

Effect of Leaching Agent Composition on Morphology, Thermal and Mechanical Properties of Bioglass® Reinforced Polyurethane Scaffold

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ABSTRACT. In this preliminary study, Bioglass® (BG) reinforced Polyurethane (PU) composite scaffolds with different composition of leaching agent were fabricated. Salt leaching technique was used to prepare the scaffolds by using different percentage of NaCl and NaHCO₃ as leaching agent to create the pore. Morphological characteristic, thermal properties and mechanical properties of the scaffold were investigated by field emission scanning electron microscope (FESEM), thermogravimetric analysis (TGA) and dynamic mechanical analysis (DMA). As revealed by FESEM images, as the percentage of NaHCO₃ increased the pore structure of the scaffold changed drastically. As the content of NaHCO₃ increased from 50%, the pore become less uniform with micropore majorly formed within the scaffold. Considering the result obtained from TGA, NaHCO₃ entrapped in the scaffold did not change the properties of the PU matrix itself. However, high percentage of NaHCO₃ greatly influenced the mechanical properties of the scaffold due to NaHCO₃ entrapped in scaffold might act as filler which further increased the storage modulus of the scaffold. Based on morphology analysis of 100C and 75C25H scaffolds, future work would attempt to consider 0-25% amount of NaHCO₃ able to further improve the microstructure of the scaffold.

Keywords: Polyurethane, Bioglass®, Salt leaching, Scaffold; NaCl, NaHCO₃;

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1. INTRODUCTION

Scaffold fabrication and design are crucial in biomaterials aspect as it plays unique role in bone tissue regeneration and repair [1]. Scaffold is defined as a 3D substrate for cells and tissue regeneration template [2]. Ideal scaffold for bone template should have the following requirements: (i) highly porous structure with interconnected pore network to allow cell penetration, as well as transport of nutrients and metabolic waste [1], (ii) biocompatible and bioresorbable with synchronize degradation rate and cell/tissue growth in vitro and in vivo [3], (iii) compatible surface chemistry for cell attachment, proliferation and differentiation [3], (iv) mechanical properties mimic with the natural bone tissue [4], (v) able to bond to host tissue as well as prevent the invasion of scar tissue [1]. Polyurethane (PU) has attracted great attention for clinical and surgical application as it has unique segmented structure [5]. Tunable soft and hard segment properties of PU make it possible to tailor its mechanical properties, thermoplasticity and durability depending on its application [6]. Therefore, there were urged among researchers to fabricate polymer scaffold reinforced with bioactive materials such as Bioglass® (BG), bioglass-ceramic and calcium phosphate ceramic [7] which is one of the alternatives to improve the bioactivity of the PU scaffold. Hence, Bioglass® 45S5 discovered by Hench in

1969-1971 with the Na₂O-CaO-SiO₂-P₂O₅ system high in calcium content with a composition closed to the Na₂O-CaO-SiO₂ diagram [8] become one of the promising candidates to improve the bioactivity of PU scaffold.

A wide variety of techniques to fabricate 3D biodegradable polymeric scaffold including thermal induced phase separation [9], salt leaching [10], freeze drying [4], solid-liquid phase separation [11] and electro spinning [12]. Salt leaching method has been chosen to construct the scaffold due to ease of fabrication, controllable pore size and porosity by varying the size and concentration of leaching agent [5], do not imply any high temperature that preserve the polymer properties and on top of that leaching agent do not release dangerous ions for the human body if trapped in the scaffold [10].

Salt leaching had been widely applied by other scholars to prepare the scaffold by means this method is effective in controlling the porosity and pore size. The leaching agent used usually sodium chloride (NaCl) salt or of an organic compound such as saccharose to create pores [13]. Cannillo et al. [10] carried out preliminary study to define the best NaCl and/or NaHCO₃ able to optimize the pore structure of the polycaprolactone scaffold. It is believed the blend of leaching agent NaCl and NaHCO₃ able to create open pore with controllable pore size as NaHCO₃ aids to form interconnected structure for the scaffold. Hence, the scaffold obtained by salt leaching technique with different percentage of salt mixtures should be further investigated as the scaffold will exhibit different structural and physical properties that will further affect the mechanical behavior of the scaffold itself. In this work, 25-100% of NaHCO₃ had been combined with NaCl as leaching agent to fabricate PU-BG scaffold and the effect of different composition of leaching agent on morphology, thermal and mechanical properties of the scaffold was studied.

