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Preparation and Properties of Biocompatible Polyurethane based on Isosorbide and Silk Fibroin

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Abstract: **Polyurethane (PU) is a dressing agent with high absorption and moisture permeability and has excellent wound healing effects. Silk fibroin (SF) is an attractive biomaterial as per clinical studies. In this study, a novel PU series was synthesized using bio-based isosorbide and SF at different ratios. The successful synthesis of the PU-SF series was confirmed by Fourier transform-infrared spectroscopy. The wettability of the polymer surface was evaluated based on the contact angle. The thermal properties were determined by thermogravimetric analysis to evaluate the thermal stability. The mechanical properties of the PU-SF series were evaluated using an Instron universal testing machine. The addition of SF significantly improved the tensile properties of the PU-based material. The mouse myoblast cell line C2C12 was used as a model for *in vitro* experiments with the PU-SF series to ensure biocompatibility and non-toxicity; the cell response improved with increasing SF content. The PU-SF series shows significant potential for specific regenerative medicine applications due to its biocompatibility and good physical properties, including in wound dressing.**

Keywords: **Polyurethane, silk fibroin, wound dressing, isosorbide, biocompatibility.**

I. INTRODUCTION

Dressing refers to the covering of wounds with a material, or dressing agent, to protect the wound surface. Wounds are covered to absorb the internal wound exudate, to prevent foreign intrusion, and to protect them from physical shock or irritation. Compared with dry dressings, wet dressings are involved in wound healing, with effects on polynuclear leukocytes, macrophages, proteolytic enzymes, and cell growth factors contained in the exudate, promoting efficient wound healing [1, 2]. Wound dressings should be non-toxic, highly absorbent, air permeable, biocompatible, and have good mechanical properties [3].

Polyurethane (PU) is a commonly used candidate for wound dressing applications. PU is frequently used in wound dressings because of its good barrier properties and oxygen permeability [4, 5]. PU can be fabricated via reactions between dialcohols and diisocyanates forming urethane linkages. PU is composed of two-segment structures, a hard segment and soft segment. Typically, the hard segment is a diisocyanate with an aromatic ring structure and a urethane bond capable of hydrogen bonding, and the soft segment is a polyether or a polyester diol [6]. Isosorbide is bio-based diol, and is the only product that can be produced on an industrial scale from starch [7]. Isosorbide is non-toxic and demonstrates thermal and chemical stability. It not only serves as a hard segment, but also has two biocyclic ring structures that exhibit biocompatibility. PUs made using such bio-based isosorbide have been studied for biomaterial, recently [8, 9].

The natural silk of the silkworm *Bombyx mori* has long been used as a surgical suture for textile materials and clinical applications in the textile industry [10]. Silkworm silk is composed of a core structural protein called silk fibroin (SF) and is coated with a gum-like protein called sericin. Because sericin causes an unwanted immune response *in vivo*, sericin-deficient SF must be used. SF is a biodegradable natural polymer with excellent mechanical properties. It is used for

artificial organs and skin because of its resilience and low inflammation rate. In addition, it degrades slowly *in vivo* like PU, and can avoid additional toxicity in tissues and cells. It has an RGD motif and its surface is activated, facilitating cell adhesion [11]. Studies have reportedly formed blended materials containing PU and SF [9]. However, SF biodegrades rapidly and, due to the strong hydrogen bonding between SF, its mechanical properties deteriorate due to phase separation with PU.

In this study, bio-based isosorbide was used as the hard segment and SF was added to synthesize chemically bonded PU. Fourier transform-infrared spectroscopy (FT-IR), contact angle determination, thermogravimetric analysis (TGA), and a universal testing machine (UTM) were used to evaluate the structural, thermal, and mechanical properties of the synthesized PU. In addition, cell proliferation experiments using the C2C12 cell line were conducted to investigate its applicability in wound dressing preparation.

