In vitro biocompatibility of diazirine-grafted biomaterials

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Title: In Vitro Biocompatibility of Diazirine Grafted Biomaterials

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Keywords: Polycaprolactone, diazirine, genotoxicity, sensitization, OECD test

Abstract

Photoactivation of aryl-diazirines is an emerging method of rapid, covalent crosslinking under ambient conditions. These attributes make those compounds candidates for grafting onto inert polymer backbones in order to produce stimuli-sensitive biomaterials. However, no risk assessments are available to gauge the toxicity of the leachable components after crosslinking activation. Herein, a stimuli-sensitive biomaterial is formulated from diazirine-grafted polycaprolactone tetrol. Also known as CaproGlu, this biomaterial undergoes UVA-activated crosslinking, with many positive attributes towards bioadhesive applications; hydrophobic, solvent-free, liquid at room temperature, and transitions into a foam biorubber after mild UVA illumination. As a model diazirine grafted biomaterial, hydrolyzed CaproGlu leachates are evaluated for genotoxicity and skin sensitization, namely: Ames test, direct peptide reactivity, and ARE-Nrf2 luciferase assays. The degradation products of diazirine mediated crosslinking observe little to no risk of *in vitro* genotoxicity or skin sensitization.

Key words: *Polycaprolactone, diazirine, genotoxicity, sensitization, OECD test*

1. Introduction

Stimuli-sensitive biomaterials are an emerging class of materials that aim to induce rapid shifts in chemical, physical, or mechanical properties.^[1] On-demand covalent crosslinking is an example, where limited chemical reactions exist in inducing rapid polymerization or liquid gelation under ambient conditions. Cyanoacrylates and acrylate-grafted macromolecules have been exploited for surface and light-activated stimuli-sensitive biomaterials, but considerable side effects are present when paired on soft tissue or wet environments.^[2] Thus, other chemical platforms of rapid, on-demand polymerization are needed. Aryl-diazirines are an emerging candidates for rapid polymerization in biomaterials, as they're relatively stable under ambient conditions, but are known to be inducible by light, voltage, heat, and ultrasound.^[3-8] Our efforts have explored aryl-diazirines on several macromolecular backbones towards multifunctional bioadhesives. Others have incorporated diazirines for skin therapies and mapping cell membranes.^[9, 10]

As these technologies relate to tissue contacting biomaterials, they require a pre-clinical assessment.^[11] Skin contacting biomaterials will have leachates that need to be assessed for genotoxicity and sensitization (irritation).^[11] If possible, *in vitro* tests should take priority to prevent needless animal sacrifices. Based on known biomaterial performance of polycaprolactone (PCL) medical devices^[12] and proprietary technology for synthetic bioadhesives,^[4, 13] this paper reports the first *in vitro* biocompatibility evaluations of diazine grafted biomaterials. The model biomaterial is CaproGlu, a hydrophobic liquid polymer formulation based on aryl-diazirine grafted onto viscous and water insoluble polycaprolactone tetrol (PCLT). Mild UVA light (365 nm; 10-20 J) irradiation results in reactive carbene and diazoalkane intermediates for covalent crosslinking (**Figure 1A**).^[14, 15] Light stimulated CaproGlu end-groups (aryl-diazirine) form intermolecular crosslinks as well as interfacial binding with protein functional groups (**Figure 1B,C**). PCL is a known biodegradable

material used in numerous medical devices,^[12] however there is a lack of data available in terms of standardized *in vitro* genotoxicity and sensitization tests recommended by International Standards Organization (ISO)^[16] and Organization for Economic Co-operation and Development (OECD). The comprehensive toxicology screening of crosslinked CaproGlu leachates is assessed by OECD tests: bacterial reverse mutation,^[17] *in chemico* direct peptide reactivity (DPR)^[18] and human-derive cell culture ARE-Nrf2 luciferase assay.^[19] Since CaproGlu formulation is based on non-toxic PCL polyester^[20] it is hypothesized that any toxic leachates would be attributed to the irradiated aryl-diazirine. PCLT (negative control) degrades into caproic acid and is not expected to cause adverse genotoxicity or sensitization detected by *in vitro* tests. Detailed description of synthesis, structural / mechanical analysis, surface morphology, sample preparation for toxicology screening and OECD-regulated methodology are available in Supporting Information.