1. MATERIALS AND METHODS

1.1 Synthesis of Bioglass® (BG).

Bioglass® 45S5 with the 45.0 wt.% SiO₂, 24.5 wt.% Na₂O, 24.5 wt.% CaO and 6 wt.% P₂O₅ were synthesized by melt quenched technique [8]. High purity powder SiO₂, Na₂CO₃, P₂O₅ (Sigma-Aldrich) and CaCO₃ (MERCK Milipore) were weighed and melted in a crucible by two melting stages at temperature 1200 °C and 1400 °C for seven hours with a heating rate 10 °C/min to ensure a homogeneous molten glass can be produced. After melting, a molten glass was quenched immediately in deionized water bath at room temperature.

1.2 Fabrication of PU-BG composite scaffold.

The PU-BG scaffold was fabricated by solvent casting / salt leaching technique followed the flow procedure by Lim et al. [14]. Neat PU (PEARLTHANE 11T80 from Duplas Marketing Sdn. Bhd.) beads were weighed and dissolved in Tetrahydrofuran (THF) (Sigma Alrich) at a concentration of 10 w/v% for 1.5 hours at 50 °C to form PU solution. Then, 10 wt.% of synthesized BG powder (mean particle size < 8µm) was added to the PU dispersion then stirred continuously for 1.5 hours to allow homogeneous dispersion of BG. Leaching agent NaCl and NaHCO₃ (MERCK Milipore) were then added into the dispersion with the ratio of 1:5 of polymer to leaching agent and mixed homogeneously for another 15 min. The dispersion was then transferred into a mold and dried in room temperature for two days to allow complete THF evaporation. The

salt in the sample was then washed out by immersing the sample in deionized water for three days. The water was refreshed every six hours to ensure salt was leached out from the sample. Finally, the obtained scaffold was dried in an oven at 80 °C for one day. Thus, five types of sample were fabricated using leaching agent NaCl, labelled as 'C' meanwhile NaHCO₃ labelled as 'H'. Table 1 tabulated the samples code with different composition of leaching agent.

Table 1 Samples of PU-BG with different composition of leaching agent

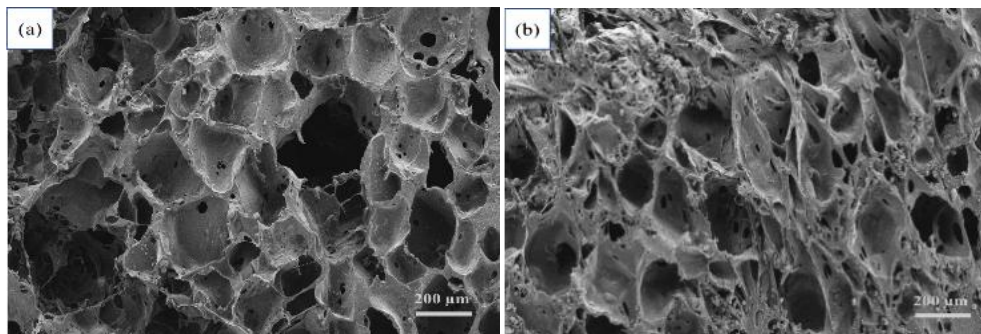
Sample Code	Composition of leaching agent
100C	100%NaCl
75C25H	75% NaCl and 25% NaHCO ₃
50C50H	50% NaCl and 50% NaHCO ₃
25C75H	25% NaCl and 75% NaHCO ₃
100H	100% NaHCO ₃

