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PHARMACEUTICALS**

**Influence of Poly (glycerol sebacate) Coating Concentration on the Structure
Property Bioactivity Relationships of Foam-Replicated 45S5 Bioactive Glass
Scaffolds**

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Declaration of Originality

I declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person except where due acknowledgement is made in the text. The experimental work reported was carried out at the Institute of Biomaterials, Friedrich-Alexander-Universität Erlangen-Nurnberg, Germany, during a research Erasmus traineeship conducted under the supervision of Prof. Aldo R. Boccaccini.

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Abstract

The polymer foam replication method was used for the fabrication of 3D 45S5 bioactive glass scaffolds with interconnected macroporosity for osteoconduction and vascularization. However, the brittleness of these scaffolds is very high due to the presence of hollow strut defects formed during the polymer burnout process, limiting their clinical application (Chen et al., 2006). In this study, the structural, mechanical, and biological effects of using the biodegradable elastomer poly (glycerol sebacate) (PGS) from endogenous monomers (Rosalia et al., 2024; Wang et al., 2002) as a reinforcing surface coating at different concentrations (0, 2.5, 5, 10, 15 and 20% w/v) were investigated. The sintered glass-ceramic scaffolds exhibited an open porosity of $94.72 \pm 0.53\%$ with interconnected pores in the range of 300 to 500 microns, which meets the criteria for mass transport in bone defect repair. Compressive strength showed a biphasic trend with a peak at 10% w/v PGS (0.0541 ± 0.0202 MPa), which was 12.5 times higher than that of uncoated controls (0.0043 ± 0.0010 MPa, $p < 0.001$) through elastomeric crack-bridging without significantly affecting the stiffness of the scaffold ($p = 0.096$). The PGS deposition altered surface wettability from 41.53° to 75.96° static contact angles and demonstrated an inverse linear relationship with mass loss on Day 7 in phosphate buffered saline (R^2 0.95 for 2.5% to 15% w/v), thereby controlling medium alkalinization. The polymeric layer showed a concentration dependent delay in the nucleation of hydroxycarbonate apatite in simulated body fluid, indicating a sequential degradation process in which structural safeguarding precedes interfacial biomineralization (Lin et al., 2015). Serum-treated glass allowed initial MG63 osteoblast adhesion, and unconditioned PGS coatings were limited in this regard because of the hydrophobic properties of the surface and the delayed mineral signals (Bacakova et al., 2011; Gómez-Cerezo et al., 2020). The 10% w/v formulation is the best compromise between mechanical stability and control of degradation and bioactivity for the regeneration of cancellous bone tissue.

Keywords: Bioactive glass 45S5; Poly (glycerol sebacate); Foam replica; Scaffold improvement; Bone tissue regeneration; Biomineralization.

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List of Abbreviations

45S5	45 wt% SiO ₂ -24.5 wt% CaO-24.5 wt% Na ₂ O-6 wt% P ₂ O ₅ bioactive glass
BG	Bioactive glass
BTE	Bone tissue engineering
FTIR	Fourier transform infrared spectroscopy
HAp	Hydroxyapatite
MG63	Human osteosarcoma cell line; widely used model for initial cytocompatibility screening of bone biomaterials
PBS	Phosphate-buffered saline
PCL	Polycaprolactone
PDLLA	Poly (D,L-lactic acid)
PGS	Poly(glycerol sebacate)
PU	Polyurethane
PVA	Polyvinyl alcohol
SBF	Simulated body fluid
SD	Standard deviation
SEM	Scanning electron microscopy
XRD	X-ray diffraction