2. Results and Discussion

¹H NMR peak assignment^[21, 22] confirms the successful grafting (~50 %; **Figure 1D**) of aryl-diazirine on to PCLT backbone, to yield a final ratio of 1:1 aryl-diazirine:-OH group. Traces of unreacted Dz-COOH are detected (< 0.1%), as seen in ¹H NMR of CaproGlu product (**Figure S1**). ¹⁹F NMR spectrum displays the singlet diazirine peak at -64.8 ppm and ~3% presence of diazoalkane intermediate at -57.4 ppm^[23, 24] (calculated from integral values in **Figure 1E**). Carbenes are known to react readily with alcohols by -OH insertion,^[25] both diazoalkane and ether peaks detected in ¹⁹F NMR possibly originate by premature activation of diazirine by ambient light.^[4, 13] Solvent-free CaproGlu is analysed with ATR- FTIR spectroscopy and the observed spectral regions are presented in **Figure 1F** (full IR spectra are shown in **Figure S2**). Peak at 1631 cm⁻¹ is assigned to diazirine (-N=N-)^[26] which disappears upon UVA activation. Carbene could not be detected in ATR mode due to the lifetime measured in nanoseconds,^[27] however, diazoalkane peak is detected at 2090 cm⁻¹ after UVA

activation^[28]. As shown in **Figure 1A** diazoalkane intermediate would eventually yield carbene and its relative stability is dependent on chemical environment (i.e. functional groups in para position from diazirine ring, solvent medium). Carbene is expected to cause intermolecular crosslinking and liquid-to-elastomer phase transition that is observed in photorheometry experiment.

Figure 2A shows the photorheological profile and time dependence of storage /loss moduli (G' and G'' respectively) of CaproGlu upon UVA activation that initiates rapidly after 2 min (marked by dashed line). CaproGlu is a viscous, syringable liquid (density $\sim 1.2 \text{ g.cm}^3$) that reaches gelation point (defined as the time point where $G' = G''$, marked with arrow in **Figure 1A**) within 15 sec of UVA activation. The crosslinking reaction continued at slower rate as the value of G' reaches 103 kPa after 1.6 min of UV activation (10 J) with the increase of G' up to 126 kPa at 3.3 min (20 J; **Figure 2A**). The amplitude sweep profile (is recorded immediately after continuous UV activation (**Figure 2B**). Both G' and G'' maintained plateau at the strain region up to 63%; within the range of 1-63% strain viscoelastic region is independent of the shear percentage.^[29-31] CaproGlu moduli begin to decrease dramatically as the G' drops for an order of magnitude within the region of 63-137% strain. Linear viscoelasticity should closely match the tissue substrates. In terms of mechanical characteristics, soft tissues could exhibit moduli in the range of 10-400 kPa.^[32, 33] **Figure 2C** shows lap shear adhesion stress vs strain profile obtained for wet collagen-CaproGlu interface (see **Figure S3**). The ultimate adhesion strength (UAS) of 120 kPa exceeds commercially available tissue sealants measured on hydrated collagen surface: fibrin-based TisseelTM (20 kPa) and cyanoacrylate-based DermabondTM (63 kPa).^[4] Both rheological profile and UAS of CaproGlu are in line with PCL-triol ($M_w = 300 \text{ Da}$) grafted with aryl-diazirine.^[4] The surface morphology of crosslinked CaproGlu results in interconnected porous structure (**Figure 2D**) with pore size in the range of 20-50 μm . This feature enables dual function of CaproGlu both

as tissue sealant and porous tissue engineering scaffold that enables three-dimensional organ regeneration.^[12]

