SB³C2022
Summer Biomechanics, Bioengineering and Biotransport Conference
June 20-23, Eastern Shore, MD, USA

FABRICATION AND DRUG RELEASE KINETICS CHARACTERISATION OF POLY(GLYCEROL SEBACATE URETHANE) ANISOTROPIC SCAFFOLDS

A. Samourides (1), Z. Xenou (1), I. Louca (1), K. Kapnisis (1), A. Anayiotos (1)

(1) Department of Mechanical Engineering and Materials Science and Engineering, Cyprus University of Technology, Limassol 3036, Cyprus

INTRODUCTION

Injuries of tendons are characterized with painful, slow healing time and usually insufficient, in terms of restoring its strength and various functions. Tendon tissue engineering has presented a promising approach to promote tendon healing or its replacement [1]. This approach is usually based on the use of three-dimensional porous scaffolds, combined with biological factors such as cells and growth factors, to support the tendon temporarily. The scaffold ideally mimics the structure and the physical properties of the native tissue until its regeneration [2].

A promising biodegradable, biocompatible material that has suitable physical properties for use in tendon tissue engineering is poly(glycerol sebacate urethane) (PGSU). Furthermore, PGSU was found to be angiogenic and to promote tissue ingrowth depending on the ratio of the reactants [3]. In this study, three different PGSU scaffolds, with different ratios of HDI and polymer concentration, were fabricated and investigated for specific physical properties such as: microstructure, pore size, swelling ratio and drug release kinetics.

METHODS

PGSU scaffolds were fabricated as described by Samourides et al. [2] with HDI ratios of 0.8 and 1.0 and polymer concentrations (w/v%) equal to 10% and 15% which were found to exhibit the highest mechanical properties between the other PGSU scaffolds that were synthesized. Briefly, the PGS pre-polymer was dissolved in 1,4-dioxane at the required w/v concentration and HDI was added at 0.8 or 1.0 ratio to glycerol. The solution was left to react for 5 hours at 55 ºC. The solution was then frozen in an in-house customized mold (see [2]) and freeze dried for 16 hours. PGSU scaffolds were characterized for their microstructure using scanning electron microscopy (SEM) (SEM Quanta 200, FEI, United States). The hydrophilicity was studied using contact angle and swelling ratio calculations. Finally, the scaffolds were studied for their ability to be loaded with bovine serum albumin (BSA) and the drug release rate was subsequently derived.

Pore size: The pore size of the samples was measured from the SEM images using ImageJ and the free hand selection tool. Images were taken from all views and 50 fully defined pores were used to determine the average pore size of the top view, cross section and bottom view of the PGSU scaffolds.

Swelling ratio: The swelling ratio of the scaffolds was measured to determine their swelling ability in phosphate buffered solution (PBS). The samples were dried, weighted and soaked in PBS for a total of 24 hours. Mass measurements of the scaffolds were taken at multiple time points to calculate their swelling ratio according to Eq. (1):

$$\mathrm{SR}\ (\%) = \frac{W_w - W_d}{W_d}\ x\ 100 \qquad \text{Eq. (1)}$$

with W_w and W_d to be the sample's wet weight at different time points and the sample's dry weight, respectively. The experiment was repeated three times in technical triplicates.

Drug loading: The PGSU scaffolds were loaded with BSA using an in-house dynamic vacuum loading method. A BSA solution was prepared at 500 μg/ml in PBS and 200 μl of the solution was placed on top of the scaffolds. The samples were moved into a vacuum chamber where the vacuum pressure was increased until air bubbles began to form and rapidly ventilated to atmospheric pressure. This cycle was repeated 12 times. The drug loaded scaffolds were then submerged in PBS and placed on a rocker at 100 rpm for 28 days. Samples were collected at various time points throughout the study and a bicinchoninic acid (BCA) assay was used to quantify the amount of protein that was released over time.

Statistical Analysis: All experiments were repeated three times and the statistical analysis was performed using two-way ANOVA in

GraphPad Prism 8. Results are shown as mean ± standard deviation and the statistical significance was considered at $p < 0.05$.

RESULTS

Figure 1 shows representative macroscopic and microscopic images of the scaffold fabricated using ice-templating and freeze-drying.

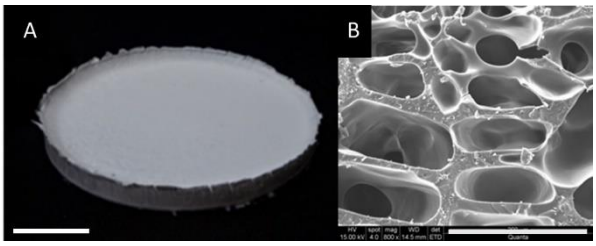

Figure 1: Representative macroscopic and microscopic images of the PGSU scaffolds. Scale bars for A = 10 mm, B = 200 µm.

