

ORIGINAL ARTICLE

Design and development of low cost polyurethane biopolymer based on castor oil and glycerol for biomedical applications

A. C. W. Tan¹ | B. J. Polo-Cambrenell² | E. Provaggi³ | C. Ardila-Suárez² |
G. E. Ramirez-Caballero² | V. G. Baldovino-Medrano^{2,4} | D. M. Kalaskar³ 

¹UCL Division of Medicine, University College London, London, United Kingdom

²GIP Grupo de Investigación en Polímeros, UIS Universidad Industrial de Santander, Bucaramanga, Colombia

³UCL Institute of Orthopaedics and Musculoskeletal Science, Division of Surgery & Interventional Science, University College London, London, United Kingdom

⁴Laboratorio de Ciencias de Superficies (#SurfSciSchoolCo), Universidad Industrial de Santander, Piedecuesta (Santander) 681011, Colombia

Correspondence

Dr. Deepak M Kalaskar, Lecturer in Nanotechnology and Biomedical Engineering, Institute of Orthopaedics and Musculoskeletal Science, Division of Surgery and Interventional Science, University College London, Royal National Orthopaedic Hospital, Brockley Hill, Stanmore, Middlesex HA7 4LP, United Kingdom.
E-mail: d.kalaskar@ucl.ac.uk

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Abstract

In the current study, we present the synthesis of novel low cost bio-polyurethane compositions with variable mechanical properties based on castor oil and glycerol for biomedical applications. A detailed investigation of the physicochemical properties of the polymer was carried out by using mechanical testing, ATR-FTIR, and X-ray photoelectron spectroscopy (XPS). Polymers were also tested in short term *in-vitro* cell culture with human mesenchymal stem cells to evaluate their biocompatibility for potential applications as biomaterial. FTIR analysis confirmed the synthesis of castor oil and glycerol based PU polymers. FTIR also showed that the addition of glycerol as copolyol increases crosslinking within the polymer backbone hence enhancing the bulk mechanical properties of the polymer. XPS data showed that glycerol incorporation leads to an enrichment of oxidized organic species on the surface of the polymers. Preliminary investigation into *in vitro* biocompatibility showed that serum protein adsorption can be controlled by varying the glycerol content with polymer backbone. An alamar blue assay looking at the metabolic activity of the cells indicated that castor oil based PU and its variants containing glycerol are non-toxic to the cells. This study opens an avenue for using low cost bio-polyurethane based on castor oil and glycerol for biomedical applications.

KEYWORDS

biomaterials, biopolymer, castor oil, glycerol, molecular structure analysis, polyurethane

1 | INTRODUCTION

Polyurethanes (PU) are the most common synthetic polymers used for various medical devices and implants.^[1] They are used in the production of medical devices and implants such as catheters, heart valves, cardiovascular devices, and artificial organs.^[2] This is possible because of their excellent structural properties, elasticity, fatigue resistance, compliance and tolerance when used in the body.^[3] In addition, they are the most blood and biocompatible polymers available today.

Structurally, polyurethanes are formed by the chemical reaction of polyols and diisocyanate, which results in a copolymer comprising soft and hard segments. Urethane and urea linkages that associate by hydrogen bonds are polyurethane hard segments, whereas high mobile polyols chains are polyurethane soft segments.^[1] Usually, the polyols used for the process are hydroxyl or amine terminated polyesters, polyethers, polycarbonates, and in some cases polyolefin or hydrocarbons.^[1] Most of these polyols are products from the petrochemical industry. The high demand for petrochemical-based raw materials has led to their steadily

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increasing cost. Alternative raw materials such as non-edible vegetable oils are thus being explored for the production of polyurethanes.^[4]

Castor oil (CO) is a relatively low cost raw material that is non-edible and is obtained from the seed of *Ricinus communis*.^[5] The interest in using castor oil for synthesizing polyurethanes is because of its high content of ricinoleic acid and its hydroxyl groups that react with isocyanate groups forming polyurethanes. On the other hand, with the rapid growth of the biodiesel industry, by-products such as glycerol (GO) are also being made available at very low cost.^[6,7] Thus, combination of CO and GO provides an opportunity for synthesis of new, low cost biopolymers. The study of GO based polymers have been carried out before in previous studies. These studies adopt complex methods in enabling dynamic mechanical changes in a polymer to observe the effects on cellular behavior.^[8,9] Novel polymers such as poly(glycerol sebacate)-*co*-aniline pentamer (PGSAP) used on peripheral cell regeneration have manipulatable mechanical properties which are regulated by polycondensation of poly(glycerol sebacate) and aniline pentamer (AP).^[8] On the other hand, the utilization of an elastic poly(glycerol sebacate) (PGS) backbone and multiple hydrogen-bonding ureido-pyrimidinone (UPy) grafts have created a novel polymer with self-healing and shape memory properties.^[9] Our study takes a simpler approach in controlling the mechanical properties of the polymer by incorporating different ratios of glycerol and castor oil which is both more efficient and time-effective method in observing the cellular effects of different mechanical properties.

The use of castor oil as a polyol for the synthesis of polyurethane resins in the absence of biocompatibility studies has been reported before.^[10–14] However, due to a lower hydroxyl density along the glyceride chain, castor oil provided polyurethanes with low crosslinking density.^[13,15] This resulted in polyurethanes with low elastic modulus and lower resultant stiffness which effected its possible industrial applications.^[10,14] Castor oil transesterification with pentaerythritol, glycerol, and other hydroxylated molecules has been investigated^[16–18] to increase the hydroxyl index of polyurethanes as well as to produce a polyurethane network with better mechanical properties. Another approach is to increase the polyol hydroxyl number using suitable polyfunctional materials as co-monomers. This is the case with glycerol.^[4,18–21] Recently, the synthesis of biodegradable elastomers with tolylene-2, 4-diisocyanate-terminated poly (ethylene adipate) (PEA-TDI) with a mixture of castor oil (CO) and glycerol (GO) has also been reported.^[22]

Current study focuses on the use of castor oil and glycerol as polyols for producing polyurethane for applications in biomaterials. The effect of glycerol addition on the physicochemical properties of PU was investigated. Further, the biocompatibility of the synthesized PU polymers was tested for their potential use for biomedical implants and devices in short term in vitro culture with human mesenchymal stem cells.

