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Synthesis and characterization of gelatin based polyester urethane scaffold

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Abstract. For tissue engineering purpose two gelatin based polyester urethane scaffolds of different compositions were prepared from lactic acid, polyethylene glycol 400 (PEG 400) and characterized by FTIR, XRD for their mechanical and morphological properties using SEM and optical microscopic analyses. Degradation and swelling studies of gelatin based polyester urethane scaffolds in phosphate buffer saline (PBS) were performed. Human keratinocyte cells were cultured within these scaffolds, which showed good cell adherence and proliferation.

Keywords. Polyester urethane; scaffold; tensile strength; swelling; degradation; cell culture.

1. Introduction

The development of tissue engineering devices and cell-based artificial organs requires a large number of cells to be cultured for the replacement of the damaged tissue. Porous biodegradable polymeric scaffolds would be ideal for these applications. In some applications, porous sponges were designed to serve as an analogue of extracellular matrix in order to provide a suitable substrate for cell attachment to enhance certain anchorage dependent processes such as migration, mitosis and matrix synthesis (Sato *et al* 1994; Gutsche *et al* 1996; Bock *et al* 2000; Chupa *et al* 2000; Aung *et al* 2002). Various biodegradable porous polymer scaffolds have been extensively used as templates for tissue regeneration (Davis and Vaccanti 1996). For these applications, porous structures with size range 100–500 μm, along with well-interconnected open pores, are required for allowing high density cell seeding and efficient nutrient and oxygen supply to the seeded cells (Park 2002). Such scaffolds also must be biocompatible, non-toxic to the cells and be capable of cell adhesion and allowing the retention of the metabolic functions of attached cells. Zhang and others (Zhang *et al* 2000, 2003) synthesized porous polyurethane scaffolds from lysine diisocyanate (LDI), glucose, and poly (ethylene glycol) (PEG), which supported the attachment, proliferation and differentiation of rabbit bone marrow stromal cells and degraded to non-toxic decomposition products (e.g. lysine and glucose) *in vitro*. These materials also induced a minimal foreign body response *in vivo*, with the formation of a capsule around the degrading implant (Zhang *et al* 2002). Guan *et al* (2002, 2004) synthesized two kinds of biodegradable polyurethane ureas, viz. poly (ester urethane) urea (PEUU) and poly (ether–ester urethane) urea (PEEUU), polycaprolactone, polycaprolactone-*b*-polyethyleneglycol-*b*-polycaprolactone, 1,4-diisocyanatobutane and putrescine, which are highly flexible and strong. Given the thermoplastic nature of these polymers, it was hypothesized that these polyurethanes could be fabricated into flexible scaffolds using a variety of techniques. Heijkants *et al* (2005) synthesized polyurethanes from poly (*e*-caprolactone) (PCL) and 1,4-butane diisocyanate with different soft segment lengths and constant uniform hard segment length in absence of catalyst for the production of a degradable meniscus scaffold. Using the recently developed surface modification technique, free amino groups have been introduced into polyester-type polyurethane scaffolds. The introduction of these free amino groups increased the surface energy and provided a convenient way to further immobilize bioactive species such as gelatin, collagen or chitosan, etc on the scaffold surface by employing glutaraldehyde as a coupling agent. These modifications are advantageous to enhance cell–material interaction (Zhu *et al* 2004). Gelatin, which is a natural polymer extracted from collagen, was used as a tissue engineering scaffold (Choi *et al* 1999a, b, 2001). Hong *et al* (2001) have shown the efficacy of cross-linked gelatin-based sponges composed of gelatin and polysaccharides for wound-dressing materials. Crosslinked gelatin sponges have also been investigated for their potential application as a component of artificial skin or tissue transplants to promote epithelialization and granulation tissue formation in wound.

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Many biodegradable polyester urethane scaffolds have been synthesized for different biomedical applications. Although there are reports on scaffolds made separately from polyester urethanes and gelatin but there is no report on gelatin based polyurethane scaffold for soft (skin) tissue engineering. So the main objective of this investigation was to synthesize some gelatin based polyester urethane scaffolds for soft tissue engineering applications, which would be biodegradable as well as biocompatible. In this paper, synthesis and characterization of some gelatin based polyester urethane scaffolds are reported. Human keratinocyte cell growth was carried out within these scaffolds. The degradation of the scaffold was also studied in phosphate buffer solution (pH = 7·4).

2. Experimental

2.1 *Materials*

The following materials were used in the present study: DL-lactic acid (88%) (LA), Quest Chemicals, India; polyethylene glycol 400 (PEG 400), E. Merck, India; dibutyltindilaurate (DBTDL), Fluka (used without purification); toluene diisocyanate (TDI), Merck, Germany (used as received); tetrahydrofuran (THF), E. Merck, India (dried over metallic sodium); benzene, Quest Chemicals, India; sulfuric acid, Quest Chemicals, India; sodium chloride, E. Merck, India; gelatin (bacteriological grade, mol. wt. 46374), Qualigens, India; glutaraldehyde, E. Merck, India; human keratinocyte (HaCaT) cells was a kind gift from Hindustan Lever Research Centre, India; Dulbecco's Modified Eagle's Medium (DMEM), Hi-Media, India; fetal calf serum (FCS), Gibco, India; penicillin-streptomycin antibiotic solution, Hi-Media, India; phosphate buffer saline (pH = 7·4) (PBS), Hi-Media, India; trypsin-EDTA (0·25% trypsin and 0·02% EDTA), Hi-Media, India; trypan blue (0·5%), Sigma Chemicals Co. USA and doubly deionized water (Milli-Q-water).