1.3 Characterization.

Field emission scanning electron microscope (FESEM) (SUPRA 35VP, ZEISS) equipped with energy dispersive X-ray spectroscopy (EDX) was performed on scaffolds to observe the pore structure of the scaffold. Thermal properties of scaffold were carried out by both thermogravimetric analysis (TGA) (1 STAR^e System, Mettler Toledo) to analyze degradation profile of scaffolds. The degradation process was analyzed from room temperature until 800 °C at a controlled heating rate of 10 °C/min under nitrogen gas. Compression test by dynamic mechanical analysis (DMA) was carried out on cylinder shaped specimens with dimensions of 9 mm height and 20 mm radius. This testing was carried out using mechanical analysis (DMA) Mettler Toledo. The temperature-dependent storage modulus of each material was evaluated with a temperature sweep of 0 °C to 100 °C, at a compression frequency of 1 Hz, 20 μm amplitude, 0.3% strain, and 0.01N static force. The storage modulus (E) value was recorded as a function of frequency.

2. RESULTS AND DISCUSSION

FESEM images of the scaffold in Fig. 1 reveal the open pore and interconnectivity of the cross-sectional structure of the scaffold. FESEM images reveals that, different ratio of salt composition gives a significant effect on the properties of the pore. Shape and size of the pore changed significantly as the proportion of the



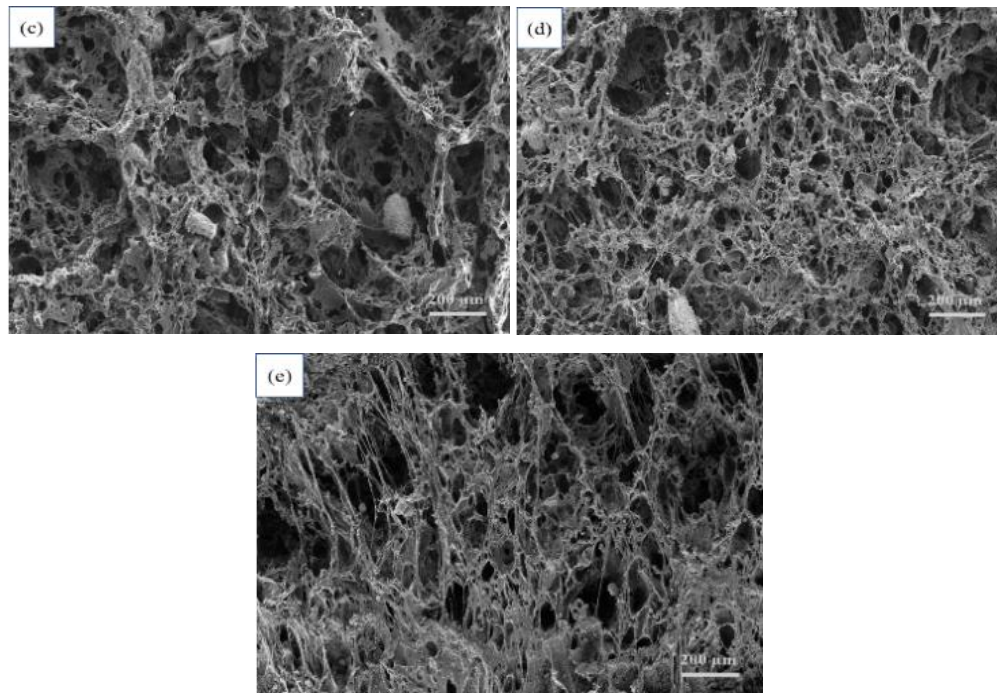


Fig. 1 FESEM images of the composite scaffolds, (a) 100C, (b) 75C25H, (c) 50C50H, (d) 25C75H, and (e) 100H