II. EXPERIMENT

A. Materials:

B. mori silkworm silk was purchased from Boeun Silk Factory (Boeun, South Korea). Anhydrous sodium carbonate (Na_2CO_3, 99%), N,N-dimethylformamide (DMF, 99.9%), isopropyl alcohol (IPA, 99%), and ethyl alcohol (EtOH, 99.5%) were obtained from Duksan Chemical Co., Ltd (Seoul, South Korea). Isosorbide (IS, 98%), hexamethylene diisocyanate (HDI, 99%), polycaprolactone diol (PCL diol, molecular weight (MW): 2,000, 98%), and calcium chloride ($CaCl_2$, 93%) were purchased from Sigma-Aldrich (St Louis, MO, USA). All chemicals were used without further purification.

B. Preparation of SF:

To remove sericin from the components constituting the cocoon, the cocoons were finely cut, dissolved in a 10 wt% Na_2CO_3 aqueous solution (190 g), and heated at 80°C for 4 h to remove sericin. To completely remove the remaining sericin, the material was washed several times with distilled water (DW) and vacuum dried. SF (20 g) was dissolved in a solution of $CaCl_2$ (57.5 g, 0.52 mmol), EtOH (47.6 g, 1.04 mol), and DW (74 g, 4.15 mmol) at 80°C for 2 h with continuous stirring. Then, dialysis tubing cellulose membrane (MW: 12,000–14,000) was used for dialysis for 1 week in the DW to remove salt and ethanol. The DW was changed daily. The final dialyzed SF solution was filtered using a 300-µm pore size glass filter to remove impurities completely and lyophilized at -10°C for 2 weeks to obtain SF sponges.

C. Preparation of the PU-SF Series:

The synthesis of the PU-SF series is shown in Figure 1. First, a four-neck round-bottomed glass flask (250 mL) was equipped with a heating mantel and a nitrogen supply line. PCL diol (59.46 g, 29.73 mmol), isosorbide (4.34 g, 29.73 mmol), and SF (0, 0.1, 0.5, and 1 g) were dissolved in DMF (60 mL) and injected into the flask. HDI (11 g, 65.40 mmol) was added to the mixture, and the reaction was continued at 120°C for 12 h. The solution was precipitated into IPA (5 L) and washed with more IPA. The synthesized PU-SF series was dried at 60°C for 24 h under a vacuum and stored in a desiccator. The formulations are listed in Table 1.

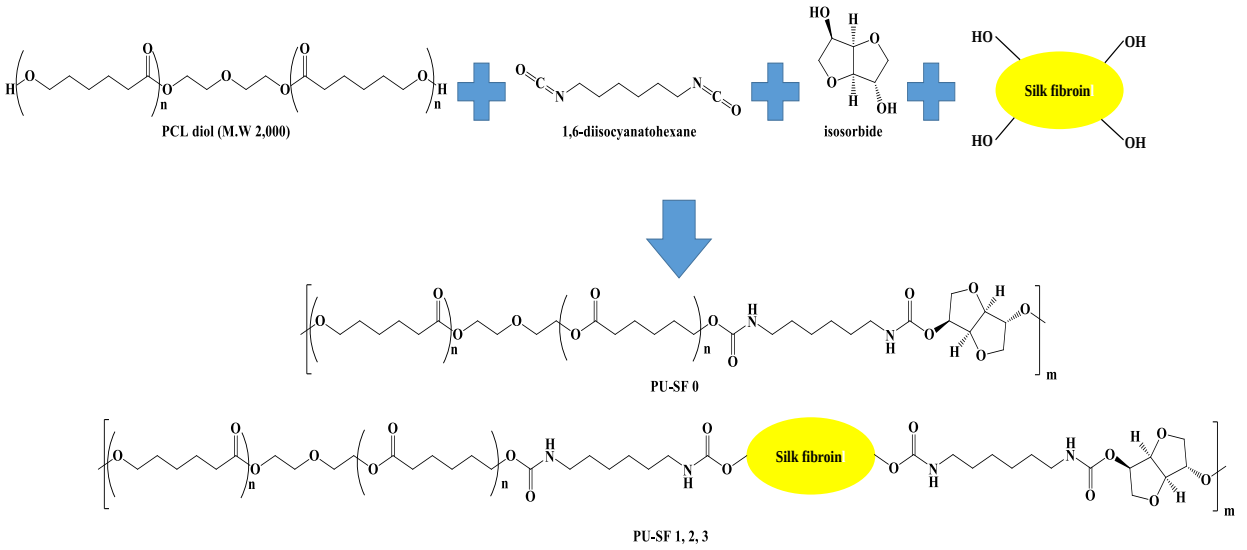

Figure 1: Schematic structure of polyurethane synthesized from polycaprolactone diol, isosorbide,1,6-diisocyanatohexane and silk fibroin.