Results for bacterial reverse mutation and sensitization of CaproGlu leachates, including the traces of unreacted Dz-COOH, detected by NMR (**Figure S1**), in PBS and 0.1 N NaOH (accelerated degradation in alkaline environment) determined by Ames bacterial mutation test are shown in **Figures 3** and **4** respectively (sample appearance and specification are shown in **Figure S4** and **Table S1**). Positive genotoxic controls for Ames test employ sodium azide and 2-aminoanthracene. **Figure 3** indicates no genotoxic effect of CaproGlu in either UVA-crosslinked or in pure (liquid) form. There is no significant difference in the number of reverted colonies in the test substances compared with negative control (PBS) measured with and without metabolic activation.^[34] Previous results reveal no general relationship between genotoxicity and skin sensitization,^[35] however, O. Mekenyan et al. reported that 83-93% of Ames-positive compounds are also found to be skin sensitizers.^[36] Therefore, toxicology screening requires both tests (genotoxicity and sensitization) for polymer leachates of novel macromolecules. *In chemico* DPR assay measures covalent binding to amino acids common in skin proteins. Cysteine and lysine residues replicate the molecular initiating event within skin sensitization biochemistry. From **Figure 4A-D**, all samples tested negative for skin sensitization effect as the binding to cysteine and lysine did not exceed mean value of analytical threshold (6.38%) of protein depletion as detected by HPLC (cinnamaldehyde,^[37, 38] is used as positive control for both *in chemico* and *in vitro* tests; **Figure 4**). Although peptide coupling effect caused by leachates from raw (uncured) CaproGlu is consistently higher (significantly different, marked as “SD”; **Figure 4**) in comparison to negative controls and internal control (pure PCLT). According to OECD guidelines, the tested sample is not considered to be sensitizer because it did not exceed the analytical threshold of 6.38%.

DPR method has some limitations. Peptide binding cannot be used to assess chemicals that are pro-haptens - i.e. chemicals that normally require metabolic conversion before interacting with skin proteins. This is because the *in vitro* protocol has no metabolic component.^[18] The combinations of non-animal methods (*in chemico* and *in vitro*) are needed to be able to substitute for the animal trials with currently available non-animal test methods.^[39-43] Immortalized cell line derived from human keratinocytes are used in ARE-Nrf2 luciferase test method (KeratinoSensTM). The cell line contains the luciferase gene that is known to be up-regulated by sensitizers. Quantitative measurement of luciferase gene induction by luminescence detection (using well established light producing luciferase substrates) is an indicator of the activity of the Nrf2 transcription factor in cells following exposure to electrophilic test chemicals. Test chemicals are considered positive if they induce a statistically significant induction of the luciferase gene above a given threshold: > 1.5-fold or 50% increase in the case of KeratinoSensTM. The maximal fold induction of the luciferase gene over negative control (I_{max}) is determined and data are presented in **Figure 4E,F**. Note that I_{max} value resulted by leachates (including the traces of unreacted Dz-COOH, detected by NMR; **Figure S1**) from crosslinked CaproGlu (50 J dose of UVA; **Figure 4F**) is between values recorded for positive and negative controls, however, this is considered to be non-sensitizing chemical according to OECD guidelines.

3. Conclusion

Diazirine grafted polycaprolactone tetrol – CaproGlu is a model material for assessing the risks of carbene-based stimuli-sensitive biomaterials. Diazirine mediated crosslinking releases reactive intermediates and molecular nitrogen that form a biorubber with interconnected porous structures. *In vitro* biocompatibility assessment of polycaprolactone tetrol, CaproGlu, and its hydrolyzed leachates revealed no genotoxic or sensitization effects under simulated physiological conditions with UVA doses up to 50 Joules. This initial assessment of aryl-

diazirine grafted polyesters suggests these emerging stimuli-sensitive biomaterials pose little to no risk for genotoxicity or skin sensitization under the limited conditions tested.

Supporting Information

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

Acknowledgements and conflicts of interest

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Figure 1. CaproGlu activation by UVA light and subsequent covalent crosslinking: (A) UVA light induces direct conversion to carbene and diazoalkane intermediate; (B) left – functional groups on Caproglu macromolecule, right – example pendant amine and carboxyl groups that originate from proteins on tissue surface; (C) proposed structures of crosslinks; (D) ¹H NMR of neat CaproGlu with peak assignments (see structure in (A)) and integral values and calculated % grafting of TPD-COOH onto PCLT from peak integration values (in brackets); (E) ¹⁹F NMR of neat CaproGlu (chemical shifts left to right; ppm): -57.14, -64.83, 71.50, -75.96, and -161.52 (HFB internal standard); (F) FTIR spectra of CaproGlu showing diazoalkane (2093 cm⁻¹) and depletion of diazirine (1632 cm⁻¹) upon UVA activation (20 J).