leaching agent varied. It can be observed that, scaffold with high percentage of NaCl (100C) had slightly rounded shape and pore size around 84-326 μm . Meanwhile, as the composition of NaHCO_3 increases, the structure of the pore changes drastically as the pore becomes less uniform with irregular pore shape within the scaffold. This observation can be distinguished from the scaffolds 25C75H and 100H which had pore size range 84-281 μm and 54-143 μm respectively. Irregular macropore exists in scaffold 25C75H and 100H was caused by the agglomeration of smaller particle of NaHCO_3 which did not dispersed homogeneously besides caused the pore wall to rupture. However, as reviewed by Karageorgiou et al. [15], general minimum pore size required to generate mineralized bone is considered $\sim 100 \mu\text{m}$. Although macroporosity (pore size $> 50 \mu\text{m}$) has big impact in osteogenic outcome, microporosity (pore size $< 10 \mu\text{m}$) and pore wall roughness play an important role as well to induce bone formation [16]. It seems that scaffold 100C is the best scaffold in this work since the scaffold exhibited a lot and well-developed porosity with pore size covers a very wide range, from tens of microns to hundreds of microns. Macropores within the range 100-400 μm are considered the ideal size for bone tissue formation meanwhile pore between 150-900 μm allows for nutrient supply and waste removal of cells grown on the scaffold [9]. Even though the pore structure for 75C25H scaffold was not well-built with a slight rupture of the pore wall, however this scaffold can be a promising candidate as it was able to create micropore as interconnected pore within the pore wall. Thus, it was suggested to continue investigating the effect of salt composition between the scaffold 100C and 75C25H.

TGA characterization was performed to monitor the weight loss of the material due to the formation of volatile compound under degradation owing to the heating and temperature rising. Fig. 2 presents the

degradation profile of PU-BG scaffold with different composition of NaCl and NaHCO₃. Based on Fig. 2, around temperature 76 until 106 °C, all scaffolds except 100C start to degrade due to the decomposition of NaHCO₃ into Na₂CO₃, water and carbon dioxide. Second step degradation was around 130 until 170 °C which can be attributed to the completion degradation of NaHCO₃ into Na₂CO₃ mostly occur around 200 °C. Eq. 1 shows the reaction of NaHCO₃ into NaCO₃, water and carbon dioxide [17]. Another degradation step befalls around temperature 240 to 480 °C which is due to the degradation of urethane bond of PU. This can conclude that, excess leaching agent entrapped in the scaffold did not alter the properties of the PU.

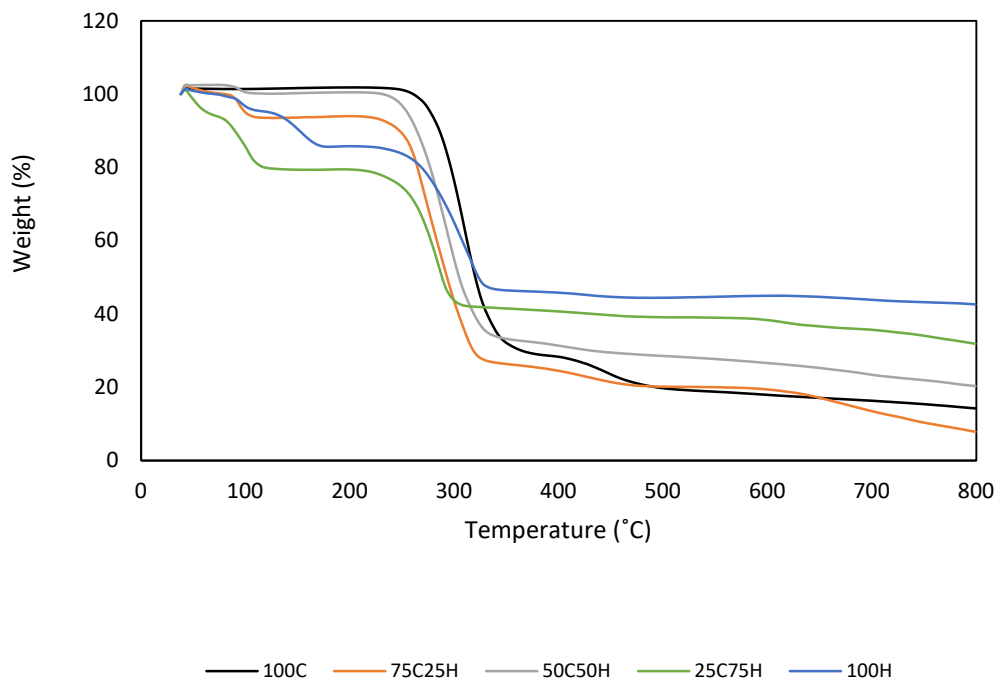
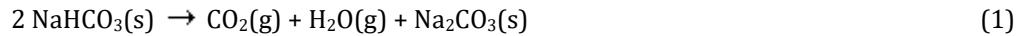