Table 1: Formulation of the PU-SF series

Samples	Weight of Raw Materials (mmol)			
	PCD diol	Isosorbide	HDI	SF
PU-SF 0	59.46 (29.73)	4.34 (29.73)	11 (65.40)	0
PU-SF 1	59.46 (29.73)	4.34 (29.73)	11 (65.40)	0.1
PU-SF 2	59.46 (29.73)	4.34 (29.73)	11 (65.40)	0.5
PU-SF 3	59.46 (29.73)	4.34 (29.73)	11 (65.40)	1

D. Instruments:

The FT-IR spectra for the synthesized PU-SF series were recorded with a Varian 640 FT-IR device (Varian Australia Pty., Ltd., Sydney, Australia) in the range of 600–4000 cm^{-1} following the attenuated total reflection method. The wettability of the PU-SF series was measured using contact angle measuring equipment (Phoenix 300, Surface Electro Optics, Korea). The polarity characteristics of the PU-SF series were measured by dropping microwave water droplets (~5 µL) onto PU-SF series films at 1-s intervals with a manual syringe. The contact angles were calculated using Phoenix 300 software. TGA tests were conducted on the samples using Shimadzu TGA 50 (Shimadzu, Tokyo, Japan) equipment operating from 30 °C to 600 °C at a heating rate of 10 °C/min and under a nitrogen atmosphere. The tensile strength and elongation were measured at a crosshead speed of 10 mm/min at room temperature using a universal testing machine (Model 3344, Instron Engineering Corp., Canton, MA, USA).

E. Cell Culture and Determination of Cell Viability:

The C2C12 cell line was purchased from American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in a growth medium (GM) consisting of Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific, MA, USA) supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific, MA, USA) and 100 units/ml penicillin/streptomycin at 37 °C and 5% CO_2.

To determine the cell viability in the presence of the PU-SF series membranes, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983) was performed. Briefly, PCL disk samples were placed in 24-well culture plate and then washed with DPBS (pH 7.4). C2C12 cells were seeded at a density of 2×10^5 cells per well and cultured for 5 days in the GM, and the MTT assay was monitored at days 1, 3 and 5. For the MTT assay, cells were incubated with 300 µL of MTT (0.5 mg/mL) for 4 h at 37 °C. After incubation, MTT was removed and 200 µL of dimethylsulfoxide (DMSO) was added to each well, and its concentration was measured at 540 nm using a microplate reader (Spectra Max M2e, Molecular Devices, CA, USA). The values were expressed as fold changes. The analysis was performed in four independent experiments using four separate wells each time.

III. RESULTS AND DISCUSSION

A. Properties of the PU-SF Series:

FT-IR is widely used to identify the structure of polymers. In this study, FT-IR was applied to confirm the synthesis of PU-SF series and SF (Fig. 2). PU-SF 0 exhibited three characteristic peaks at 3387, 1721, and 1528, which may have been attributed to N-H stretching, N-H bending, and C=O stretching absorption bands of the urethane bond, respectively. A comparison of the spectra of PU-SF 0 and PU-SF 3 showed that new absorption bands at 1654 cm^{-1} appeared, which corresponded to the N-H absorption band of SF. This was indicative of the successful synthesis of the PU-SF series and of the covalent bonding of SF to the PU molecular chain.