2 | MATERIALS AND METHODS

2.1 | Materials

Castor oil was purchased from a local provider (Laboratorios León, Colombia) and glycerol (85% for analysis EMSURE Reag. Ph Eur) was

purchased from Merck (Germany). These reactants have hydroxyl numbers: 160 mg KOH/g and 1815 mg KOH/g, respectively, as determined by standard ASTM D 4274. Methylene Diphenyl Di-isocyanate (MDI) under the trade mark name Rubinate 5005 was purchased from Huntsman (USA) with a functionality of 2.7.

2.2 | Synthesis of polyurethane and its variants

A family of PU polymers was synthesized using castor oil and glycerol as polyols. The castor oil and glycerol % ratio (CO: GO) was varied: 100:0; 80:20; 60:40; and 40:60, to produce four different variants of PU. Initially, castor oil and glycerol polyols were mixed under vacuum and under continuous stirring at 70°C and then the MDI was added in equimolar ratio. The reaction was allowed to be continued for 2 min. The mixture was then poured into the module to cast thin films of the required thickness and shape.

2.3 | Characterization of the physicochemical properties of PU polymers

PU polymers were analyzed by different techniques to assess their molecular structure (Infrared spectroscopy), mechanical properties, and surface chemical composition and speciation (X-ray photoelectron spectroscopy).

2.3.1 | Mechanical properties

The mechanical properties of all PU samples were measured using Dynamical Mechanical Analysis (DMA) in a Q800 instrument. PU samples were placed under tension between a fixed and a movable clamp under a strain rate of 5% min⁻¹ and at a constant temperature of 30°C. Mechanical properties were obtained averaging samples in triplicates ($n = 3$). The crosslinking density (n) was calculated according to the following equation: $n = E0/(3RT)$, where $E0$ is the Young's modulus, R is the universal gas constant and T is the test temperature. The n calculated was considered as the combined density of the chemical and effective physical crosslinks. In the equation is assumed that the internal energy remains constant under length variation. Also, the polymer chains are freely joined.^[8,23]

2.3.2 | Molecular structure

The molecular structure of PU polymers was evaluated with Attenuated Total Reflection Fourier Transform Infrared spectroscopy (ATR-FTIR) using a Nicolet iS50 FT-IR instrument (Thermo Scientific). All spectra were collected with 32 scans and at a resolution of 4 cm⁻¹. The raw data was analyzed with the software OMNICTM provided with the instrument.

2.3.3 | Surface elemental composition

Surface elemental composition and surface chemical speciation of PU polymers were assessed by X-ray photoelectron spectroscopy (XPS). XPS experiments were carried out with the A Centeno-XPS/ISS/UPS surface characterization platform built by SPECS (Germany). The platform is provided with a PHOIBOS 150 2D-DLD Energy Analyzer Package. A monochromatized Al K α X-ray source (FOCUS 500) operated at

100 W was employed for measurements. The pressure in the analysis chamber was $\sim 1 \times 10^{-8}$ Pa. The pass energy of the hemispherical analyzer was set at 100 eV for the General spectra and to 60 eV for high resolution spectra. Polymers were cut to 1 cm \times 1 cm squares and mounted on carbon conductive tape over metallic sample holders for analysis. Surface charge compensation was controlled with a Flood Gun (FG 15/40-PS FG500 device) operated at 100A and 4.0 eV. General spectra were recorded first for all samples followed by high resolution spectra: C 1s + Mg KLL, O 1s, N 1s, Si 2p, Ca 2p, Na 1s, S 2p. Among these elements, Mg, Si, Ca, Na, and S are low concentration contaminants ($< 1.0\%$) firstly found in the general spectra (not shown here) of the samples. The stability of surface charge compensation was verified by recording the C 1s peak at the end of the analyses. Though the C 1s peaks were displaced by a few eV during analyses, they did not lose their original shapes. Data analysis was performed with the CasaXPS program (Casa Software Ltd) using the SPECS Prodigy library for R.S.F. values. A Tougaard base line was employed for background modelling together with a 15% Gaussian-Laurentzian line shape for peak decomposition. The binding energy (BE) scale of the spectra was corrected taking the C-(C, H) component of the C 1s peak at 284.8 eV as a reference.^[24] At least two independent samples of each material were tested in these measurements.

2.4 | *In vitro* characterization of PU

Protein adsorption was measured from 10% serum using BCA assay. The adhesion, morphology, and proliferation of Human Mesenchymal Stem Cells (HMSC) were investigated to study *in vitro* biocompatibility of all PU samples.

2.4.1 | BCA protein assay

For BCA assay, polymer samples were incubated in 10% serum containing Dulbecco's Modified Eagle Medium (DMEM) at 37°C for 4 h. Control samples were incubated in serum-free medium. The basal media was then removed and the samples were transferred into fresh well plates to only account for protein deposited on the test samples. Samples were then washed three times with PBS to remove any loosely adsorbed proteins. Then 150 μ L of BCA reagent (Thermo Scientific) was added to each well plate containing the PU samples and incubated at 37°C for 2 h. Then 200 μ L of reacted reagent was transferred to a sterile 96 well plate. The absorbance of the samples was measured at 562 nm on a colorimetric plate reader (Biotek Instruments). The standard curve was prepared using Bovine Serum Albumin (BSA) as per manufacturer's instructions, and the amount of protein adsorbed was calculated from optical density measurements carried out for the corresponding samples ($n = 6$).

2.4.2 | Cell cultures and seeding

Human bone marrow-derived mesenchymal stem cells (hMSC) were purchased from Lonza (UK). Cells were cultured and maintained using low glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Life Technologies) and 1% antibiotics (Sigma-Aldrich). Cells were trypsinized and passaged every 3

days. For cell seeding on PU samples, samples were placed into 24 well plates and sterilized using 70% ethanol, followed by washing with sterile PBS three times, before cell seeding. Samples were seeded with 1×10^4 cells per cm^2 . The media was changed every 3 days to maintain the cells.