1 Introduction

1.1 Clinical need for bone graft substitutes and tissue engineered scaffolds

Bone is the second most transplanted tissue after blood, and an estimated 2.2 million bone grafting procedures are performed around the world each year to repair defects caused by trauma, tumor resection, osteoporotic fractures and degenerative diseases (Zhang et al., 2025). However, autologous bone grafts are the clinical gold standard due to their osteogenic, osteoinductive, and osteoconductive properties (He et al., 2023; A. H. Schmidt, 2021; Zhang et al., 2025) but their use is limited by donor site morbidity, limited graft availability, infection risk, and increased operative time. Allogeneic grafts do not display complications at the donor site but have issues such as immunogenicity, infection, chronic inflammation, and delayed integration with the host (He et al., 2023). The above limitations have resulted in the pursuit of synthetic biomaterials capable of promoting bone regeneration without using biological donors. The fabrication of three-dimensional porous scaffolds is a key issue in bone tissue engineering (BTE). Scaffolds serve as a temporary matrix for the attachment, proliferation and differentiation of cells and degrade along with the formation of new tissue (Gerhardt & Boccaccini, 2010). For efficacy, scaffolds must be highly interconnected in their porosity to allow cell and tissue infiltration, vascularization and metabolite exchange, and they must have mechanical stability and a degradation rate compatible with tissue regeneration. Furthermore, cellular responses are strongly influenced by surface and material properties (Gerhardt & Boccaccini, 2010; He et al., 2023).

1.2 Bioactive glasses, and that 45S5 composition

Bioactive glasses have been the subject of much attention as biomaterials, due to their unique ability to chemically bond to bone tissue when implanted. (Jones, 2013) refers to the seminal work of Hench (1969), who prepared the 45S5 composition (45 wt.% SiO_2 , 24.5 wt.% Na_2O , 24.5 wt.% CaO , 6 wt.% P_2O_5) and showed stable, direct contacts between synthetic materials and biological bone. The bioactive glass 45S5 exhibits reactivity with physiological fluids through a sequence of surface reactions. Such as rapid ion-exchange and silanol group synthesis. This results in the creation of a silica-rich layer. This layer ensures the nucleation of an amorphous calcium phosphate phase. This stage ultimately leads to crystallization into hydroxycarbonate apatite (HCA), which bears a close resemblance to the natural bone

mineral and is the basis of the strong interfacial bonding (Islam et al., 2017; Kokubo & Takadama, 2006). Moreover, release of physiologically active ions (Si, Ca, P, Na) regulates osteoblastic gene expression, cellular proliferation, angiogenesis, and inflammatory responses to promote bone repair (Hoppe et al., 2011). However, due to the intrinsic brittleness and low fracture toughness (0.5-1 MPa.m^{1/2}) of the 45S5 bioactive glass, its utility in load bearing applications is limited (Fu et al., 2013). The manufacturing of very porous scaffolds also reduces mechanical strength; however, with optimization of composition and processing, compressive qualities similar to trabecular bone can be attained (Boccardi et al., 2017; Fu et al., 2013).

1.3 The foam replica method and structural requirements for bone tissue engineering scaffolds

Porous bioactive glass 3D scaffolds can be fabricated using robocasting, freeze casting, sol-gel foaming, and foam replica technique (Boccardi et al., 2017). A common method is the foam replica technique, firstly reported by (Chen et al., 2006) for 45S5 scaffolds. The approach is simple, easy and reproducible. It can generate interconnected porous structures very similar to the trabecular bone. The procedure uses an open cell polyurethane foam as a template, which is immersed in a slurry created from bioactive glass. The excess slurry is drained off, and the sample is then dried and sintered at high temperature to burn out the foam template. What remains is a porous scaffold that retains the foam shape, frequently with porosity above 85% (Chen et al., 2006). Templates set the pore features and can be designed to incorporate cells and capillaries and to help scaffold promote new bone formation (Fiume et al., 2021; Gerhardt & Boccaccini, 2010). Pores need to be more than 100 µm for bone formation and around 300-500 µm for the best osteogenesis and vascularization. 70-90% of porosity is said to be the best, so long as it is not so porous to compromise the mechanical strength of the implant (Huang et al., 2024; Karageorgiou & Kaplan, 2005) previously mentioned. The upper 90% of porosity is suggested to be the most ideal porosity of the implant (Karageorgiou & Kaplan, 2005). The best performance of biological scaffolds is achieved by the greatest porosity and the highest percentages of the upper 90% of total porosity (Karageorgiou & Kaplan, 2005). The design of the scaffold must not only be able to perform the desired biological task but also be able to perform the mechanical strength required to hold its desired position within the body; thus, more research is being done in this regard (Miri et al., 2024).