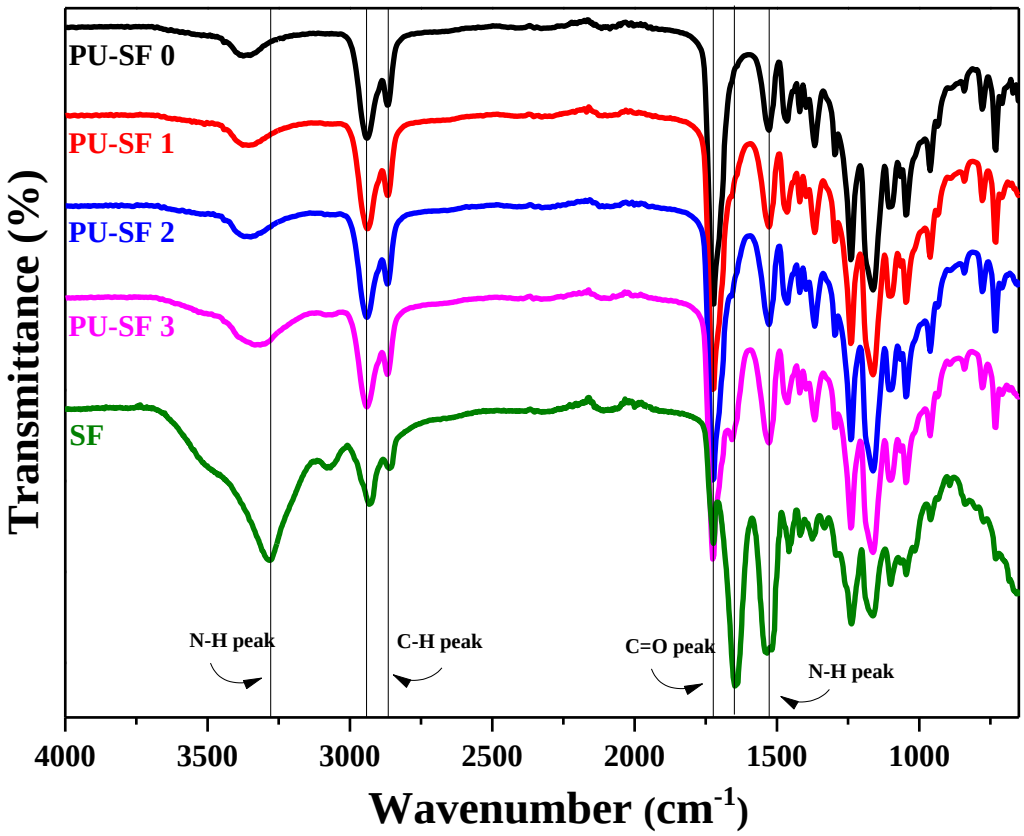

Figure 2: FT-IR spectra of PU-SF series and SF.

The surface structure and properties of biocompatible polymeric materials are very important, because the functional groups that comprise the surface have a major role in the interaction between biomolecules and cells. The contact angle was measured to confirm the hydrophilicity or hydrophobicity of the sample surface (Fig. 3). PU-SF 0, 1, 2, and 3 had contact angles of 70.01°, 69.39°, 68.41°, and 65.16°, respectively. These results revealed that higher silk contents resulted in smaller contact angles, indicative of greater hydrophilicity.