Figure 2. Photorheological profile, adhesion strength and surface morphology of CaproGlu: (A) dynamic change of storage and loss moduli (G' and G'' respectively) upon UVA activation of 100 mW.cm^{-2} with dynamic viscosity (calculated from G'') and gelation point ($G' = G''$); (B) amplitude sweep: G'/G'' vs strain; (C) representative lap shear adhesion stress vs strain profile and ultimate adhesion strength before failure (UAS; $n = 5$); (D) SEM image of UVA-crosslinked (20 J) CaproGlu (magnification bar = $50 \mu\text{m}$).

Figure 3. *In Vitro* Bacterial reverse mutation (Ames OECD 471) assay of leachate components from raw / crosslinked CaproGlu and pure PCLT at 37 °C in aqueous medium: (A and B) PBS buffer (pH = 7.4) with and without metabolic activation respectively; (C and D) 0.1N NaOH with and without metabolic activation respectively ($p < 0.05$; $n = 3$).

Figure 4. Skin sensitization analysis of leachate components from raw / crosslinked CaproGlu and pure PCLT (control) extracted at 37 °C in PBS and 0.1N NaOH: (A-D) *in chemico* direct peptide reactivity assay with cysteine and lysine-OECD 442C: (E and F) *in vitro* cell culture assay KeratinoSens™ ARE-Nrf2 Luciferase-OECD 442D ($p < 0.05$; $n = 3$).

290 **The table of contents entry:** Aryl-diazirines are an emerging strategy to induce rapid
291 covalent crosslinking and polymerization under ambient conditions upon light and voltage
292 activation. Aryl-diazirine grafted polyester-CaproGlu rapidly undergoes liquid-to-elastomer
293 transition with low-energy UVA activation. Hydrolyzed leachates from CaproGlu are
294 assessed for genotoxic and sensitization risks with respect to UVA dose and degree of
295 hydrolysis. Biocompatibility results compare favorably to the non-toxic polyester,
296 polycaprolactone tetrol.

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298 **Keyword:** bioadhesive; diazirine; carbene; polycaprolactone

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Supporting Information

Title: In Vitro Biocompatibility of Diazirine Grafted Biomaterials

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1. Materials

4-[3-(Trifluoromethyl)-3H-diazirin-3-yl] benzyl Alcohol ($216.16 \text{ g.mol}^{-1}$; Dz-MeOH) and Hexafluoro benzene ($186.06 \text{ g.mol}^{-1}$; HFB) are purchased from TCI Chemicals, Japan. Polycaprolactone tetrol (PCLT; $M_w = 1000 \text{ Da}$) is kindly donated from Perstorp. Other reagents and solvents: KOH, KMnO_4 , HCl, MgSO_4 , 1,1- carbonyldiimidazole (CDI), deuterated and pure dichloromethane (DCM), diethyl ether (Et_2O) are purchased from Sigma (Singapore).

2. CaproGlu synthesis

4-[3-(Trifluoromethyl)-3H-diazirin-3-YL] Benzoic acid ($230.14 \text{ g.mol}^{-1}$ Dz-COOH) is synthesized from Dz-MeOH. Solution of Dz-MeOH (1 mol. eq.) and potassium hydroxide (1.55 mol. eq.; 0.6 M KOH solution) is dissolved in a mixture of water/dioxane (5/1), cooled down to 0°C in an ice bath and potassium permanganate (1.5 eq.) is added portion-wise. The biphasic reaction mixture is vigorously stirred at room temperature overnight (18 hours). The suspension

