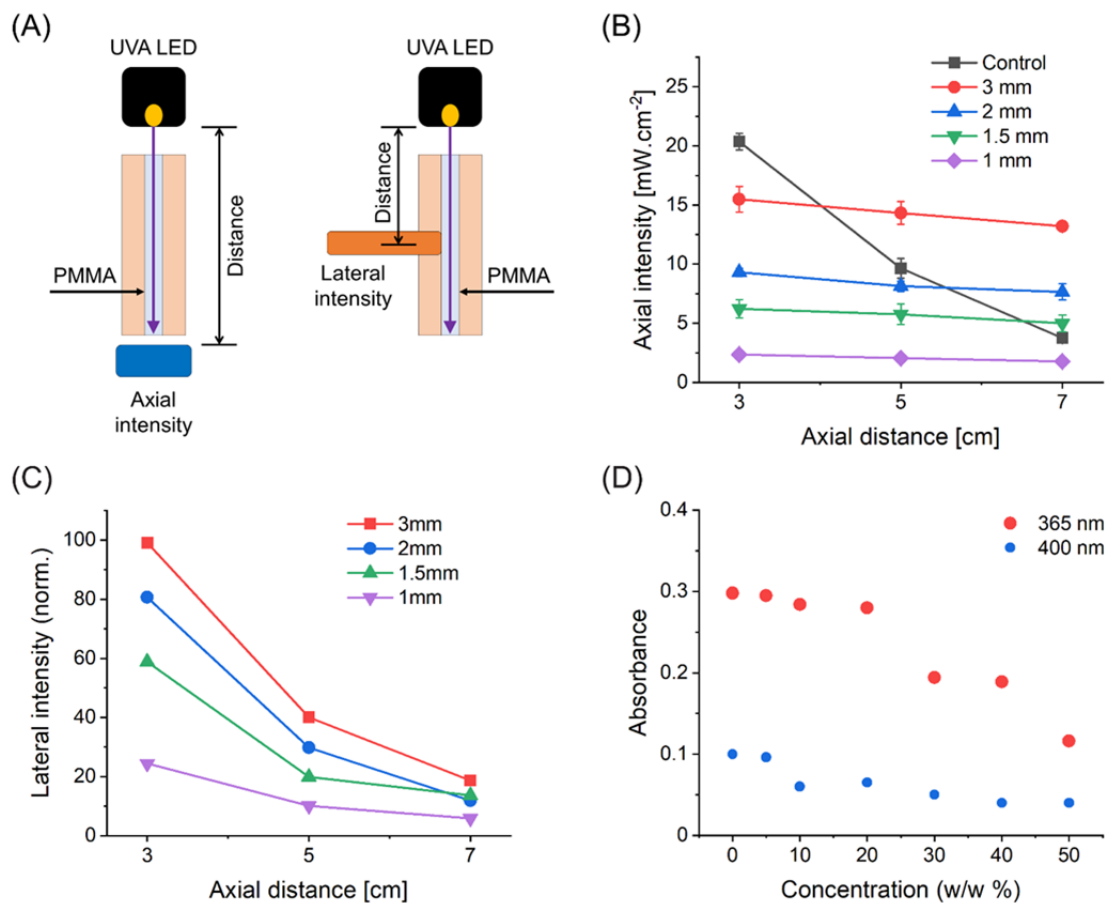
Measurement thickness	Additive concentration (w/w)	Bioglass 45S5 (BG)	Hydroxyapatite nanopowder (HNP)	Hydroxyapatite coarse powder (HMP)
0.1 mm	0 %	36.4 ± 0.33		
	5 %	56.9 ± 6.97	112 ± 2.60	71.7 ± 16.6
	10 %	78.3 ± 8.65	113 ± 4.79	84.4 ± 13.1
	20 %	100 ± 9.38	127 ± 9.51	155 ± 2.93
0.2 mm	0 %	16.5 ± 2.11		
	5 %	31.2 ± 4.02	88.7 ± 3.79	40.7 ± 20.0
	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
0.4 mm	0 %	12.1 ± 1.52		
	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

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309 **3.2 Light transmission properties of PMMA optical fiber**

310 Optical fiber-grade PMMA of different diameters 1 mm, 1.5 mm, 2 mm, and 3 mm are cut
 311 into different lengths 3 cm, 5 cm, and 7 cm. The UV LED is fitted to a custom 3D-printed
 312 adapter to direct the light onto the 3mm diameter PMMA pin. Axial and lateral intensity
 313 measurements are performed to assess pin transparency (intensity loss) and length dependent
 314 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
 320 different distance, and the highest intensity achieved is $20 \text{ mW}\cdot\text{cm}^{-2}$ at 3 cm. With increasing
 321 distance, the intensity reading reduces slightly. For lateral intensity measurement performed
 322 using a spectrometer, the results are plotted as a normalized relative light unit (**Fig. 4C**).