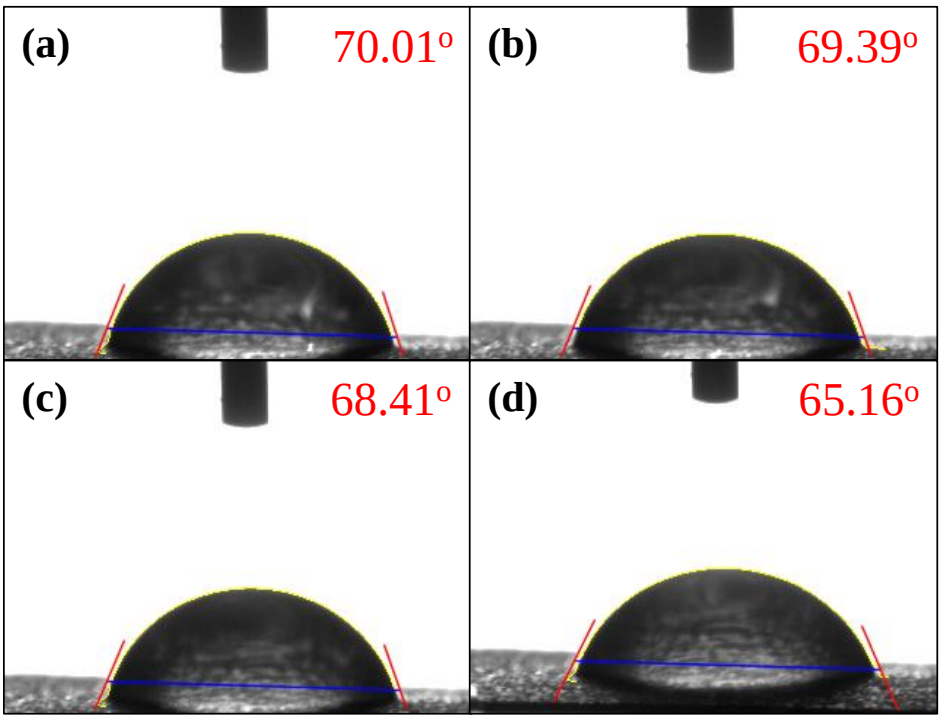

Figure 3: Water contact angles and optical images of PU-SF series and SF.

The mechanical properties of the PU-SF series are shown in Figure 4. The tensile stress of PU-SF 0 was 12.34 MPa. Meanwhile, the tensile stress of PU-SF 1 increased to 13.31 MPa and that of PU-SF 2 increased to 14.17 MPa with the addition of SF. However, the tensile stress of PU-SF 3 was significantly lower (8.48 MPa). This was indicative of the excess addition of SF, resulting in the deterioration of physical properties. The tensile strain of PU-SF 0 was 1273.70%, which showed a tensile strain about 12 times that of PU alone. The tensile strains of PU-SF 1, 2 and 3 containing SF were 1355.18%, 1305.18%, and 1090.36%, respectively. When 0.1% SF was added, tensile deformation increased and showed good characteristics. However, tensile deformation decreased when more than 0.5% SF was added. In general, increasing addition of SF acted to inhibit tensile strength and tensile strain. However, optimizing the amount of added SF can contribute to improving the mechanical properties of tensile strength and tensile strain.

It is important to understand the thermal properties of biomedical materials because the thermal properties determine applicability and processing characteristics. The TGA profile of the PU-SF series is shown in Figure 5. The temperatures at which PU-SF 0, 1, 2, and 3 began to lose 10% weight were 302.7, 310.3, 131.15 and 331.36 °C, respectively. Thus, it can be concluded that the addition of silk to the PU increases the thermal stability.