2.2 *Synthesis of gelatin-based polyester urethane scaffold*

Gelatin based polyester urethane was prepared in three steps. In the first step, an ester diol was synthesized in benzene by reacting lactic acid (LA) with polyethylene glycol 400 (PEG 400) (1 : 2 molar ratio) in presence of sulfuric acid as catalyst in a round-bottomed flask with an attachment of Dean and Stark apparatus. Benzene was added in such a quantity as to have a 50 wt% of the reagents. The reaction mixture was refluxed for 10 h at 80°C for maximum conversion of lactic acid and PEG 400 to the polyethylene lactate ester diol. In this reaction 95% conversion of lactic acid was achieved. The polyethylene lactate ester diol was collected by vacuum distillation. Subsequently, gelatin solutions (2·5% and 5%) were prepared by dissolving in doubly deionized water. In the second step, a prepolyurethane was synthesized by reacting the ester diol (4·1884 g) with 2,4-toluene diisocyanate (TDI) (2·438 ml) (in the molar proportions (NCO : OH = 2 : 1)) taken in dry THF (50 wt% of the reactants) in presence of DBTDL (0·05 wt%) as catalyst. The prepolyurethane was obtained as a viscous solution in THF. In the third step, viscous prepolyurethane solution in THF and 5 wt% glutaraldehyde were added to the gelatin solution (1·25 g and 2·5 g gelatin in 50 ml water) and the reaction mixture was stirred. Within 5 min the mass became more viscous as a result of some reaction among prepolyurethane, gelatin and glutaraldehyde. The viscous mass was then poured on a flat base glass petri-dish. The petri-dish was then inserted into a vacuum chamber made of glass. Within the vacuum chamber a small quantity of water was kept deliberately for supplying water vapour required for curing of polyurethane scaffold during simultaneous foaming of the polyurethane by solvent evaporation. Then by slow evacuation (gas foaming method), porous spongy polyurethane was formed and maintained in this inflated spongy form atleast for 10 h with simultaneous curing by moisture within the closed glass chamber. Two different gelatin based polyurethane scaffolds were prepared by varying the gelatin contents and designated as PUG1, which contained 5% gelatin and PUG2, which contained 2·5% gelatin. The spongy polyurethane slabs were taken out and finally sterilized by boiling in deionized water for 20 min.

2.3 *Characterization*

2.3a *FTIR spectroscopic analysis*: For structural analysis, FTIR (in ATR mode) spectra of the gelatin based polyurethane scaffolds were taken using Thermo Nicolet FTIR, Model Nexus 870. The polyurethane scaffolds were dried and kept in a vacuum desiccator for 24 h. The spectra were taken in the frequency range 400–4000 cm^{-1}.

2.3b *XRD analysis*: To know whether the prepared gelatin based polyurethane scaffolds are crystalline or amorphous, X-ray diffraction study was done. The samples were prepared and conditioned as done for FTIR analysis. The XRD study was done using Phillips XRD Analyser (Model PW 1729), Holland, for the range $2q$ = 10–80° using Cu as target.

2.3c *Contact angle measurement*: In order to have some idea about the hydrophilicity of the synthesized gelatin based polyester urethanes, the contact angles of the scaffold slabs were measured by using Rame Hart Goniometer (model 100–00–230).

2.3d *Swelling behaviour in PBS*: Swelling of the gelatin based polyurethane scaffolds in PBS solution at 37°C was measured by immersing the samples till equilibrium swelling (the samples achieved equilibrium swelling within

24 h). The swollen samples were taken out from PBS solution and its surface was wiped with tissue paper followed by immediate weighing in a previously weighed weighing bottle. The swollen weight was recorded and % equilibrium swelling was calculated using the following formula

$$\%\ \text{Equilibrium swelling} = \frac{(W_f - W_i)}{W_i} \times 100,$$

where, W_i is the initial weight of the dry gelatin based polyurethane sample and W_f the weight of the swollen gelatin based polyurethane sample.

2.3e *Optical microscopy*: Optical microscopy of the gelatin based polyester urethane scaffolds was done using a light optical microscope (Leica). The samples were prepared and conditioned as done for FTIR analysis.

2.3f *Scanning electron microscopy*: Scanning electron microscopy of the gelatin based polyester urethane scaffolds was done using JEOL SEM, model JEOL–JSM 5800. The samples were prepared and conditioned as done for FTIR analysis. The polymer films were gold coated before the study. Photographs were taken at 100 and 1000 magnifications.

2.3g *Tensile strength measurement*: For measurement of tensile strength the gelatin based polyester urethane scaffold (PUG1 and PUG2) samples were prepared by the following method. The polyurethane scaffolds were kept in a vacuum desiccator overnight. Then the scaffolds were cut into rectangular strips of 5 mm width and were subjected to test of various mechanical properties like tensile strength, elongation at break and modulus using a Hounsfield UTM tensile testing machine according to ASTM D 424 standard. The strips were well gripped using thick paper during the measurement of tensile strength and elongation at break.

2.4 *Degradation study in phosphate buffer solution*

Gelatin based polyester urethane scaffold (PUG1 and PUG2) samples were cut into rectangular strips of 10×50 mm and weights were taken. Each sample was boiled in water for 20 min to remove any unreacted isocyanate. Then the samples were sterilized by 70% alcohol followed by washing with sterile water for five times. The sterilized samples were put into the phosphate buffer saline solution (pH 7·4) at 37°C in a BOD incubator shaker and after different time intervals samples were taken out, washed thoroughly with deionized water, dried in a vacuum oven at 60°C and kept overnight in a vacuum desiccator. Finally the change in weight of the polymer samples was measured. The percent degradation of the polyurethane scaffold was then calculated using the formula

$$\%\ \text{Degradation of scaffold} = \frac{(W_0 - W_d)}{W_0} \times 100,$$

where W_0 is the initial weight of the dry sample and W_d the weight of the degraded sample.