323

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

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

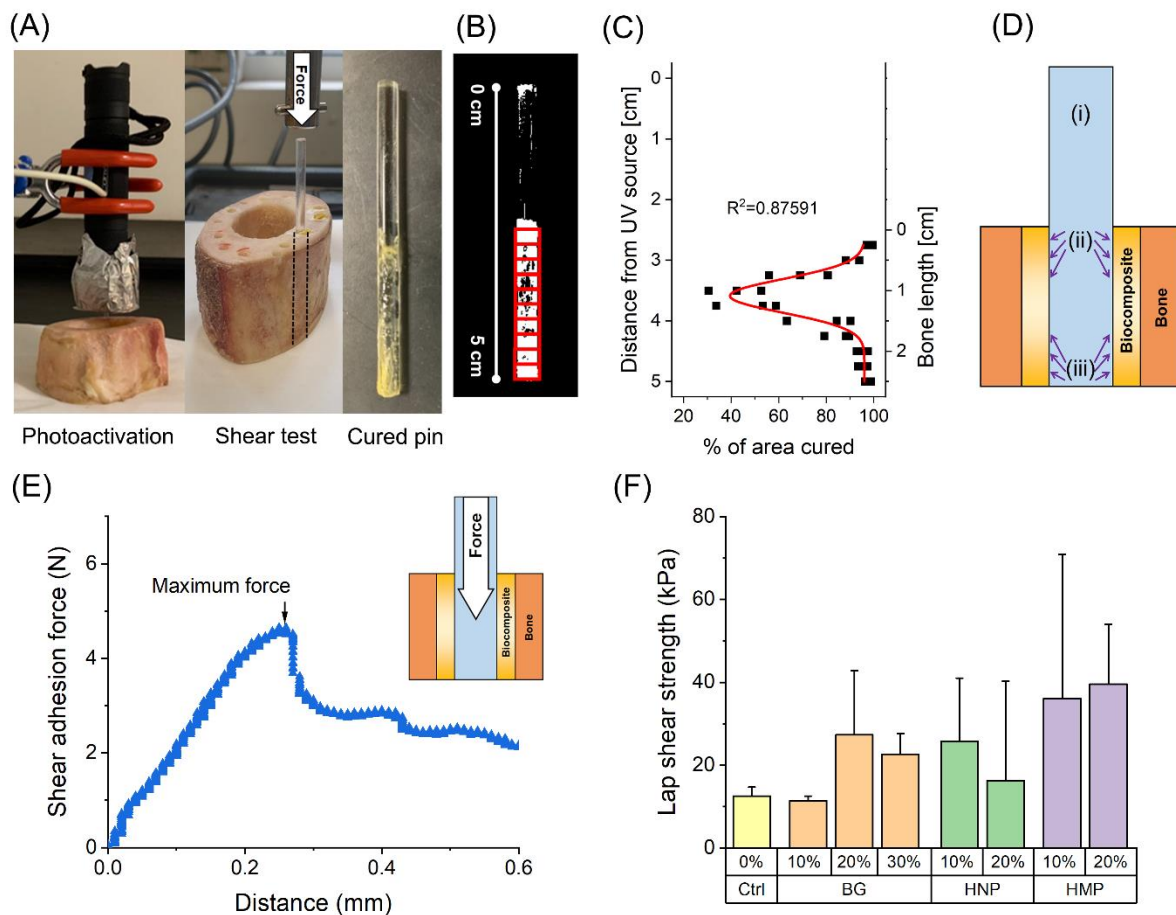
332

333 The results demonstrate that the longer the distance is, the difference between intensity
334 readings are getting closer as dispersion starts taking effect. In both directions, the larger
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
344 bone. UV activation is performed by exposing the PMMA optical fibers with UV for 5
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
351 air/PMMA interface, UV light is internally reflected (42° critical angle, refractive index of
352 1.49; **Fig. 5D, i**). Internal reflection no longer occurs at the CaproGlu interface because
353 polycaprolactone (major constituent of CaproGlu) has refractive index of 1.46, similar to

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
 360 creates a second virtual light source (**Fig. 5D, iii**), which is responsible for curing from the
 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**.