1.4 Polymer coating as a strategy to reinforce 45S5 scaffolds

Highly porous scaffolds composed of 45S5 bioactive glass possess considerable potential for applications related to the regeneration of bone. However, their potential is lessened by their brittle nature. To address this shortcoming, complementary studies by several groups have demonstrated that the application of coatings composed of biodegradable polymers is beneficial. Such coatings serve to bridge microcracks, reinforce connections between struts, and overall serve to distribute stresses within the structure of the scaffold, while maintaining the open porosity of the original scaffold (Chen et al., 2006). A particularly relevant coating material, poly (D, L-lactic acid) (PDLLA), has been shown to increase the compressive strength of the scaffold by 2.3 MPa while still exhibiting over 90% porosity and bioactivity (Chen & Boccaccini, 2006). These attributes closely match the microstructure and strength of human trabecular bone which spans a range of 0.1-16 MPa (Gerhardt & Boccaccini, 2010). In other studies, poly(3-hydroxybutyrate) P(3HB) coatings and cellulose nanowhiskers were shown to improve mechanical attributes and support the proliferation of osteoblasts (Li et al., 2014). Beginning with the polymer based coatings, numerous improvements to the conceptual influences have been shown. They include the use of carbon nanotubes along with several other coatings like PCL/zein, genipin-crosslinked gelatin and zein/Manuka honey that combine the polymer with other bioactive materials improving strength and biological functions (Arango-Ospina et al., 2021; Boffito et al., 2021; Fereshteh et al., 2015; Kumar et al., 2023; Li et al., 2015). Typical polymer-coated 45S5 scaffolds designed to achieve high porosity and bioactivity tend to meet the mechanical and biological requirements of bone, assuring suitability for use in biological tissue engineering applications.

1.5 Poly (glycerol sebacate) as a candidate elastomeric coating

Poly (glycerol sebacate) (PGS) biodegradable elastomers are synthesized by polycondensation through mixing and heating glycerol with sebacic acid, both of which are endogenous monomers. Sebacic acid is an important metabolic BAP (biocompatible acid precursor), as it is metabolized to acetyl-CoA, while glycerol is important for both the lipid and energy cycles. Sebacic acid and glycerol yield elastomers that are safe when metabolized in vivo. Sebacic and glycerol elastomers have previously been used in medical applications that have undergone FDA approval (Rosalia et al., 2024). The PGS elastomers properties are controlled by synthesis

conditions and include elasticity and degradation rate. Processing conditions such as heating duration and temperature are used to adjust crosslinking and curing times to generate materials with controllable stiffness. For example, polycondensation at 110–130°C for 24–120 hours, followed by a vacuum curing at 120–150°C (Rosalia et al., 2024). PGS elastomers were originally designed for cardiac tissue applications due to their nonlinear elasticity. They exhibit cytocompatibility, degradation, and the stiffness is controllable. These properties make PGS a good candidate for reinforcing brittle bioactive glass scaffolds (Wang et al., 2002). A recent review presented the importance of PGS processing conditions in demonstrating control of elastomer stiffness, strength, and crosslink density (Rosalia et al., 2024). PGS has been widely researched in combination with bioactive glass since it is biodegradable, and it imparts strength, controlled protein release and osteogenic function to the scaffolds it coatings with suggested optimum efficiency of uncrosslinked PGS in the region of 2-12 MPa (Lin et al., 2015). Bioactive glass was used in PGS printable fiber composites which were previously blended with typical PGS-zein formulations in prior investigations to be 3D printed, and the scaffolds showed improved mechanical properties, bioactivity and cytocompatibility (Ruther et al., 2022; Souza et al., 2017). PGS, however, has not been tested for its applicability as a solvent cast PGS coating for foam replicated 45S5 bioactive glass scaffolds. This includes how varying coating concentrations affects porosity, mechanical characteristics, degradation, bioactivity and wettability of the scaffold. This is reportedly the first systematic study where PGS has been used in this way on 45S5 bioactive glass scaffolds.