2.5 *Cell culture study*

Human keratinocyte cells (HaCaT) were maintained in complete DMEM medium in presence of 10% FCS at 37°C and 5% CO_2. These were subcultured once in every 3–4 days using trypsin-EDTA (0·25% trypsin and 0·02% EDTA).

The scaffolds were cut into desired size that fit into 24 well culture plates. These were then immersed overnight in DMEM medium for absorption of medium by the scaffolds. Trypsinized cells from the main culture were adjusted to a concentration of 5×10^4 cells/ml and were seeded into the 24 well culture plates containing the scaffolds. Cells were allowed to grow for 48 h before analysis. Culture conditions were maintained at 37°C in an atmosphere of 5% CO_2 in humidified incubator (Heraeus CO_2 Incubator, 37°C, 5% CO_2). After 48 h, the culture plates were analysed under microscope for cell growth pattern as well as their adhesion and spreading.

3. Results and discussion

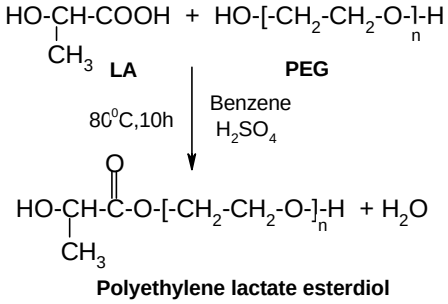
3.1 *Synthesis of gelatin based polyester urethane scaffold*

The main objective of this investigation was to design and synthesize some novel biodegradable polyurethane scaffold using low cost starting materials. For this purpose incorporation of ester linkage in the polyurethane backbone was considered using lactic acid and polyethylene glycol (PEG 400). It is obvious that the extent and rate of biodegradation could be governed by the frequency of the biodegradable ester linkages in the polyurethane chain. In view of this we selected DL-lactic acid and low molecular weight polyethylene glycol (PEG 400) for making low molecular weight hydroxy terminated polyesters (esterdiols). The synthesis reaction of ester diol formation is shown in scheme 1. Gelatin solutions (2·5% and 5%) were prepared by dissolving in doubly deionized water. Then prepolyurethane was synthesized by reacting the ester diol with 2,4-toluene diisocyanate (TDI) in the molar proportions (NCO : OH = 2 : 1) taken in dry THF in presence of DBTDL (0·05 wt%) as catalyst at room temperature (29°C) for 2 h in stirring condition. The polyurethane formation was found to be facile in this condition. The prepolyurethane solution in THF and 5 wt% glutaraldehyde were added to the gelatin solution and the reaction was continued for 15 min. Two different polyester urethane scaffolds were prepared by varying the amount of gelatin.

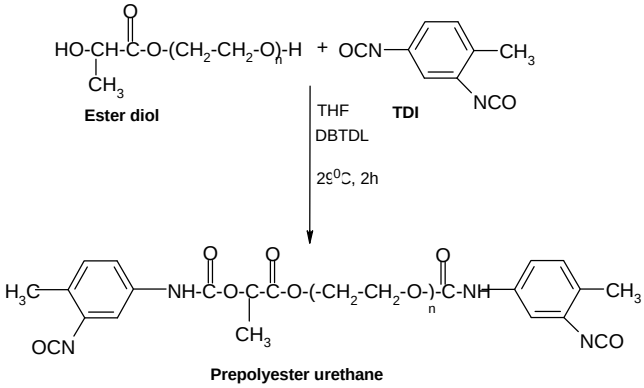
PUG1 contained 5% gelatin and PUG2 contained 2·5% gelatin.

Lactic acid and PEG 400 based polyurethanes were synthesized in two steps. In the first step, the ester diol was synthesized by reacting lactic acid with PEG 400 in presence of H_2SO_4 as a catalyst. One hydroxyl group of PEG 400 was reacted with the carboxyl group of lactic acid to form polyethylene lactate leaving two unreacted hydroxyl groups. Although there might be a self-condensation of lactic acid to polylactic acid but by keeping a higher proportion of PEG, it was greatly minimized. The esterification reaction was continued at a reflux temperature of benzene (80°C) in presence of H_2SO_4 as catalyst for 10 h.

Step 1. Reaction of LA with PEG 400



Step 2: Reaction of ester diol with TDI (excess)



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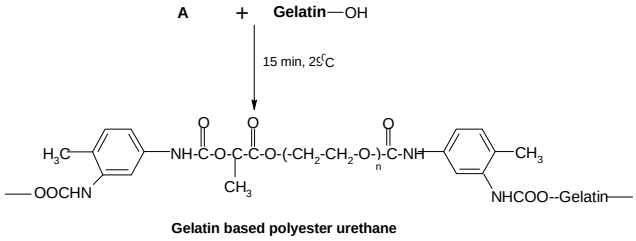
Step 3. Reaction of gelatin with polyester urethane

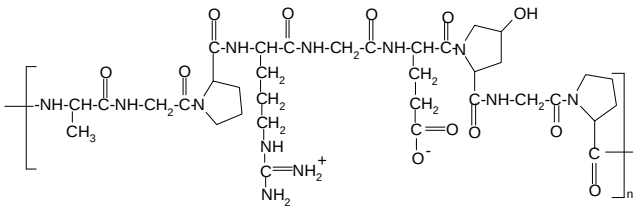


Scheme 1. Synthesis of ester diol and gelatin based polyester urethane.

The esterification was facilitated by removing water as azeotrope with benzene. Pure polyethylene glycol lactate was obtained through fractional distillation to remove the unreacted lactic acid and PEG-400 followed by vacuum distillation of the remaining liquid in the distilling flask. Polyethylene glycol lactate was collected as a colourless viscous liquid.