The assessment of the pore size showed that the scaffolds exhibited a range of pore sizes, from 54.6 ± 18.3 to 66.1 ± 23.7 µm depending on the polymer concentration and HDI ratio (see Figure 2). The PGSU scaffolds fabricated with 1.0 HDI ratio had the most uniform pore sizes between the different viewing angles.

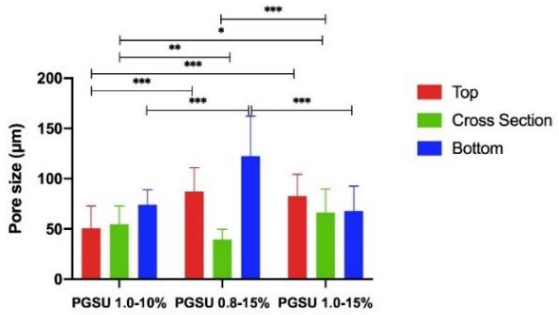

Figure 2: The pore sizes of the PGSU scaffolds viewed from different angles using SEM. * when $p < 0.05$, ** when $p < 0.01$ and * when $p < 0.001$.**

The measurements for the swelling ratio are shown in Figure 3 and at 24 hours it ranged between 71.3 ± 7.3%, 115.9 ± 7.1% and 140.8 ± 15.5%. for PGSU 1.0-10%, 0.8-15% and 1.0-15% respectively. The samples showed statistical difference and the swelling ratio increased with the increase of polymer concentration and HDI ratio.

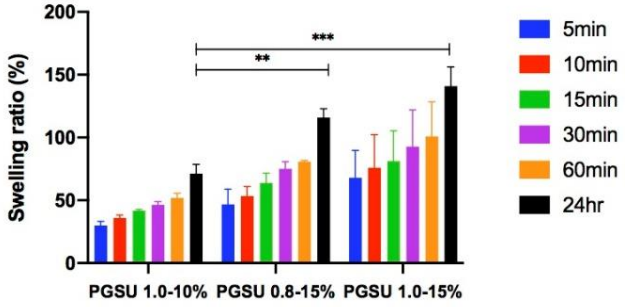

Figure 3: The swelling ratio measurements for the samples. ** when $p < 0.01$ and * when $p < 0.001$.**

Finally, the drug release rate of BSA loaded into the PGSU scaffolds was examined using a BCA assay. A linear release rate was found and almost all samples withheld and released the protein over a period of at least 19 days.

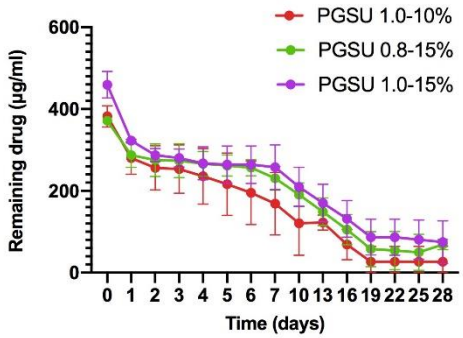

Figure 4: Drug release rate of the PGSU scaffolds over a period of 28 days using BCA assay.

DISCUSSION

Large PGSU scaffolds were fabricated, and a controlled pore orientation was achieved using ice-templating and freeze drying. The scaffolds exhibited significantly different pore sizes between sample groups. However, a uniform microstructure in terms of pore size was achieved from the scaffolds that were synthesized using 1.0 HDI (PGSU 1.0-10% and PGSU 1.0-15%). Furthermore, a linear swelling rate was also observed from the swelling testing of the scaffolds which is considered an advantage in drug loading because a uniform distribution of the drug within the scaffold construct is promoted. The PGSU scaffolds have also shown a fairly linear release of the BSA over time which correlates with our previous swelling and degradation studies in [3] and further adds to the advantages of the anisotropic PGSU scaffolds that were fabricated in this study. The loading and steady release of proteins that can stimulate the biological response for tendon regeneration indicated no significant difference between the examined PGSU scaffolds. Finally, the drug loading technique that was developed by our group for this purpose allowed us to load BSA at approximately the 3-folds the mass of the scaffold with an average of 75% loading efficiency. This method can be modified to be used with almost any scaffold.

ACKNOWLEDGEMENTS

This work was co-funded by the European Regional Development Fund and the Republic of Cyprus through the Research and Innovation Foundation (Grant number: OPPORTUNITY/0916/MSCA/0017).

REFERENCES

1. Testa, S., et al., *Combination of biochemical and mechanical cues for tendon tissue engineering.* Journal of cellular and molecular medicine, 2017. **21**(11): p. 2711-2719.
2. Samourides, A., et al., *Fabrication of Hierarchical Multilayer Poly(Glycerol Sebacate urethane) Scaffolds Based on Ice-Templating.* Applied Sciences, 2021. **11**(11): p. 5004.
3. Samourides, A., et al., *The effect of porous structure on the cell proliferation, tissue ingrowth and angiogenic properties of poly(glycerol sebacate urethane) scaffolds.* Materials Science and Engineering: C, 2020. **108**: p. 110384.