2.4.3 | Cells adhesion and morphology

Cells adhesion and morphology were assessed by F-actin staining of actin cytoskeleton. For cytoskeleton staining, the cell culture media was removed from the wells at 4 and 24 h post cell seeding. Samples were washed three times with PBS and cells were fixed using 4% (w/v) paraformaldehyde (Sigma-Aldrich) for 20 min. Samples were again washed in PBS and permeabilized by adding 0.1% Triton X-100 (Sigma-Aldrich) in PBS for 5 min. Alexa Fluor 488® Phalloidin (Life Technologies) was used to fluorescently label actin stress fibers in the ratio of 1:40 in PBS by incubating with samples at room temperature for 30 min. Cells were then washed thrice in PBS. DAPI (1:1000 to PBS) was added to the cells to label nuclei at room temperature for 15 min. Samples were washed and mounted using Prolong® Gold Antifade agent (Life Technologies). Samples were observed using a fluorescent LSM 800 confocal microscope by Zeiss. Images were taken at 20 \times magnification for each sample in order to carry out further quantitative analysis on the cellular morphology of cells on PU samples. The images captured were analyzed using image analysis software ImageJ (<https://imagej.nih.gov/ij/>). The perimeter of each individual cell was traced and the mean cell area and circularity were calculated. Approximately 30 cells per sample were measured ($n = 30$).

2.4.4 | Metabolic activity of the cells

Metabolic activity of the cells was measured using an Alamar blue assay kit (Molecular Probes, UK). PU samples were placed into 24 well plates and sterilized using the method previously described. Cells were then seeded on the polymer sample at a concentration of 1×10^4 cells. At specific post cell seeding time points; day 1, 3, and 7, PU samples were moved to a new sterile 24 well plate to ensure that the Alamar blue readings only accounted for the cells attached to the polymers. The Alamar blue assay was performed in accordance with the manufacturer's protocol. In summary, 1 mL of 10% Alamar blue in DMEM were added to each well and incubated at 37°C for 4 h. Hundred microliters of the reagent was then transferred into 96-well plates and the fluorescence was measured at excitation and emission wavelength of 530 nm and 620 nm respectively using the Fluoroskan Ascent FL plate reader (Thermo LabSystem, UK). The same set of cells in each 24 well plate of a specific time point was then washed with PBS and 500 μ L of distilled water was added to each well. The well plates were then stored in a freezer at 6°C for future use.

2.4.5 | Cell growth using DNA quantification

To measure the cellular growth on PU samples, Fluorescence Hoechst DNA Quantification Kit (Sigma, UK) was utilized. The assay was performed in accordance with the manufacturer's protocol. The fluorescence was measured at specific post seeding time points; day 1, 3, and 7, using the Fluoroskan Ascent FL (Thermo LabSystems) at excitation

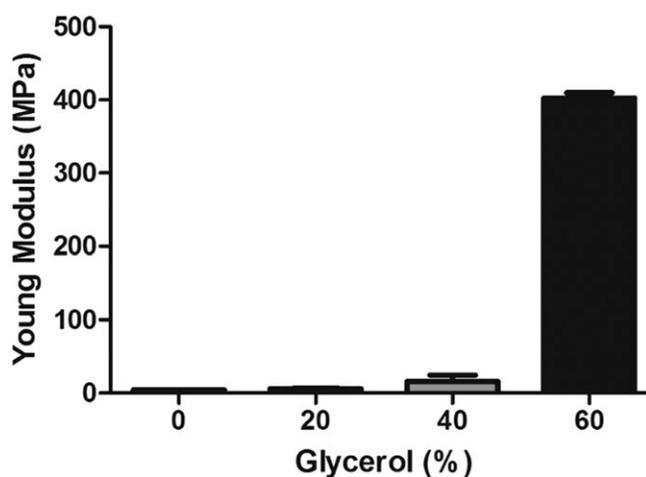


FIGURE 1 Effect of glycerol addition on mechanical properties of castor oil based polyurethane (PU). ($N = 3$)

and emission wavelengths of 360 nm and 460 nm, respectively. A standard curve was prepared with a known concentration of DNA and later used to calculate DNA content for test samples.

2.5 | Statistical analysis

All error bars on data are expressed as the standard error of the mean (SEM). The statistical significance was determined at 95% level using a one-way ANOVA procedure. In this case, * represents $p < 0.05$, ** represents $p < 0.01$ and *** represent $p < 0.001$. Post-hoc statistical analysis of the means of individual groups was performed using Tukey's test.

3 | RESULTS AND DISCUSSION

3.1 | Effect of glycerol addition on the mechanical properties of castor oil based PU

The effect of glycerol addition on the mechanical properties of the castor oil based polyurethane is presented in Figure 1. Young's modulus of PU polymer increased with glycerol content. PU with only castor oil and 0% glycerol showed the lowest Young' modulus (4.4 MPa). Young's modulus of the PU polymers slightly increased for glycerol contents of 20% (6 ± 3 MPa) and 40% (16 ± 8 MPa). In contrast, for 60% glycerol, the synthesized PU displayed the highest modulus: 403 ± 7 MPa. These results show that the use of glycerol promotes the mechanical properties of the PU polymer. It has been reported that castor oil-based polyurethanes exhibit low mechanical properties compared with commercial fossil-derived ones due to its low hydroxyl number leading to polyurethanes with low crosslinking density.^[25] To improve the Young's Modulus of castor oil based PU some authors have employed nanofillers such as graphene and clays.^[26] Yang et al.^[27] introduced hydroxyapatite to a castor-oil polyurethane matrix to increase the bio-activity of the polymer. However, the addition of these fillers could affect the polyurethane behavior for biomedical applications. Another alternative to improve mechanical strength is the addition of a comonomer during polyurethane synthesis. For example, Nguyen Dang