In the next step, gelatin based polyester urethane scaffolds were prepared by the reaction of ester diol with TDI in dry THF medium at room temperature (29°C) for 2 h with DBTDL as a catalyst and that prepolyurethane solution in THF and 5 wt% glutaraldehyde were added to the gelatin solution (2·5% and 5%) and mixed. Gelatin gets chemically linked through its hydroxyl group with one NCO group of prepolyurethane. Since the gelatin is reported to be crosslinked through its hydroxyl groups by glutaraldehyde (Kang *et al* 1999), it was used as a crosslinking agent for gelatin. It was observed that the viscosity of the mass rapidly increased immediately after the addition of gelatin solution and glutaraldehyde to prepolyurethane solution due to simultaneous reaction of gelatin with the prepolyurethane and crosslinking of gelatin by glutaraldehyde.

Preparation of a scaffold from a viscous solution of this gelatin based polyester urethane is a rather tricky process. The viscous polyester urethane solution was poured onto a flat glass petri-dish and the petri-dish was inserted in a vacuum chamber. Then by slow evacuation (gas foaming method), the THF solvent and water (from gelatin solution) were released out from the viscous solution forming porous spongy polyurethane, which was simultaneously crosslinked by water molecules. The spongy form was maintained atleast for 10 h with simultaneous curing by moisture within the closed glass chamber. Finally a 1–1·26 mm thick spongy gelatin based polyester urethane sheet was obtained. Some physical properties of the two prepared scaffolds are included in table 1.



Structure of gelatin

3.2 *FTIR analysis*

FTIR spectra of gelatin based polyester urethane scaffolds were taken for structural characterization (figure 1). The assigned peaks for gelatin based polyester urethanes (PUG1, PUG2) are given in table 2. Bands for –NH (urethane N–H stretching and N–H stretching of peptide linkage in gelatin) and C=O groups of the urethane bonds appear at

Table 1. Physical properties of gelatin based polyester urethane scaffold.

Scaffold	Gelatin content (%)	Thickness (mm)	Contact angle (degree)	Work of adhesion (ergs)	Pore size[a] (μm)
PUG1	5	1·256	35 ± 1·0	131	131 ± 54
PUG2	2·5	1·023	39 ± 0·5	128	122 ± 35

[a]Pore size measured from SEM photographs.

Table 2. FTIR peak assignments for gelatin based polyester urethanes.

Wave numbers (cm^{-1}), PUG1	Wave numbers (cm^{-1}), PUG2	Peak assignment
3299	3286	Urethane N–H stretching and N–H stretching of peptide linkage in gelatin
2868	2862	C–H stretching
1730	1730	C=O str. of urethane and ester linkage
1637	1637	C=O str. of amide (peptide) linkage in gelatin
1530	1530	N–H bending of amide (peptide) linkage in gelatin

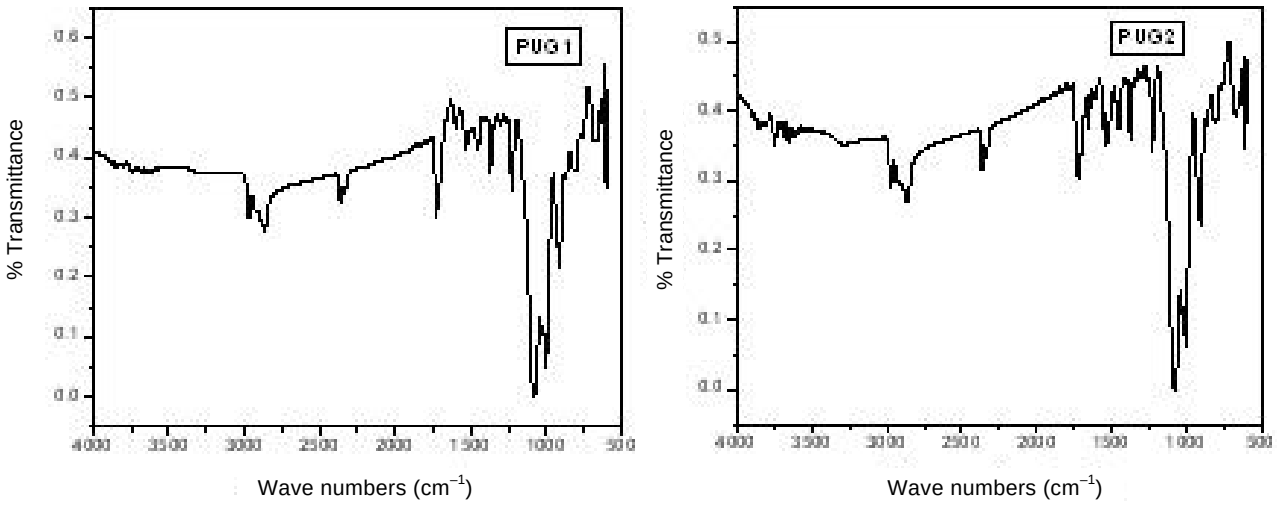


Figure 1. FTIR spectra of gelatin based polyester urethane scaffolds.

about 3299 cm^{-1} and 1730 cm^{-1}, respectively for PUG1 and 3286 cm^{-1} and 1730 cm^{-1}, respectively for PUG2. Band for –NHCOO–gelatin also appears at 1730 cm^{-1} region for PUG1 and PUG2. In polyester urethane (without gelatin), C=O groups of the urethane bonds appear at about 1700 cm^{-1} and this peak is shifted to 1730 cm^{-1} in gelatin based polyester urethane due to –NHCOO–gelatin linkage. C=O stretching of amide (peptide) linkage in gelatin and N–H bending of amide (peptide) linkage in gelatin appear at 1637 cm^{-1} and 1530 cm^{-1}, respectively for PUG1 and PUG2. Since there is no peak at 2270 cm^{-1} region it may be said that gelatin based polyester urethane scaffold does not contain free –NCO groups of TDI.