1.6 Research hypothesis

Hypothesis: Poly(glycerol sebacate) (PGS) coating of foam-replicated 45S5 bioactive glass scaffolds will increase the scaffold's compressive mechanical strength through elastomeric crack-bridging while maintaining open macroporous interconnectivity. Coating concentration will determine overall performance. In particular, intermediate PGS concentrations will achieve an optimal compromise between mechanical reinforcement, degradation stability and surface wettability. Higher coating concentrations will act as a physical diffusion barrier that enhances surface hydrophobicity, slows down hydroxyapatite nucleation in simulated body fluid and restricts early MG63 cell attachment.

2 Aim and Objectives

2.1 Overall aim

The main goal of this thesis is to fabricate foam replicated 45S5 bioactive glass scaffolds and analyze the effects of different concentration levels of PGS on the physical, mechanical, degradation, and biological properties of 45S5 foam replicated glass scaffolds for bone tissue engineering.

2.2 Specific objectives

- a) Use a foam replication technique to prepare highly porous 45S5 BG scaffolds and analyze and characterize their initial physical properties.
- b) Formulate a process to synthesize PGS through thermal polycondensation and to uniformly coat scaffold surfaces with different concentration levels of PGS.
- c) Characterize and determine the effects of the scaffold coating on the surface morphology using scanning electron microscopy (SEM)
- d) Measure scaffold surface wettability using static contact angle analysis and understand how it is influenced by PGS coating concentration.
- e) Formulate the concentration effects of PGS coating on compressive mechanical properties of scaffolds (strength and modulus).
- f) Monitor mass loss and pH changes to quantify the effects of PGS coated scaffolds in phosphate buffered saline (PBS).
- g) Follow up early stage in vitro bioactivity through monitoring hydroxyapatite formation with SEM, FTIR, and XRD to quantify the effects of PGS coated scaffolds on the formation of apatite mineral in SBF.
- h) Quantify the effects of PGS on cell-scaffold interactions by evaluating the morphology of MG63 cells and their attachment to the uncoated and coated scaffolds.

3 Materials and Methods

3.1 Fabrication of 45S5 scaffolds by the foam replica method

Table 3.1 shows the core materials that were used to fabricate 45S5 bioglass scaffolds by foam replica method. A commercial 45S5 bioactive glass powder, with particle size under 4 micrometers, was used for the scaffold fabrication. For the slurry preparation, PVA served as a binder, while the crosslinked PU foam was the sacrificial template. For PGS synthesis, glycerol and sebacic acid were used.

Table 3.1 Materials, suppliers, and chemical information used in this study.

Material	Supplier	Specification
45S5 bioactive glass powder (Vitryxx)	Schott AG, Mainz, Germany	Particle size < 4 μm
Polyvinyl alcohol (PVA)	Merck Millipore, Darmstadt, Germany	MW 30 000 g/mol, fully hydrolyzed
Polyurethane (PU) foam	Eurofoam Deutschland GmbH, Germany	45 ppi
Glycerol	Sigma-Aldrich, Germany	Purity > 99 % (BioXtra)
Sebacic acid	Sigma-Aldrich, Germany	Purity > 99 %
Ethanol	VWR, Germany	99 % absolute
Phosphate-buffered saline (PBS)	Sigma-Aldrich, Germany	pH 7.4, 1x
Ultrapure water (MilliQ)	In-house, FAU Erlangen	18.2 MOhm-cm
NaCl, NaHCO ₃ , KCl, K ₂ HPO ₄ ·3H ₂ O, MgCl ₂ ·6H ₂ O, CaCl ₂ , Na ₂ SO ₄	Sigma-Aldrich, Germany	For SBF preparation (Kokubo protocol)

3.1.1 Polyurethane template preparation

Cylindrical polyurethane foam templates (45 pores per inch (ppi)) were cut to some standardized sizes, 10 mm in diameter and 12 mm in height, using a cylindrical punch. The templates were then washed with isopropanol to get rid of any remaining surfactants and manufacturing oils, and then they were dried at 60°C in a lab oven until completely dry before being used.