et al.^[28] incorporated polydimethylsiloxane polyol (PDMS) during the synthesis of a castor oil-based polyurethane and showed improved mechanical strength. In current study, the polyurethanes are formed by the reaction of three chemical constituents: a long chain diol obtained from castor oil, a small diol obtained from glycerol and diisocyanate. The resulted polyurethanes can be considered as copolymers formed by soft and hard segments. Hard segments are formed by urethane and urea linkages associated by hydrogen bonds. Soft segments are formed by high mobile castor oil chains. Addition of a small molecule with three hydroxyl functional groups as glycerol increased hard domain density. Moreover, the addition of glycerol increased physical contributions that are produced by the monodentate and bidentate hydrogen bonding between urethane and urea groups respectively.^[18,23,29] Yilgör et al. reported that the H-bonding in urea groups displays stronger interactions than urethane groups,^[29] which is augmented if high levels of glycerol are added. These results are according to the Wu et al. work in which the mechanical properties of synthesized materials are dramatically increased due to the addition of ureido-pyrimidinone (UPy).^[9] Results suggested that when the content of glycerol increased 60% the increased hard domains density and physical crosslinking of hydrogen bonding introduced by presence of glycerol start to play a dominate role in final mechanical properties resulting in a dramatically change in young's modules. This is further confirmed by the crosslinking density of PUs samples with various concentration of glycerol (Table 1). Crosslinking density goes from 577 ± 1.8 mole/m³ for 0% glycerol to $53,255.9 \pm 919.4$ mole/m for 60% glycerol. This thus, further confirms that the significant increase in Young's modulus of 60% glycerol sample are related with changes in crosslinking density introduced by presence of glycerol.

3.2 | Effect of glycerol addition on the molecular structure of castor oil based PU

The molecular structure of the synthesized bio-polymers was investigated with ATR-FTIR analysis. Figure 2A,B shows ATR-FTIR spectra for the synthesized PU bio-polymers. In Figure 2A, the absence of a band at 2225 cm^{-1} corresponding to $\text{N}=\text{C}$ stretching indicates lack of detectable amounts of unreacted MDI. On the other hand, the appearance of the bands corresponding to $\text{C}=\text{O}$ stretching from urea and urethane functional groups and the stretching of the NH bond ($3330\text{--}3450 \text{ cm}^{-1}$) characteristic of urethane functional group, confirm the formation of urethane and urea bonds. When $\text{C}=\text{O}$ functional groups are interacting with hydrogen bonds, the carbonyl bond length is

TABLE 1 Crosslinking density of PU polymer as a function of glycerol concentration

Glycerol content (%)	Young's modulus (MPa)	Crosslinking density (mol/m ³)
0	4.4	577.1 ± 1.78
20	6.1	808.5 ± 34.69
40	16.2	2141.1 ± 1095.97
60	402.7	$53,255.9 \pm 916.43$

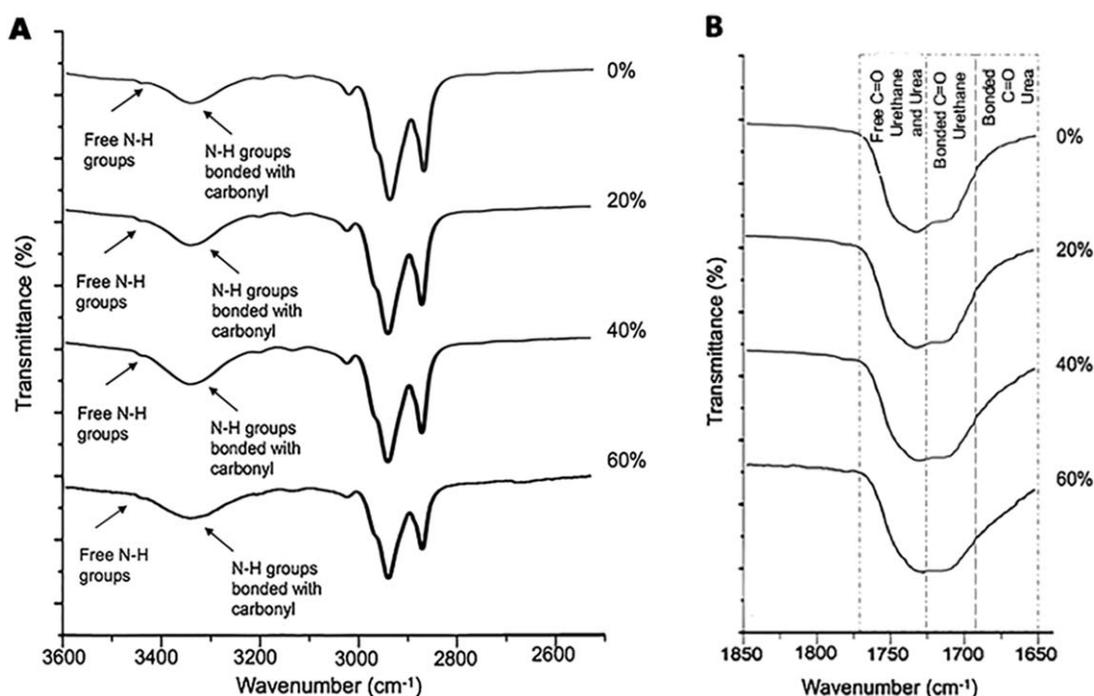


FIGURE 2 ATR-FTIR spectra for castor oil based PU with various concentration of glycerol. A) General spectra showing free and carbonyl bonded N-H groups. Shift in carbonyl bonded N-H group is observed with increase glycerol content. B) High resolution IR spectra showing free and bonded urethane and urea groups

modified leading to a reduction of its stretching vibration frequency.^[30] Figure 2B shows an asymmetrical shape of the band around 1725 cm^{-1} , indicating that a free C=O stretch is overlapping with a hydrogen bonded C=O.^[31] Free C=O from urethane and urea groups are centered at $\sim 1725\text{--}1740\text{ cm}^{-1}$ and at $1710\text{--}1725\text{ cm}^{-1}$, respectively. Hydrogen bonded C=O linkages are centered approximately at $1670\text{--}1685\text{ cm}^{-1}$ and at $1644\text{--}1655\text{ cm}^{-1}$. All PU compositions showed a low intensity band corresponding to N—H stretching at 3332 cm^{-1} . This confirms the presence of hydrogen bonding interactions,^[32] Figure 2A. These results show that glycerol may act as a crosslinker for PU. The increment in the shoulder located around 1670 cm^{-1} corresponding to C=O stretching from urethane and urea groups interacting by hydrogen bonds, as the glycerol content in PU increases can be considered as further evidence of crosslinking. Such crosslinking with short carbon chains correlate with an increase in hard domain density self-associated via hydrogen bonding. The interaction of functional groups by hydrogen bonds is a physical crosslinking that increases materials stiffness. In consequence, the enhancement in mechanical resistance of glycerol/castor oil PU reported earlier can be ascribed of crosslinking as described above.