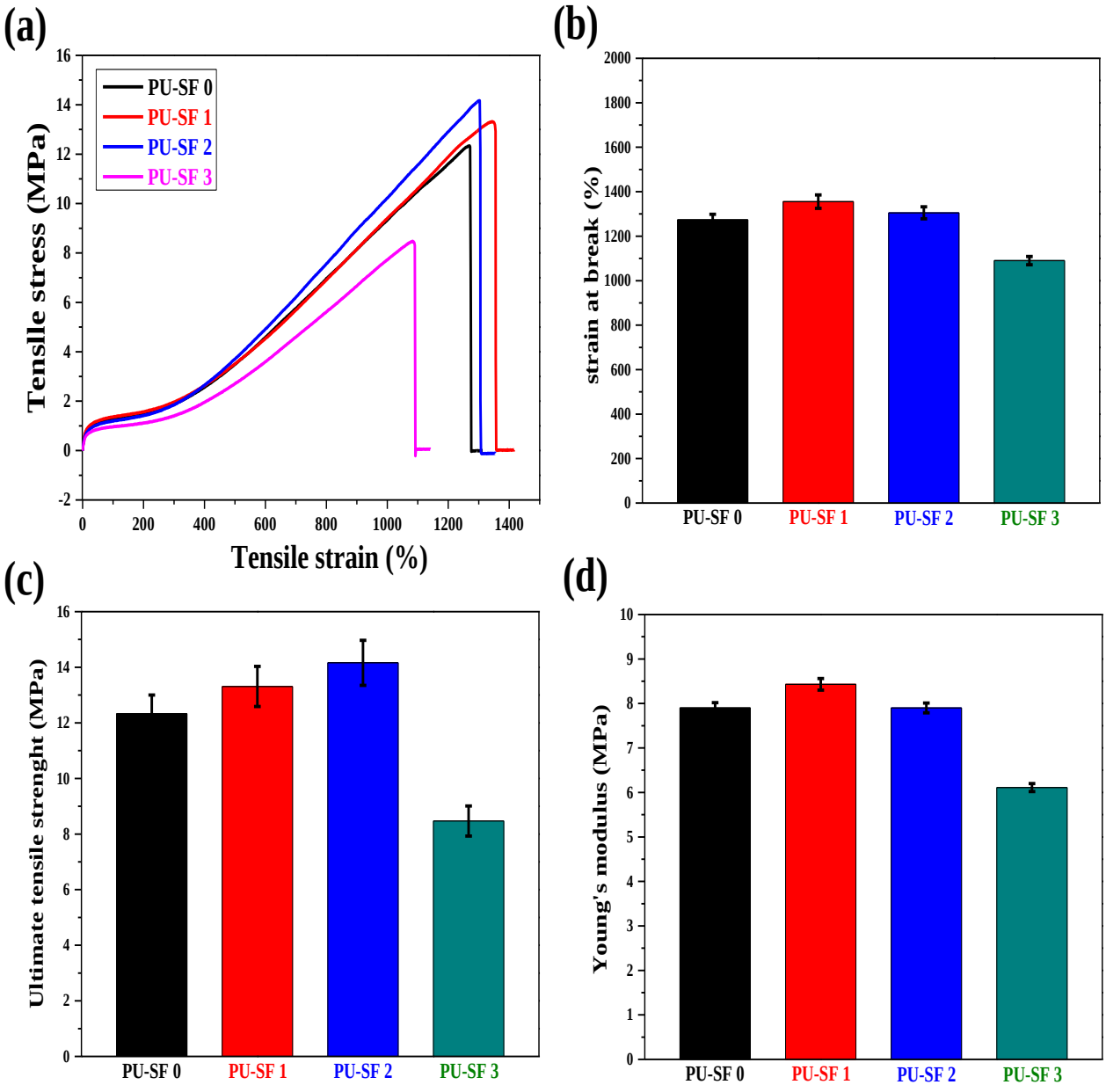

Figure 4: Mechanical properties of PU-SF series: (a) tensile stress-strain curves, (b) ultimate tensile strength, (c) strain at breaking and (d) Young's modulus.

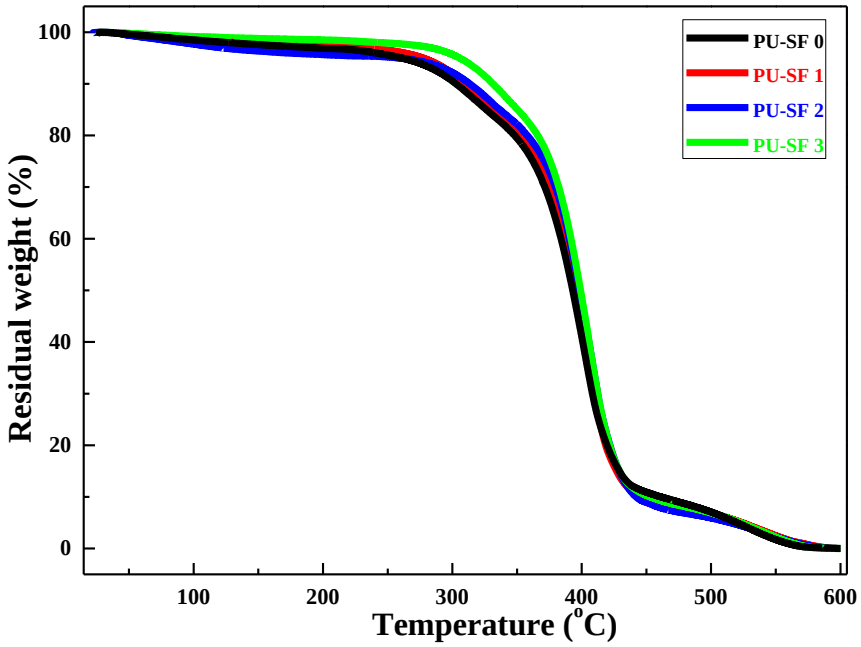

Figure 5: TGA thermograms of PU-SF series.