3.3 *XRD analysis*

The X-ray diffraction patterns of the gelatin based polyester urethane scaffolds are shown in figure 2. From the XRD patterns presented in figure 2, it appears that the synthesized

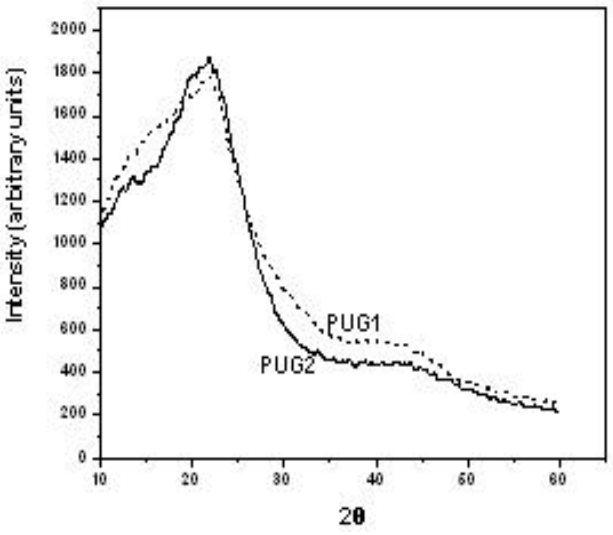


Figure 2. XRD of gelatin based polyester urethane scaffold (PUG1, PUG2).

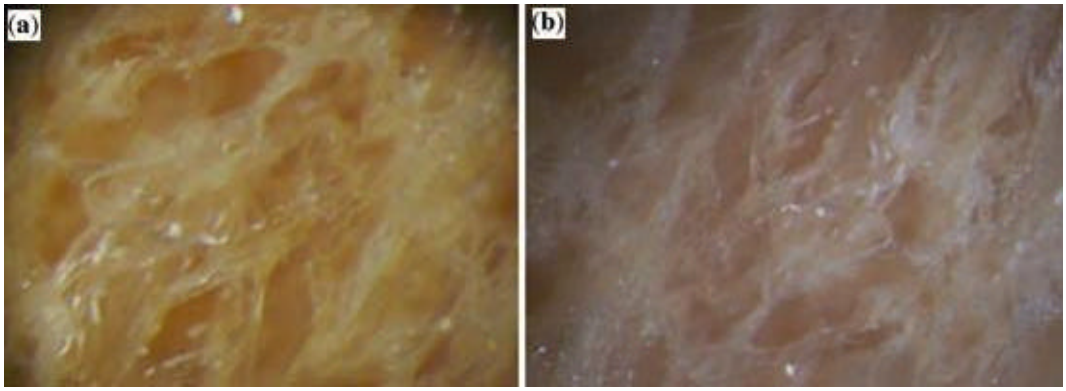


Figure 3. Optical microscopic photographs of (**a**) PUG1 scaffold and (**b**) PUG2 scaffold, at 160X.