3.3 | Effect of glycerol addition on the surface properties of castor oil based PU

Static water contact angle (WCA) measurement on all the samples shows no significant changes (Supporting Information Figure S1). All samples show WCA between $80 \pm 4^\circ$, representing hydrophobic interface. Detailed surface analysis is however established using XPS analysis. Table 2 shows normalized carbon, oxygen, and nitrogen surface

concentrations for the prepared materials. In general, surface carbon concentration decreased after incorporation of glycerol for PUs prepared in the absence of this polyol. Conversely, surface oxygen concentration increased when glycerol was employed in PU synthesis. Concerning the concentration of surface nitrogen, its concentration slightly increased in glycerol modified PUs but it was rather constant as a function of the glycerol content. An assessment of surface chemical species can be derived from a decomposition of the recorded high-resolution C 1s, O 1s, and N 1s spectra. Supporting Information Figure S2 (A,B) feature typical O 1s and N 1s spectra, respectively, of the synthesized polyurethanes. In general, contributions from inorganic and organic oxygen were distinguished in the O 1s peak (Supporting Information Figure S2).

Inorganic oxygen species may be related to the presence of contaminants already mentioned in the experimental section. Concerning nitrogen, the N 1s peak displayed a single contribution centered on 400.0 eV . This peak is typical of nitrogen linked to carbon.^[33] A

TABLE 2 XPS data of Elemental decomposition of C, O and N for glycerol content in castor oil based PU 0%, 20%, 40% and 60% of glycerol content, respectively.

Glycerol (%)	mole (%)		
	C	O	N
0	86.0 ± 1.00	13.8 ± 1.00	0.12 ± 0.04
20	66.5 ± 3.70	33.3 ± 3.70	0.16 ± 0.03
40	58.5 ± 2.80	41.4 ± 2.60	0.16 ± 0.01
60	79.8 ± 11.40	20 ± 13.10	0.16 ± 0.05

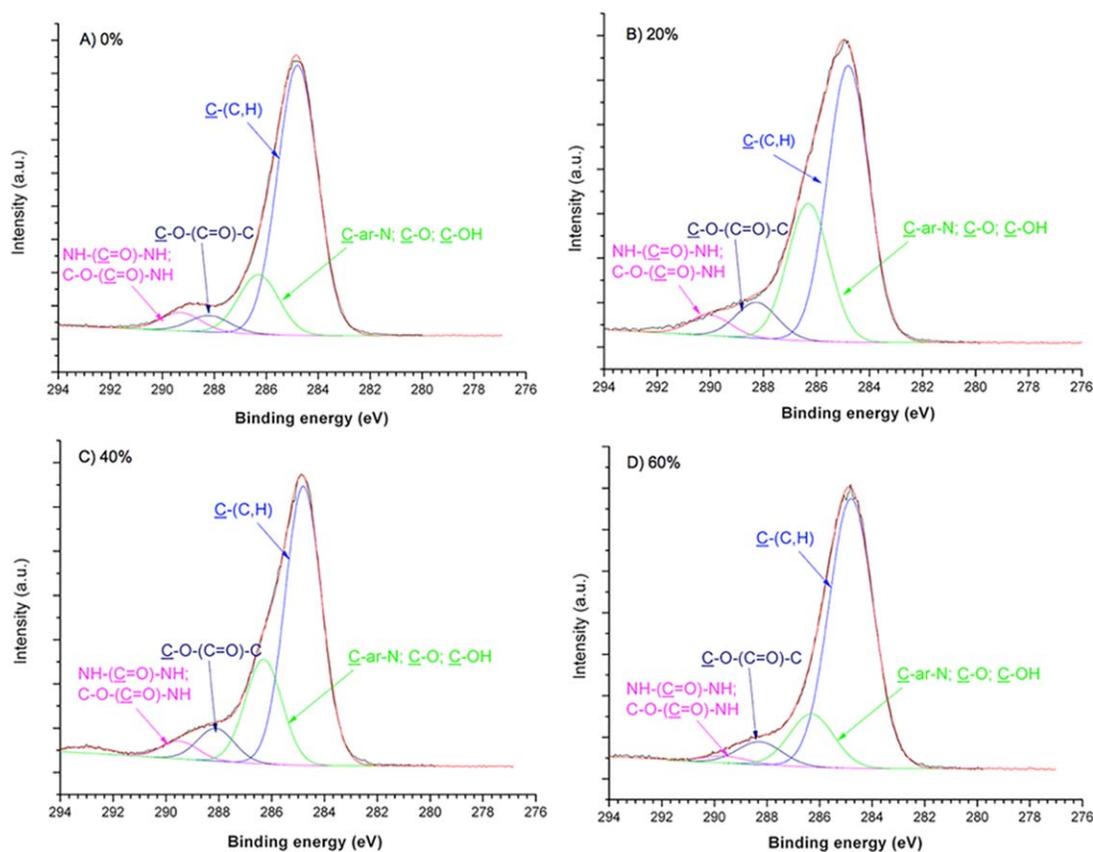


FIGURE 3 Decomposition of the C 1s peaks recorded in XPS analyses for various concentration of glycerol in PU. The C-(C, H) component at 284.8 eV was taken as a reference for calibrating the Binding energy scale

decomposition of the recorded C 1s peaks of the materials is presented in Figure 3 (A) = 0%; 3(B) = 20%; 3(C) = 40%; and, 3(D) = 60% of glycerol content.