B. Biocompatibility:

Biocompatibility of the polymers was confirmed by MTT analysis. If cellular proliferation in the polymer is maintained, the polymer can be considered cytocompatible and biocompatible. The reliability and sensitivity of the C2C12 cells were evaluated with the MTT analysis. The cytotoxic efficacy of PU-SF series toward C2C12 were assessed by the MTT assay at 1,3, and 5 days, as shown in Figure 6. The cytotoxic effect was evaluated according to the EN ISO 10993-5 standard. In general, compared with the control, samples with viability above 70% are considered non-cytotoxic. MTT analysis showed that the viability of C2C12 up to 5 days in the PU-SF series was 79.3, 81.4, 84.3, and 82.7% in PU-SF 0, 1, 2, and 3, respectively. The PU-SF materials were considered to be non-cytotoxic and the cells showed higher survival rates in PU containing silk, indicating that silk significantly affects cell adhesion and proliferation. However, the rate of cell proliferation was slower than that of the control. Overall, this experiment demonstrated the non-toxicity and biocompatibility of the PU-SF series.

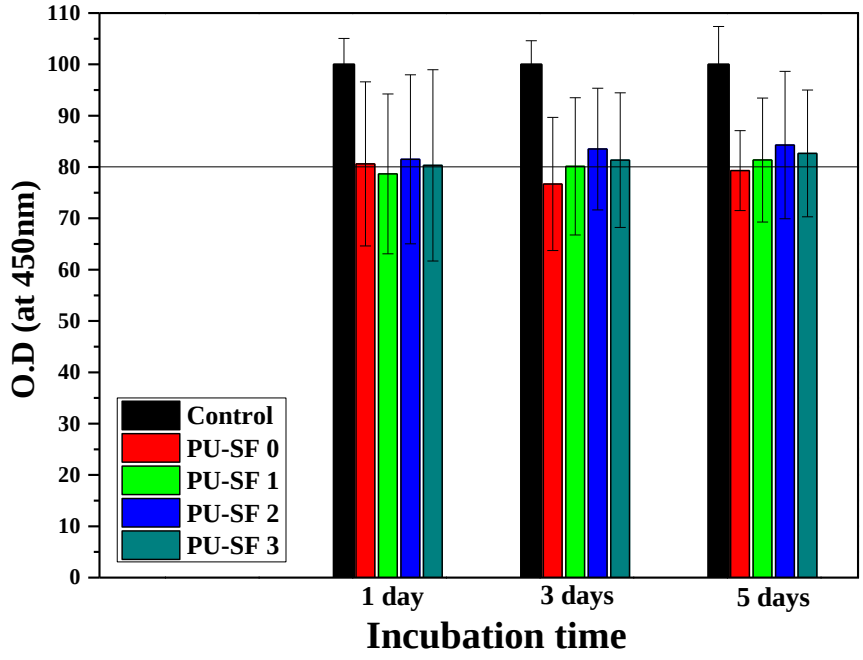

Figure 6: MTT assay of mouse C2C12 cultured on PU-SF series wells for 5 days.

IV. CONCLUSION

In this study, a novel PU-SF series was prepared with SF at different ratios to realize enhanced biocompatibility and physical properties. The PU-SF series was synthesized with HDI using bio-based isosorbide as the hard segment and PCL diol as the soft segment. The synthesized PU-SF series was analyzed by FT-IR, and it was confirmed that the PU surface containing silk had a hydrophilic group, indicated by the reduced contact angle. The thermal stability of the PU containing silk was confirmed by TGA results, and the physical properties were also determined by using a UTM. C2C12 cells cultured in PU-SF series exhibited remarkable adhesion and proliferation. Thus, the PU-SF series produced in this study may be a beneficial material due to the excellent biocompatibility and good physical properties that are needed in regenerative medicine applications, including in wound dressings.

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