363
 364 **Figure 5.** *Ex vivo* investigation of PMMA fixation by UV-activated CaproGlu biocomposite
 365 formulations: (A) *Left:* UV-curing setup of composites on PMMA optical fiber surface,
 366 inserted into holes drilled onto bovine femur bone. *Middle:* setup of shear test on bovine
 367 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
371 Gaussian distribution with R^2 value of 0.87591. **(D)** Schematic presentation for proposed
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.

379

380 **Figure 5E** shows a representative result of this experiment on a pure CaproGlu as shear force
381 reading at the pin-bone interface contributed by cured CaproGlu versus PMMA pin
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
385 ultimate shear stress value represents the adhesion (shear) strength of cured CaproGlu
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,
389 and therefore no lap shear adhesion results are available. As BG has high refractive index of
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.

393 This work was inspired by previous investigations of polymer waveguides that elucidated the
394 structure activity relationships of deep tissue light delivery, transparent biopolymers, and

395 photochemical tissue bonding.³¹ With 900 J of visible irradiation, they demonstrated a
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
411 of the pin.³² Ultimately, the lower crosslinking density is likely to cause faster resorption of
412 polycaprolactone component.³³

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

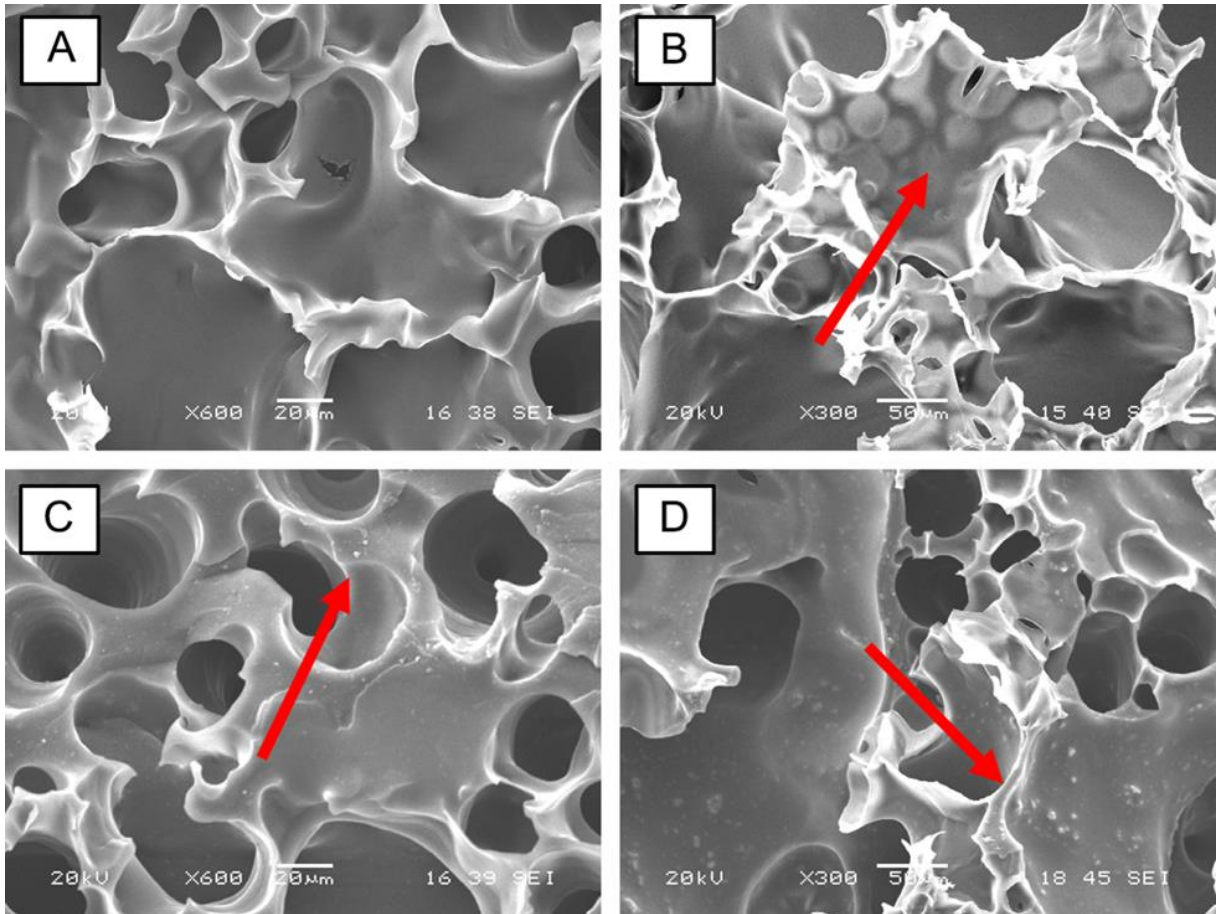
Additive concentration (w/w)	Bioglass 45S5 (BG), RI = 1.55	Hydroxyapatite (HNP & HMP), RI = 1.64
CaproGlu	1.485 ± 0.005	
5 %	1.49	1.49
10 %	1.49	1.50
20 %	1.50	1.52
30 %	1.50	1.53