Fig. 2 TGA thermogram of PU-BG scaffold

The excess of NaHCO₃ entrapped in the scaffold had been proved by the EDX result in Fig. 3. The amount of sodium (Na) contain in the sample 100H is much higher (12.68 wt.%) as compared to sample with 100C (0.80 wt.%). High percentage of Na in 100H scaffold indicates the presence of NaHCO₃ which did not fully leached out during the immersion in deionized water. This finding suggested the difficulty to leached out NaHCO₃ as NaHCO₃ had lower water solubility which is only 96 g/L as compared to NaCl with 358 g/L (according to SDS provided by Merck Milipore).

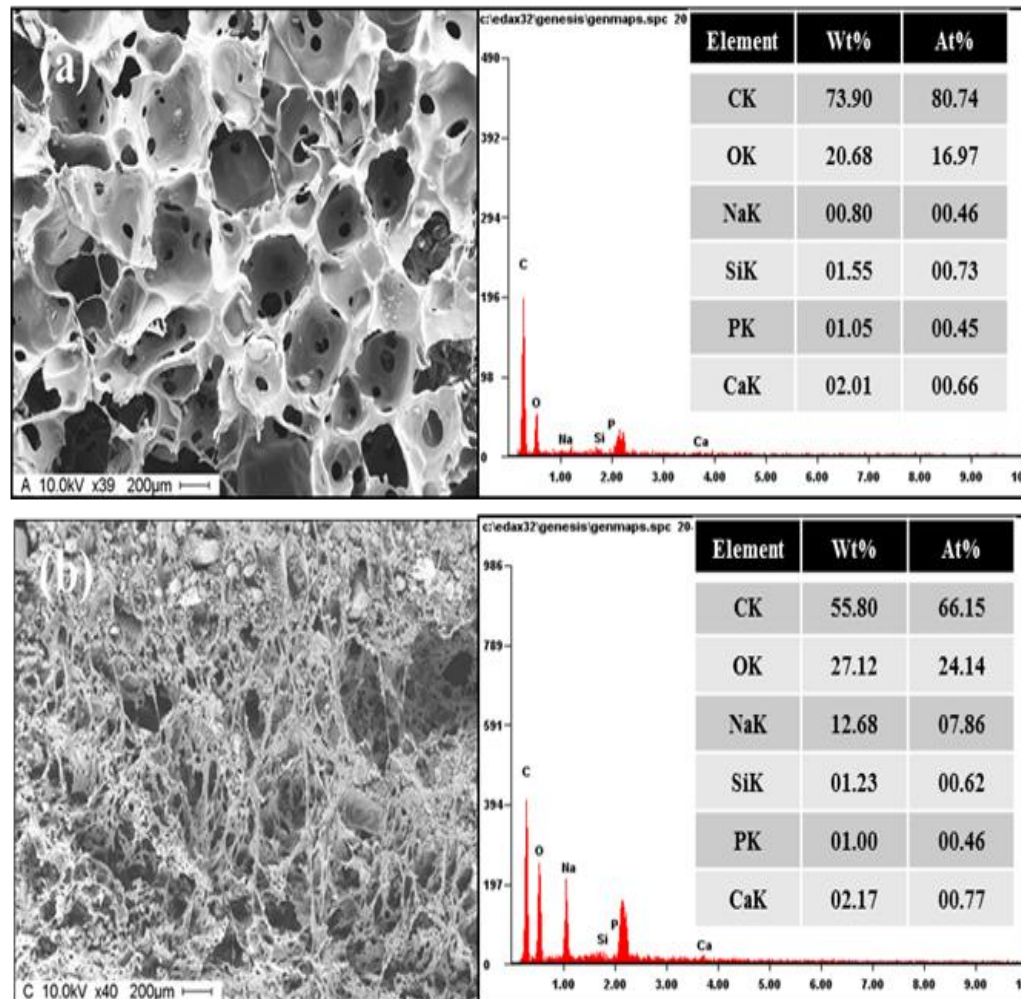


Fig. 3 FESEM images and EDX analysis for scaffold (a) 100H and (b) 100C

Fig. 4 represents the storage modulus for PU-BG scaffold at 27 °C and 37 °C. Such temperatures were selected to investigate the behavior of the samples at room temperature and human body temperature since the application is related to bone tissue engineering. According to Fig. 4, the data showed scaffold 100H exhibits the highest storage modulus compared with others scaffold which do not have significant difference in storage modulus. Due to the smaller particles size of NaHCO_3 , micropores was formed in the 100H scaffold resulted with higher density scaffold. In addition, the presence of leaching agent NaHCO_3 that had not been completely leached out during immersion in deionized might act as filler and improved the storage modulus of the PU-BG scaffold. Scaffold 50C50H and 25C75H used NaHCO_3 leaching agent 50 and 75% respectively, however the value of storage modulus of 50C50H (14.5 kPa) is higher than 25C75H (9 kPa) suggesting that the formation of more homogeneous foam structure in scaffold 50C50H had allowed for higher resulting storage modulus of the scaffold. Other study also demonstrated storage modulus of PU scaffold within the similar range with the obtained result [18].