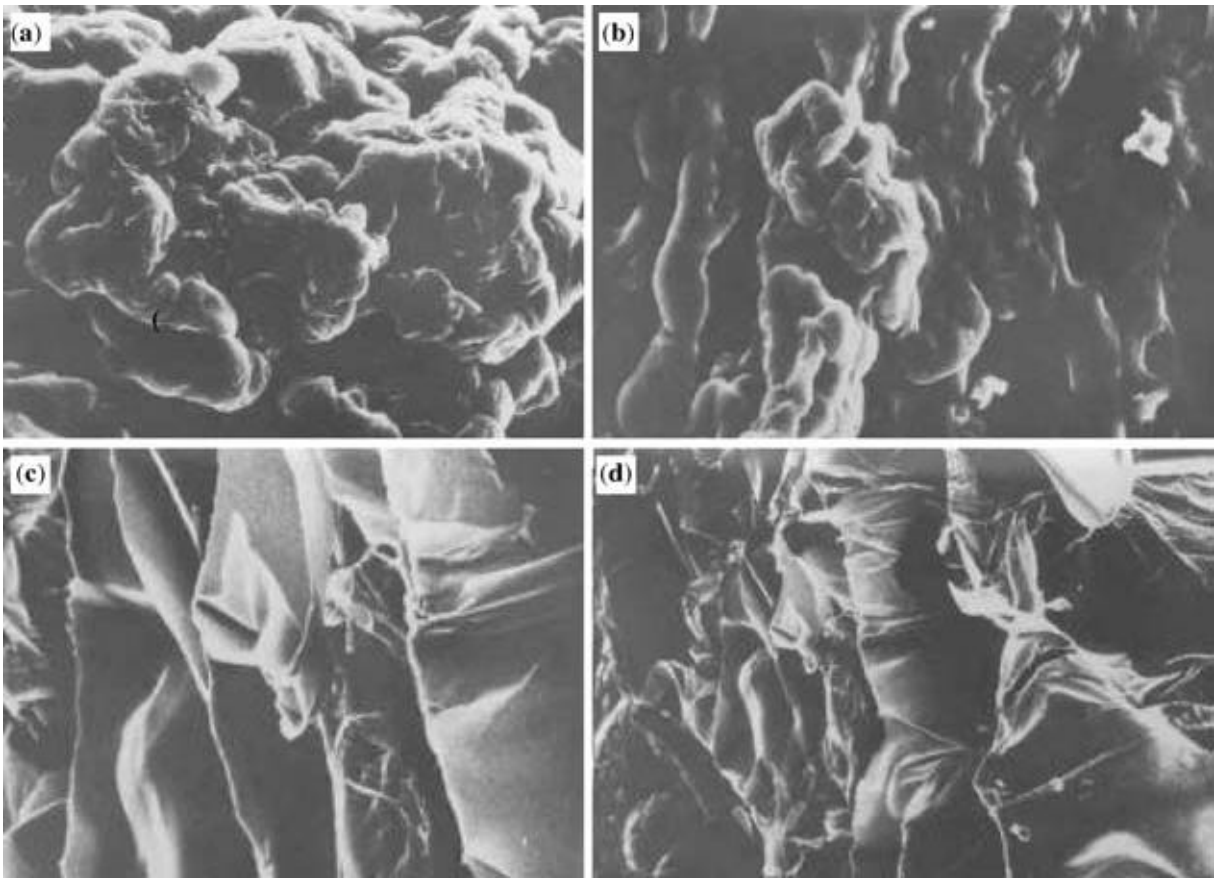


Figure 4. SEM photographs of external surfaces of (**a**) PUG1, (**b**) PUG2 scaffolds (at 1000 X) and cross sections of the (**c**) PUG1 and (**d**) PUG2 scaffolds (at 100 X).

Table 3. Percent swelling of gelatin based polyester urethane scaffold in phosphate buffer saline solution at 37°C.

		Percent swelling in PBS after					
Scaffold	Initial wt (g)	2 h	5 h	10 h	24 h	48 h	72 h
PUG1	0·11265	222	315	391	462	462	462
PUG2	0·12356	180	216	264	296	296	296

Table 4. Mechanical properties of gelatin based polyester urethane scaffolds after degradation in PBS solution at 37°C.

Scaffold	Degradation time (days)	Tensile modulus (GPa)	Tensile strength (T.S.) (MPa)	% Loss of T.S.	Elongation at break (EB) (%)	% Loss of EB
PUG1	0	0·0008	1·26	–	289	–
	7	0·015	1·17	7·14	197	31·83
	14	0·028	1·04	17·46	175	39·44
	21	0·042	0·93	26·19	156	46·02
	30	0·065	0·44	65·07	128	55·70
PUG2	0	0·0011	1·72	–	188	–
	7	0·020	1·45	15·69	163	13·29
	14	0·034	1·14	33·72	154	18·08
	21	0·047	1·05	38·95	131	30·31
	30	0·071	0·81	52·90	119	36·70

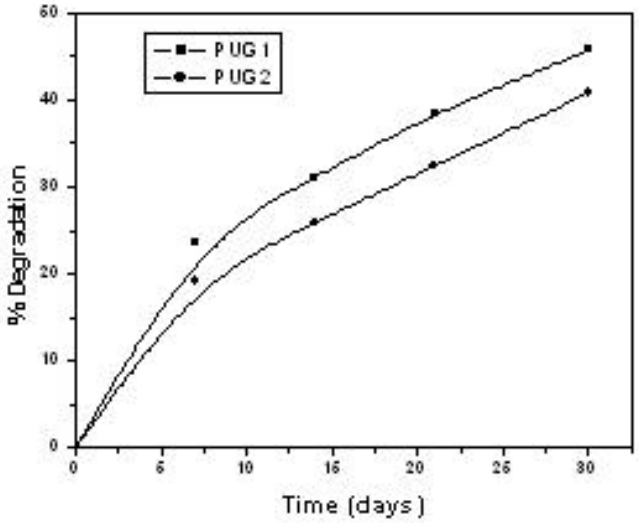


Figure 5. Degradation in terms of weight loss of gelatin based polyester urethane scaffolds in phosphate buffer saline solution at 37°C.

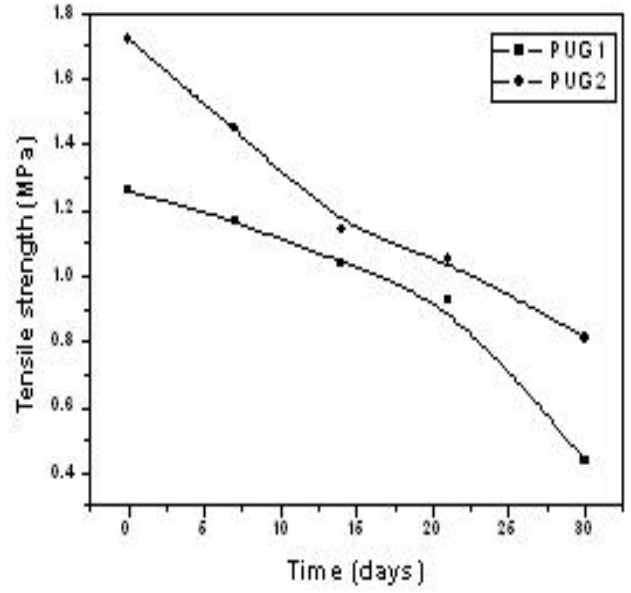


Figure 6. Change of tensile strength of polyester urethane scaffold during PBS degradation.

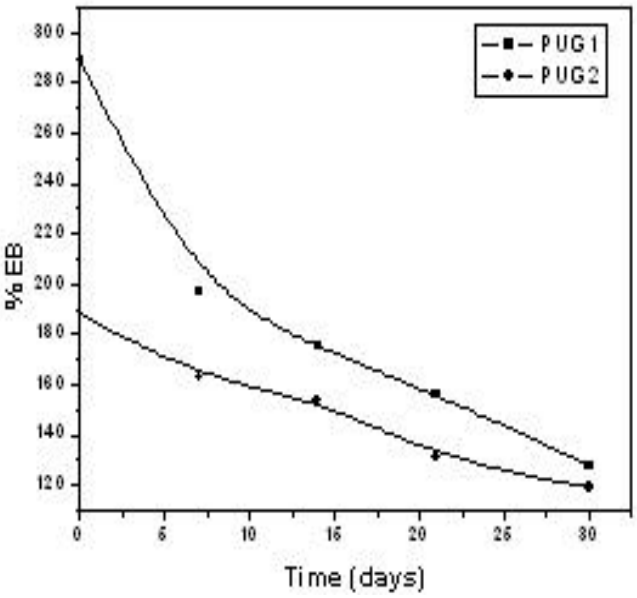


Figure 7. Change of elongation at break (%) of polyester urethane scaffold during PBS degradation.