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

415 While shear stresses are evaluated, we speculate the broad standard deviation results from
416 irregular photocuring and therefore no statistical significance can be gained with respect to
417 additive comparison. Given that the hardest part of the bone is near the surface (cortical bone),
418 bone adhesion may not be warranted within the bone marrow and optical flux may instead be
419 minimized within the bone marrow. Part of our future work will continue to refine the optical
420 setup to achieve precise control over light flux to reach conclusive shear adhesion test results
421 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
427 demonstrate the same porous morphology of pure CaproGlu bioadhesive formulation.¹⁷ In
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
433 previously reported $\sim 50 \mu\text{m}$ pore size of CaproGlu³⁴. It should be noted that nanoparticle
434 load (HNP) caused significantly lower pore size in comparison to both control and
435 microparticle-embedding composite (HMP and BG; **Fig. S4**).



436

437 **Figure 6.** Morphological analysis of crosslinked CaproGlu biocomposites (UV; 10 J) by
 438 scanning electron microscopy (SEM; arrows indicate embedded mineral particles in polymer
 439 matrix): (A) pure CaproGlu (control). CaproGlu composites with: (B) Bioglass 45S5 (10%);
 440 (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
 445 cranium substrates.¹⁶ Generation of molecular nitrogen as byproduct of diazirine activation
 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
454 formed by screws or pressure-fit pins: the Young's modulus of bone changes during healing
455 in the range of 20 – 6,000 MPa⁷, and residual compressive stresses could form because of
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,
458 therefore minimizing risk of complications.³⁴⁻³⁵ The expanding matrix may act as a porous
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.

463 Additives to liquid polymers can plasticize the matrix^{19, 36} while solid additives improve the
464 modulus and adhesive strength of photocured CaproGlu (**Fig. 2; Table 2**). Inorganic
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
475 fixation by commercial cements, such as acrylate (i.e. Cemex[®], Simplex[™]) or ceramic (i.e.
476 Norian[®], HydroSet[®]) formulations.³⁷⁻³⁹ Although the clinical use of modern acrylates dates
477 back to 1943⁴⁰ the next generation of fixatives seeks to avoid acrylates-based polymerization
478 due to their unresorbable nature, immunological rejection, and further injury due to
479 mechanical mismatch with native osteo-tissues.⁴¹ Free-radical polymerization can be
480 activated by light-based mechanisms or two-part mixing, but the bulk of these adhesives
481 requires free radical initiators and preservatives that leach into surrounding tissues. The
482 exothermic reactions can heat up to 100°C⁴² if no cooling is factored into the application.
483 Modulus can only be grossly controlled, further exacerbating tissue sensitivity.⁴¹ Bone
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
486 implantation syndrome) caused by initiator / monomer leachates from the shrinking acrylate
487 resins.⁴³ Calcium phosphate-based cements (CPCs) were developed to overcome acrylate
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
491 exhibit low interfacial adhesion with hydrated tissues.⁴⁴ Thus, there is still an unmet clinical
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.

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

499 forward and simplified application on hard-to-reach areas, and (4) bone healing mediation.⁴⁵
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
513 available for transparent waveguides.⁴⁶⁻⁴⁸

514 *In vivo* investigation of CaproGlu has previously demonstrated moderate immunological
515 response¹⁶. CaproGlu was also assessed by OECD-regulated *in vitro* tests that demonstrated
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.
520 polymerization time, molecular weight).^{33, 49-50} In our previous *in vivo* work (rabbit model)
521 we have observed CaproGlu resorption within 1-3 weeks due to the porous nature of UVA-
522 activated CaproGlu bioadhesive layer in close contact with blood vessels.¹⁶ Like all
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 from SEM images (**Fig. S4**) and EDX results (**Table S2**).

546

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-
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561 Bioadhesives-preventing catheter extravasation and skin infections.

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