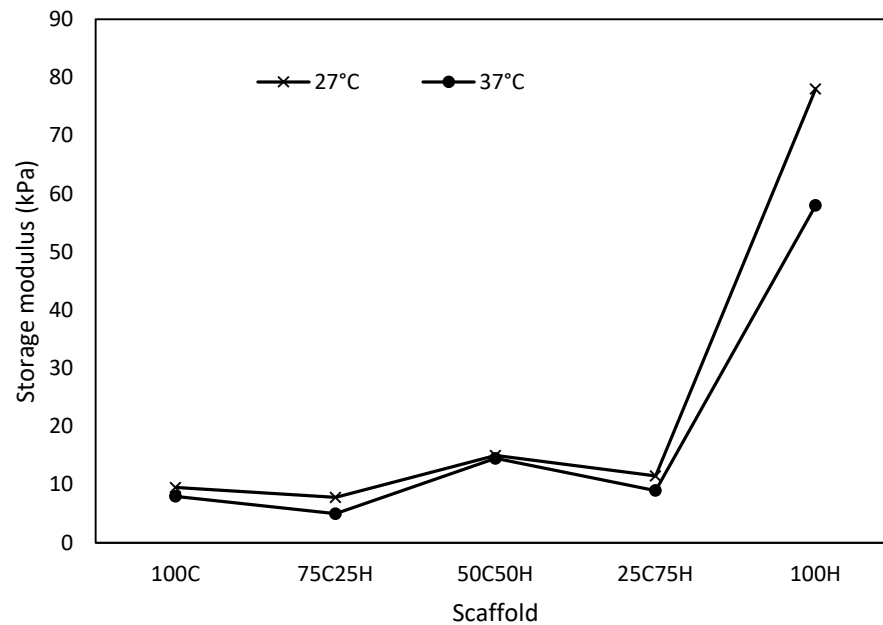


Fig. 4 Storage modulus of PU-BG scaffold with different composition of leaching agent

3. SUMMARY

In this study, porous Bioglass® (BG) reinforced Polyurethane (PU) scaffolds were fabricated using salt leaching technique with different composition of NaCl and NaHCO₃ as leaching agents. It was found that, the composition of leaching agent gives a profound impact on the scaffold morphology and mechanical properties. As the composition of NaHCO₃ increased, pore formation changed drastically with irregular shape of pore formed and smaller pore size. The 100C scaffold showed better morphology with a well-developed pore structure compared with others composite. However, 75C25H scaffold showed promising candidate as it was able to create micropore as interconnected pore within the pore wall. Thus, further study should be conducted to investigate the effect of salt composition within the range 100C and 75C25H. It was also found that, scaffolds with higher composition of NaCl aid to the leaching process as TGA analysis showed no traces of leaching agent left at the final product of scaffold. Storage modulus of the scaffold indicate that, scaffold using high percentage of NaHCO₃ composition possessed higher value of storage modulus resulted from the smaller pore size of the scaffold besides the entrapped leaching agent may act as filler that increased the storage modulus.

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