is filtered through a pad of Celite®. The filtrate is cooled in an ice bath and acidified with 2M HCl to pH=1. The obtained white precipitate is filtered off and dissolved in ethanol. The organic layer is washed 5 times with water and one time with brine, dried with anhydrous MgSO₄, and the solvent is evaporated in vacuum to yield pure Dz-COOH as a pale-yellow solid. Proprietary CaproGlu synthesis method¹ is the esterification reaction between PCLT (polyol) and Dz-COOH (acid) with 1,1- carbonyldiimidazole (CDI) used as a coupling agent²⁻⁴. The molar ratio of Dz-COOH/PCLT = 2/1 is deliberately chosen to yield ~50% diazirine conjugation (grafting: **Figure 1A**). The reactants are placed in round-bottomed flask with a magnetic stir bar. The flask is immersed in water bath placed on the top of the heater equipped with magnetic stirrer. DCM is added dropwise and stirred at room temperature for 1 h. Solution of PCLT in DCM is added into reaction mixture slowly and the round bottom glass is fitted with condenser. The reaction mixture is heated to 40 °C and stirred for 18 h. After completion DCM is removed on rotavap and the product CaproGlu is purified by water / Et₂O extraction (the water phase contained saturated NaHCO₃ in order to remove unreacted Dz-COOH). The organic layer is dried with anhydrous MgSO₄ and filtrated. Et₂O is removed with rotavap. Traces of solvent from CaproGlu product are removed on high vacuum for 6 h at 40 °C.

3. Nuclear magnetic resonance (NMR) spectroscopy

¹H and ¹⁹F NMR spectra of CaproGlu are recorded on a JEOL ECA400 NMR spectrometer at 400 MHz in CDCl₃ solution. NMR samples are prepared by dissolving 10 mg of CaproGlu in 0.6 mL of CDCl₃. The chemical shifts are given in ppm, referenced to TMS (0 ppm for ¹H NMR spectra) and C₆F₆ (-164.9 ppm for ¹⁹F NMR spectra). The peak assignment and peak integration are performed with SpinWorks software.

4. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR)

spectroscopy

FTIR analysis is performed using PerkinElmer Frontier IR equipped with ATR sampling accessory. Liquid CaproGlu sample is directly placed onto ATR crystal. Spectra are recorded over accumulation of 8 scans at resolution 4 cm^{-1} , at range of 4000 cm^{-1} to 600 cm^{-1} . CaproGlu is UVA-activated with UVA LED Thorlabs SOLIS 365C of dominant wavelength at 365 nm: constant UVA intensity of $100\text{ mW}\cdot\text{cm}^{-2}$ is applied over 200 sec duration for a total dose of 20 J. Raw FTIR spectra are collected and compared (neat/UVA-activated) and processed in OriginPro 2016 software.

5. Dynamic mechanical analysis (DMA) by photorheometry

Rheometry measurements are conducted with Anton Paar Physica MCR 501 rheometer fitted with parallel plate probe PP10 and UVA transparent glass plate. The applied UVA intensity (365 nm) is calibrated to 100 mW cm^{-2} with IL 1400 Radiometer through handheld UVA LEDs or by Thorlabs SOLIS-365C High-Power LED. Dynamic oscillatory strain has the following parameters: 0.1 mm measuring gap, amplitude of 1% and frequency of 1 Hz. The storage moduli (G') and loss moduli (G'') are evaluated in the first 2 minutes, followed by UVA irradiation for 5 minutes, and an amplitude sweep on the photocured sample of 0.1-1000% at 1 Hz.

6. Lap shear adhesion test

PMMA (2.5 mm thickness) is cut into 5 cm x 2.5 cm sheets and collagen/tissue samples (2.5 cm x 2 cm) are glued with cyanoacrylate onto PMMA slides. CaproGlu sample is applied on the top of the substrate. Prepared plates are fixed in sandwich structure with paper clips prior to curing with S1000-1B-3188 UV initiator (OmniCure, Canada) with 365 nm UV filter (019-01036) and power gauge ($20\text{-}120\text{ mWcm}^{-2}$). The curing time and the distance from the substrate is adjusted to provide a 20 J dose. After curing, the sandwich structure is evaluated

for shear adhesion strength with Series Force Measurement System (Chatillon Force Measurement Products, USA) equipped with 100 N loading cell ($n = 5$). Obtained values of force (N) are normalized to sample area and strain is normalized to sample thickness (50 μm). The results are plotted as lap shear adhesion stress (kPa) vs strain (mm/mm).

7. Scanning electron microscopy (SEM)

Surface morphology of crosslinked CaproGlu is analyzed by SEM using a JEOL 5500LV electron microscope. Samples are subjected to platinum coating (90 s, chamber pressure <5 Pa at 20 mA). Images are obtained by JSM 5510 SEM at an acceleration voltage of 5–20 kV and a working distance of 15 mm.