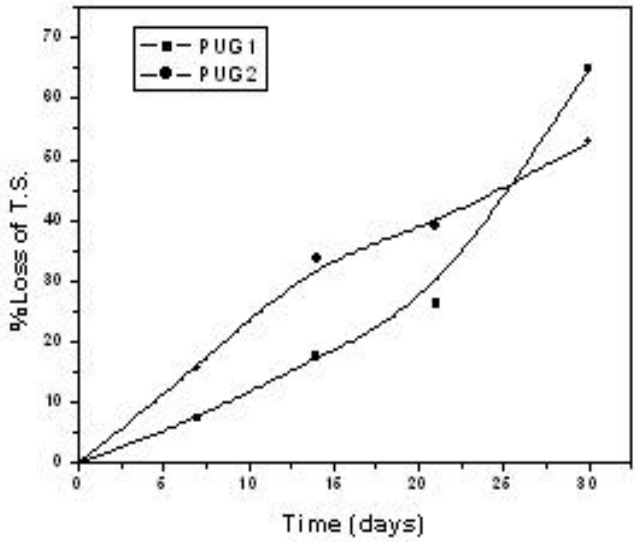


Figure 8. % Loss of T.S. of polyester urethane scaffold during PBS degradation.

gelatin based polyurethanes are amorphous in nature. Although the polyester urethane scaffold contains urethane hard segments but due to lack of proper alignment of the molecules no crystalline phase was formed. This happened due to the easy onset of cross-linking between the segments of the molecules. Not only this, molecular orientation was hindered on the airside of the spongy sheet due to rapid solvent evaporation and moisture curing. From the XRD pattern it is observed that PUG1 with higher amount of gelatin is more amorphous than that of PUG2 with less gelatin content.

3.4 *Contact angle and swelling properties*

The hydrophilicity of biomaterials is very important for biocompatibility. PUG scaffolds surface hydrophilicity, as characterized by contact angle measurement and is reported in table 1. Contact angle value of PUG2 scaffold (39°) is higher than that of PUG1 scaffold (35°) because gelatin content (soft segment content) is more in case of PUG1. Bulk water absorption or % swelling plays an important role on degradation rate of the scaffold. Percent swelling of gelatin based polyester urethane scaffolds in PBS is given in table 3. Equilibrium swelling is maximum in case of PUG1 scaffold (462%) than that of PUG2 scaffold (296%) because gelatin content is more in case of PUG1 scaffold. So PUG1 shows maximum swelling in PBS with maximum hydrophilicity.

3.5 *Optical and scanning electron microscopy (SEM)*

Pore morphology of gelatin based polyester urethane scaffolds was characterized by optical and scanning electron microscopies. For viewing under optical microscope the spongy scaffold sheets were placed in water-swollen condition and the optical micrographs of the swollen gelatin based polyester urethane scaffolds are shown in figure 3. The optical microscope was properly focused to view the porous structure of the scaffolds. Figure 3 shows the interconnected porous structure of PUG1 and PUG2 scaffolds.

Using SEM the surface morphology of the dry gelatin based polyester urethane scaffold sheets was examined by placing the external surface of the scaffold sheet at 1000 magnification and the bulk morphology of the scaffold sheet was examined using the cross-section at 100 magnification. The SEM micrographs of the scaffolds are shown in figure 4. On the surface side (figures 4a and b) of the scaffold vacant spaces between irregular aggregate masses are visible, whereas open spaces in between membrane like walls are visible at the cross-section of the scaffold (figures 4c and d).

3.6 *Tensile properties*

The mechanical properties of the synthesized gelatin based polyester urethane scaffolds such as tensile strength, elongation at break (%) and tensile modulus have been measured, and the results are shown in table 4. In the synthesized polyester urethane there are two types of domain present. One is a hard domain, which is formed by the aromatic isocyanate groups, and the other a soft domain formed by the polyol (polyethylene lactate diol) and gelatin. It is expected that with the increase of soft domain the tensile strength of the polyurethanes should decrease and elongation at break (%) should increase. In fact it has happened in case of PUG1, which contains higher amount of gelatin (5%) than that of PUG2 (2·5%). So PUG1 has exhibited lower tensile strength and higher elongation at break than those of PUG2.

3.7 *Degradation study in phosphate buffer saline*

The degradation of the gelatin based polyester urethane scaffolds in PBS was carried out for a maximum period

Table 5. Degradation of gelatin based polyester urethane scaffolds in phosphate buffer saline solution at 37°C.

Scaffold	Degradation time (days)	% Degradation
PUG1	0	0
	7	23·6
	14	31·1
	21	38·5
	30	45·7
PUG2	0	0
	7	19·2
	14	25·9
	21	32·4
	30	40·8

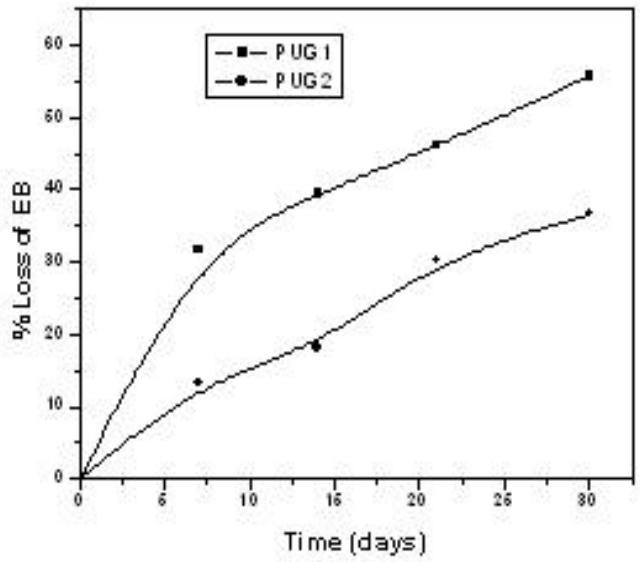


Figure 9. % Loss of E.B. of polyester urethane scaffold during PBS degradation.