The C 1s peak was decomposed into four main contributions; namely, $\underline{\text{C}}-(\text{C,H})$ at 284.8; $\underline{\text{C}}-\text{O}-\text{C} + \underline{\text{C}}-\text{OH} + \underline{\text{C}}-\text{ar}-\text{N}$ at 286.3; $\underline{\text{C}}-\text{O}-(\text{C}=\text{O})-\text{O}$ at ca. 288.3; and $\text{NH}-(\text{C}=\text{O})-\text{NH} + \text{C}-\text{O}-(\text{C}=\text{O})-\text{NH}$ at ~ 289.5 eV.^[34,35] Where, ar stands for an aromatic structure. Overlapping of these contributions with adventitious carbon peaks^[36] is inevitable. Herein, we assume that such contamination is similar for all measured samples. Therefore, our analyses are restricted to comparing trends regarding changes in the relative percentages of the above samples within the samples analyzed herein. Table 3 shows results from C 1s peak decomposition performed as announced above.

Results show that glycerol incorporation to polymers leads to a surface enrichment of carbon oxidized species. Particularly, of those species related to the aliphatic chains of the polymer: $\underline{\text{C}}-\text{O}-\text{C}$, and of

those from polymer precursors: $\underline{\text{C}}-\text{O}-(\text{C}=\text{O})-\text{O}$ from ricinoleic acid and $\underline{\text{C}}-\text{OH}$ from glycerol. These species are linked to the chemical reaction between ricinoleic acid and glycerol during the polymerization process. Concerning the surface chemical species associated to urethane; $\text{NH}-(\text{C}=\text{O})-\text{NH}$, and urea; $\text{C}-\text{O}-(\text{C}=\text{O})-\text{NH}$, their concentration decreased with the increase in glycerol concentration.

Particularly, surface analysis showed that as the glycerol content increases during the synthesis of PU, the concentration of polymer aliphatic chains increases on PU surface (soft domains) whereas species associated to urethane and urea decreases (hard domains). This result suggests that hard domains interaction by hydrogen bonding favored by glycerol as crosslinking agent propitiated segregation of mobile soft aliphatic chains toward surface. Furthermore, if hard domains occupy large areas of the surface of the polymer, their relative surface concentration as derived from XPS will decrease since in the technique, smaller structures have stronger signals. This is due to the fact that the

TABLE 3 XPS data of C Contributions species for glycerol content in castor oil based PU 0%, 20%, 40% and 60%.

Glycerol (wt.%)	$\underline{\text{C}}-(\text{C,H})$	$\underline{\text{C}}-\text{ar}-\text{H}; \underline{\text{C}}-\text{O}-\text{C}; \underline{\text{C}}-\text{OH}$	$\underline{\text{C}}-\text{O}-(\text{C}=\text{O})-\text{C}$	$\text{NH}-(\text{C}=\text{O})-\text{NH}; \text{C}-\text{O}-(\text{C}=\text{O})-\text{NH}$
0	76.4	14.5	4.1	5.0
20	62.6	26.3	6.6	4.5
40	63.8	24.8	7.4	4.0
60	69.2	22.1	6.3	2.5

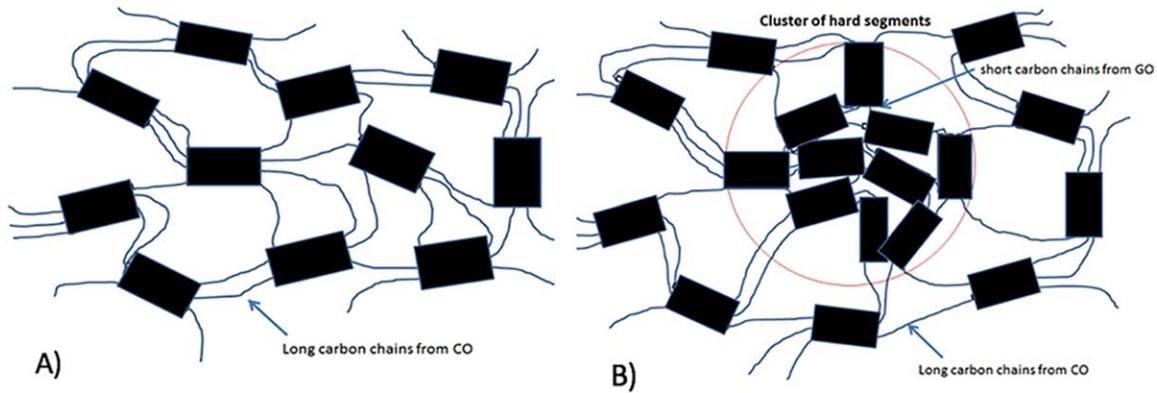


FIGURE 4 Schematic representation of molecular arrangements of hard and soft domains in castor oil based PU polymer formed without and with addition of glycerol. A, Represents PU formed by crosslinking of long carbon chains like those of castor oil. B, Represents PU formed by crosslinking of long and short carbon chains similar to those of castor oil and glycerol respectively

latter are better distributed on the surface of a given sample. Schematic representation of polymer segments (hard and soft) organization within PU polymer without and with addition of glycerol is shown in Figure 4.

3.4 | Biocompatibility of castor oil based PU

Cells interact with materials through an adsorbed layer of proteins deposited through blood serum.^[26] Figure 5A shows protein concentration as a function of glycerol wt. % in PU. A correlation between protein adsorption with the increase in glycerol content when incubated with serum containing media is evidenced. This trend correlates with the mechanical stiffness of PU as shown in Figure 5B. Water contact angle do not change with increase in concentration of glycerol during polymer synthesis (Supporting Information Figure S1). Most samples show WCA between $80 \pm 4^\circ$. This suggests that changes in protein adsorption may not be related to hydrophilicity of the polymer, instead in this study protein adsorption is the true reflection of changes in stiffness of the polymer. XPS analysis suggests that aliphatic (soft segments) increases as glycerol (%) increases in polymer backbone, providing stiffer polymer. Type of available aliphatic groups made available by unique chemistry during these PU polymer synthesis might be responsible for more protein adsorption on the polymer surface. However, exact reason for this effect remains unclear. The effect of increase in protein adsorption due to polymer stiffness is however evident on cellular morphology.