8. Sample preparation for *In Vitro* and *In Chemico* assays

According to ISO 10993⁵ extraction of polymer leachates should be carried out at temperatures that simulate the highest temperatures the polymer may be exposed to during use. Extraction conditions should not cause deformation or degradation of the test or control articles⁶⁻⁹. CaproGlu sample (0.5 g) is placed in capped glass vial (10 ml) as shown and subsequently crosslinked with UV lamp, through the bottom of the glass vial with UV energy in the range of 0-50 J (Table S1). All the samples in glass vials, including crosslinked CaproGlu, raw CaproGlu and control PCLT (pure, non-conjugated), are topped with 5 ml of aqueous mediums prepared for the experiment (PBS and 0.1N NaOH). Vials with CaproGlu (and controls) and medium are incubated in water bath at 37°C. The medium is pipetted out of the vial after 24h of incubation and stored in freezer prior to sending for analysis. All the aqueous mediums with leachates from CaproGlu and control samples are topped-up to 10 ml in centrifuge vials. Samples soaked in 0.1N NaOH are titrated with 0.2N CA to pH = 7, topped up to 10 ml in centrifuge vials and stored in the freezer. Each experiment was performed in triplicates. PBS and pure PCLT are used as negative controls.

9. *In Vitro* OECD biocompatibility assay protocols: mutagenicity and sensitization

Blind analysis is performed according to OECD guidelines¹⁰⁻¹² by third-party GLP-compliant laboratories. The test samples and negative controls items are sterilized using 0.2 µm filtration unit prior to test.

9.1 OECD 471: *In Vitro* bacterial reverse mutation test (1997-07-21; ISO/TR 10993; biological evaluation of medical devices; part 33: Supplement ISO 10993-3. First edition 2015-03-01)

Bacteria strain: salmonella typhimurium TA 100 is cultured overnight in 37 °C shaking incubator at 100 rpm. Positive controls: sodium azide (CAS No: 26628-22-8) and 2-Aminoanthracene (CAS No 613-13-8). Triplicate plates are used for each test, negative control and positive control respectively. The minimal glucose agar base (1.5% agar, Vogel-Bonner E Medium with 2% glucose) is used for each plate. The study is conducted both with and without the presence of metabolic activation system. A co-factor supplemented post-mitochondrial fraction (S9) from the liver of Aroclor 1254 induced Sprague-Dawley rats is used as metabolic activation system. Based on plate incorporation method, 2 ml of molten top agar are dispensed to each culture tube. 0.1 ml of a fresh over-night culture of the test strain, 0.1 ml of the test substance / negative control / positive control, 0.2 ml Biotin/Histidine/Tryptophan and 0.5 ml of 0.2 M PBS (or 0.5 ml of cold S9 mixture) are then dispensed to each agar tube. The concentration of S9 fraction in S9 mixture used in each plate is 10%. Student's t-test is used to assess statistically significant differences between the number of revertant colonies of the test substance and its corresponding negative control.

9.2 OECD TG 442C: *In Chemico* Skin Sensitisation

Method: Direct Peptide Reactivity Assay (2015) and EURL ECVAM DB-ALM Protocol 154: Direct Peptide Reactivity Assay (DPRA) for Skin Sensitisation Testing (2013). Protocol: Test

substances are analysed as received (composition and concentration unknown). Cinnamic aldehyde (100 mM in acetonitrile) is used as a positive control. Solvent reference controls are run in parallel to the test substances. 0.534 mM solutions of cysteine and lysine peptides are prepared in phosphate (pH 7.5) and ammonium acetate (pH 10.4) buffers, respectively. The test substances are incubated with the peptide solutions for 24 hours at 25 °C. The ratios of test substance: peptides are 1:10 and 1:50 for cysteine and lysine respectively. Peptide depletion is monitored by HPLC coupled with UV detection at 220 nm. The mixture of top agar is vortexed and then poured on to a minimal glucose agar plate. The plates are allowed to harden and the inverted plates are incubated at 37 °C in a dark and vented incubator. After 48-hr incubation, the plates are checked and the revertant colonies are scored.