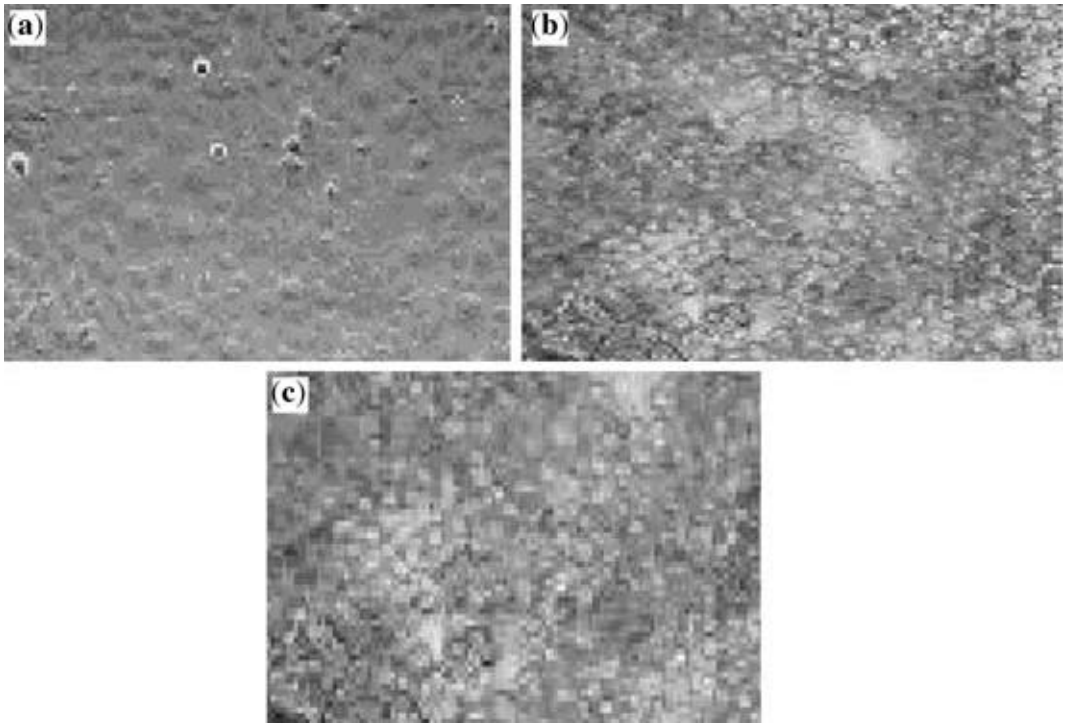


Figure 10. Optical microscopic photographs of (**a**) HaCaT cell growth on control (40 X), (**b**) HaCaT grown on PUG1 scaffold (40 X) and (**c**) HaCaT grown on PUG2 scaffold (40 X).

of 30 days in BOD incubator shaker at 37°C. The degradation of the scaffolds was evaluated by loss in weight of the scaffold sheet with the progress of degradation. The results of degradation in PBS solution in terms of weight loss of the scaffolds are presented in table 5 and figure 5, which clearly show higher degradation nature of PUG1 compared to that of PUG2. Higher degradation rate of PUG1 is attributed to more gelatin content.

Further to this, changes in tensile strength and elongation at break of the gelatin based polyester urethane scaffold sheets with the progress of degradation were also measured and the results are presented in table 4 and figures 6–9. It is evident from figures 6–9 that the higher rate of degradation of PUG1 is reflected in the progress of degradation, measured in terms of mechanical properties, due to the presence of higher amount of gelatin. The weight loss occurred due to the hydrolytic degradation of the gelatin based polyester urethane scaffold by PBS solution. It is seen from table 5 that PUG1 shows higher degradation (45·7%) than that of PUG2 (40·8%) in PBS in 30 days because PUG1 has higher gelatin content. The same trend of loss in tensile strength of the gelatin based polyester urethane has been observed in case of weight loss due to degradation in PBS solution. PUG1 has shown maximum loss in T.S. (65%, table 4, figure 8) due to its higher gelatin content. With the progress of degradation up to 30 days the loss in elongation at break gradually increased in each scaffold (table 4, figure 9).

3.8 *Cell culture study*

In order to see the viability of gelatin based polyester urethane scaffold for cell proliferation and adhesion, human keratinocyte (HaCaT) cells have been inoculated and cultured in DMEM medium in presence of 10% FCS at 37°C in a CO_2 incubator. Cells were morphologically analysed by light microscopy (Leica). Figure 10 shows optical microscopic photographs of HaCaT cell growth on control (without polymer scaffold) of PUG1 and PUG2 scaffolds (40X). It has been observed from figure 10 that cells are well seeded and adhered on the gelatin based polyester urethane scaffold surface and good fibroblast cell proliferation is found.

4. Conclusions

For tissue engineering purpose, two gelatin based polyester urethane scaffolds of different compositions were prepared and characterized. The gelatin based polyester urethane scaffolds are mechanically strong as well as degradable in PBS. Within these scaffolds human keratinocytes cells were cultured and good cell adherence and proliferation was observed. This study indicates that the synthesized gelatin based polyester urethane scaffold might find use as a material for soft tissue engineering because it is biodegradable as well as biocompatible. It can be said that such

polymers will degrade in actual biological environment and can be tried for drug delivery devices also.

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