When the cellular response to polymers composition was studied in short term cultures, cells showed a variable cell morphological behavior depending on polymer compositions. Figure 6A shows the morphology of human MSC cells on castor oil based PU with varying concentration of glycerol as polyol and the quantification of cell morphology in terms of circularity (Figure 6B) and mean cell area (Figure 6C) for $n = 50$, respectively. Cells on the polymer without glycerol, i.e. the softest one, showed a limited cell spreading as shown by the corresponding circularity index and mean cell area. Cell spreading increased with glycerol content which is also related to the increase in stiffness of the polymer. PU with the highest concentration of glycerol (60%) showed the highest mean cell area which was similar to the one found

over the control glass surface. This suggests that cell spreading was proportional to the stiffness of the polymer. Effect of material stiffness is well documented in the literature. Results seen in this study are in line with previously reported data, where material stiffness has been shown to have effect on cellular morphology.^[37] Predominantly, soft

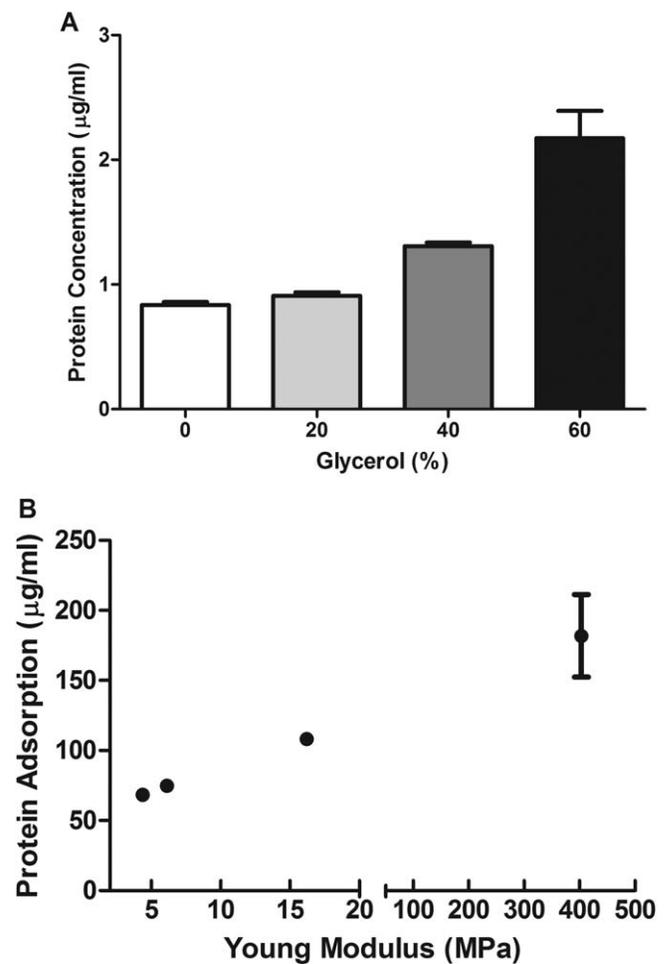


FIGURE 5 A, Effect of glycerol addition on serum protein adsorption on castor oil based PU ($n = 4$). B, Correlation between protein adsorption and mechanical stiffness of PU polymer

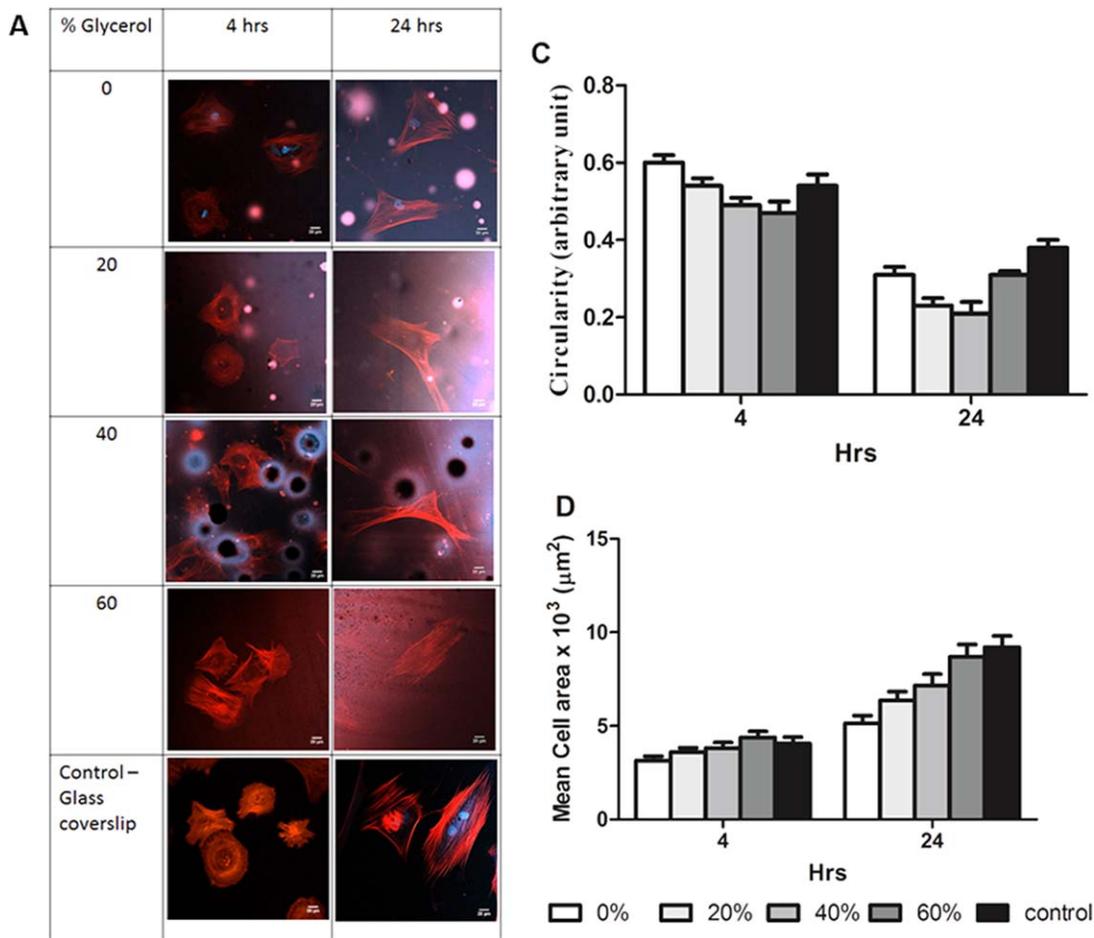


FIGURE 6 A, Morphology of human MSC cells on castor oil based PU with varying concentration of glycerol as polyol, quantification of cell morphology in terms of B, Circularity and C, Mean cell area where ($n = 50$)