9.3 OECD 442D: *In Vitro* sensitization evaluation test: KeratinoSens™ (ARE-Nrf2 Luciferase)

Test samples are first serially diluted in buffer PBS and subsequently at 40% (w/v) in culture medium (Gibco™ 21885025). Each sample in three replicates are assayed, in two independent repetitions. Positive control: cinnamic aldehyde at five concentrations from 4 – 64 µM. Negative control: solvent for the positive control (DMSO) and solvent for the samples (PBS). Protocol: KeratinoSens cell line is derived from the human keratinocyte culture. It contains a stable insertion of a Luciferase gene under the control of the ARE-element of the gene AKR1C2. The KeratinoSens cell line is cultivated in DMEM supplemented with 10% FBS and incubated at 37°C under a humidified atmosphere with 5% CO₂ and 95% air. Cells are grown for 24 h in 96-well plates. The culture medium is then replaced with medium containing different concentration of assayed samples. Each sample is tested at 12 concentrations in the range of 0.195-400 µg/ml. Each test plate contains wells with the solvent control for positive control (DMSO; wells with no cells are used for background value) and wells with a dose response of the positive control cinnamic aldehyde (4-64 µM). PBS buffer

is used as solvent for analytes and negative control. The test samples and their dilutions are assayed in three replicates and in two independent repetitions for luciferase induction and cytotoxicity determination.

10. Statistics

One-way ANOVA statistical analysis is performed by Tukey's comparison and $P < 0.05$ was set as significant in all the tests. Data are presented as mean values $\pm$ standard deviation. All the calculations and graphs are produced in OriginPro software.

Figure S1. ¹H NMR spectral regions and chemical shifts of aromatic peaks: (A) pure Dz-COOH; (B) neat CaproGlu as isolated and purified product of grafting esterification (50% conjugation) reaction of Dz-COOH onto PCL-tetrol; arrow indicates traces of unreacted Dz-COOH - Integration values (I) quantify Dz-COOH < 0.1%.

Figure S2. FTIR spectra of CaproGlu before and after UVA activation (20 J).

Figure S3. Lap shear adhesion test experiment design: (A) schematic view of liquid CaproGlu application onto collagen surface and fixation into collagen + CaproGlu + PET sandwich structure for subsequent UVA crosslinking and mechanical test; (B) digital photograph of representative test sample before and after UVA crosslinking (20 J).

Figure S4. Digital photographs of samples prepared for biocompatibility analysis of leachates from polymer matrix (raw CaproGlu, crosslinked CaproGlu and internal control PCLT 1000; numbers correspond to nomenclature in **Table S1**); Samples #7-9 are of identical appearance as #10.

Table S1. Sample specification prepared for genotoxicity and sensitization of CaproGlu leachates (pure PCLT 1000 is used as internal control). “CaproGlu 2:1” represents diazirne-grafted polycaprolactone tetrol (PCLT; 1000 Da) with grafting concentration of 50%.

#	Samples	M (g)	UV (J)	Medium	Dillution	pH before titr.	pH after titr.
1	CaproGlu 2:1	0.512	50	PBS	0	7.25	7.25
2	CaproGlu 2:1	0.525	20	PBS	0	7.32	7.32
3	CaproGlu 2:1	0.539	0	PBS	0	7.42	7.42
4	CaproGlu 2:1	0.502	50	0.1N NaOH	Titrated with 0.2N CA	12.49	7.52
5	CaproGlu 2:1	0.5246	20	0.1N NaOH	Titrated with 0.2N CA	12.47	7.1
6	CaproGlu 2:1	0.522	0	0.1N NaOH	Titrated with 0.2N CA	12.5	7.2
7	Pure PCLT 1000	0.564	0	PBS	0	7.38	7.38
8	Pure PCLT 1000	0.522	50	0.1N NaOH	Titrated with 0.2N CA	11.58	7.15
9	Pure PCLT 1000	0.516	20	0.1N NaOH	Titrated with 0.2N CA	11.6	7.04
10	Pure PCLT 1000	0.521	0	0.1N NaOH	Titrated with 0.2N CA	11.85	7.26
	Control-1	7.45	PBS				
	Control-2	7.41	Water + NaOH + Citric acid				

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