material retain round, whereas stiff materials facilitated spreading of the cells.^[37–39] Cells interact with surface via adsorbed protein layer, and it is this layer which governs intra and extra cellular behavior. When cell attached strongly to the protein rich layer via integrin's, they are able to form clusters of focal adhesions points. Cytoskeleton organization of cells and actin stress fiber formation is closely governed by the density these focal adhesion points on the surface.^[26] It is also responsible for the shape or morphology of cells. Especially with stem cells, it is shown that changes in cells shape can predict their differentiation lineage.^[26] It is possible that increase in protein adsorption on PU polymers with increase stiffness, facilitated higher integrin's clusters and help in organization of actin cytoskeleton leading to more spread morphology.

The metabolic activity of the cells was also studied using Alamar blue in short term culture to ascertain biocompatibility of PU polymers with human mesenchymal stem cells. Figure 7 presents the quantification of metabolic activity using Alamar blue assay of human MSC cells on castor oil based PU with varying concentration of glycerol (%) as polyol. The metabolic activity of the cells did not show any significant changes with respect to each other or to the control on day 1. On day 3, cells showed similar level metabolic activity on all PU samples, and were significantly different with respect to control (p -value < 0.05). On

day 7, the PU sample with the highest concentration of glycerol (60%) showed a significantly higher metabolic activity (p -value < 0.05) as compared with 0%, 20% and 40% glycerol containing PU samples. However, 60% glycerol containing samples remained non-significant

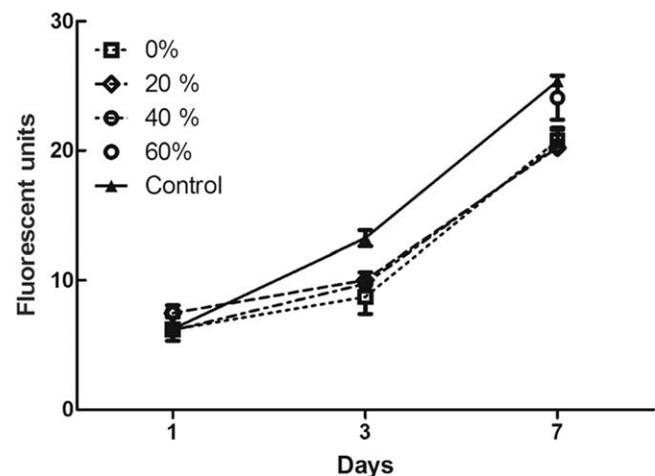


FIGURE 7 Quantification of metabolic activity using alamar blue assay of human MSC cells on castor oil based PU with varying concentration of glycerol (%) as polyol ($N = 4$)

with respect to control. Overall, the metabolic activity increased with time for all PU samples, suggesting a viable cell growth on all samples. Hence, all PU polymer compositions can be considered to remain non-toxic to the human mesenchymal stem cells.

The use of castor oil based PU for medical applications and studies focusing on its use for cells/tissue contacting medical devices have been poorly reported in the literature. In terms of scaffold fabrication in tissue engineering, a great variety of natural and synthetic materials have been investigated. Although natural polymers have favorable properties of biodegradability, low toxicity and environmental compatibility, the lack of control over mechanical properties lead to less control over the resorption rates *in vivo*.^[40] On the other hand, the current widely used synthetic polymers with FDA approvals include PGA and PLA. These synthetic polymers have proven to be advantageous due to their adjustable physical and mechanical properties. However, these synthetic polymers release degradation products which lead to a strong inflammatory response.^[41] In our previous work synthetic nanocomposite polyurethane polymer has been extensively tested for various tissue engineering applications. However, higher cost of raw material and complex chemical process of synthesizing this material significantly increases its cost. Apart from this, this synthetic polymer still needs further bulk or surface^[2,42,43] modification to improve its biological properties. The ability to tailor the bulk mechanical properties of castor oil based biopolymer PU by adding various amount of glycerol thus provides a unique opportunity to produce low cost polyurethane for biomedical applications. This also reduces need for further manipulation of surface or bulk properties, making whole application process simple and easy.

5 | CONCLUSIONS

This study shows that the addition of glycerol provides polyurethanes with the ability to tailor their bulk mechanical properties. From an interpretation of ATR-FTIR and XPS data, it was possible to postulate that mechanical strength enhancement was linked to the formation cross-linked hard and soft domains within the polyurethanes and to a promotion of hydrogen bonding after glycerol addition. The increase in glycerol loading during synthesis would thus promote the formation of a larger proportion of hard polymeric domains over soft polymeric domains. These hard domains are associated with a promotion in the biocompatibility of castor oil-glycerol based polyurethanes. Indeed, short term *in vitro* studies showed a positive correlation between glycerol content, mechanical strength and protein adsorption, and cell morphology. The effect observed on protein adsorption and cell morphology did not affect the metabolic activity of the cells. Therefore, this investigation provides the confidence that castor oil based PU with and without glycerol is compatible for cellular activity and thus can be further explored for specialized medical applications. Especially, the ability to tailor the mechanical properties of the polymer via the addition of glycerol provides a basis for future studies directed to the differentiation of stem cells in particular lineages by alteration of the bulk mechanical properties of the polymers.

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ORCID

D. M. Kalaskar  <http://orcid.org/0000-0002-0576-1816>

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