3.1.2 Bioactive glass slurry preparation

A 6% w/v PVA solution was prepared by dissolving 0.6 g PVA in 10 mL of ultrapure water in a preheated 80°C water bath for 1 h with the help of a magnetic stirrer in an effort to ensure uniform heat transfer. The temperature was decreased after dissolution, and 10 g of 45S5 bioactive glass powder (Vitryxx, <4 µm) was added in steps to obtain a 50 wt% slurry. The suspension was stirred for another hour until homogeneous.

3.1.3 Green body formation and sintering

The PU templates were dipped into a continuously stirred bioglass slurry for 1 min. By a gentle pressure, the excess slurry was removed to obtain the most uniform coating possible on the pore walls. The templates were dried at 60°C for one hour. They were then dipped and dried 2-3 times to build up the bioactive glass layer as applied by the methods of (Chen et al., 2006) and (Boffito et al., 2021). Then the green bodies were sintered in a muffle furnace. First, the PU and the binder were burnt out by heating the temperature to 400°C at a rate of 2°C in a minute and holding it for an hour. The green bodies were heated to 1050°C in 1 hour and kept at this temperature for 1 hour to sinter and partially crystallize the glass (Lefebvre et al., 2007). The scaffold was allowed to cool by itself to room temperature before it was removed.

3.2 Physical characterization of sintered scaffolds

The sintered scaffolds were characterized for bulk density, porosity and dimensional shrinkage. All the measurements were done with n = 10 scaffolds. Scaffold volume was calculated from caliper readings of the diameter (d) and height (h) assuming a cylindrical geometry:

$$V = \pi d^2 h / 4$$

Then bulk density (ρ_{bulk}) was calculated by taking the scaffold mass right after sintering (m_{after}) and dividing it by the corresponding scaffold volume (V_{after}):

$$\rho_{\text{bulk}} = m_{\text{after}} / V_{\text{after}}$$

Scaffold porosity was calculated from the measured bulk density versus the theoretical density of dense 45S5 bioactive glass. $\rho_{45S5} = 2.7 \text{ g/cm}^3$ was considered, according to (Chen et al., 2006):

$$\text{Porosity (\%)} = [1 - (\rho_{\text{bulk}} / \rho_{4555})] \times 100$$

Linear, diametric, and volumetric shrinkages were calculated from the dimensions and the volume of the green bodies “before sintering,” compared to the values after sintering:

$$\text{Shrinkage (L) (\%)} = [(L_{\text{before}} - L_{\text{after}}) / L_{\text{before}}] \times 100$$

$$\text{Shrinkage (D) (\%)} = [(D_{\text{before}} - D_{\text{after}}) / D_{\text{before}}] \times 100$$

$$\text{Shrinkage (V) (\%)} = [(V_{\text{before}} - V_{\text{after}}) / V_{\text{before}}] \times 100$$

3.3 PGS synthesis

Poly (glycerol sebacate) was produced by equimolar polycondensation of 0.1 mol glycerol (Sigma-Aldrich) and 0.1 mol sebacic acid (Sigma-Aldrich) following the process of (Rosalia et al., 2024). The reaction was done in a round-bottom flask with a stir bar and thermocouple. The monomers were first added to the flask, and then a gentle constant nitrogen flow of about one bubble per second was employed to reduce oxidation. The reaction conditions were based on guidelines for PGS synthesis that are outlined in the similar protocol by (Rosalia et al., 2024). The flask was then heated to 120°C in an oil bath. The reaction was left to partially polymerize for 24 hours at 120°C under nitrogen with continuous stirring. The viscous pale yellow prepolymer material was then poured into a polypropylene container at room temperature for future use.

3.3.1 Pilot coating strategy using pre-polymer

A PGS coating was applied to a scaffold surface, and then it was cross linked in a vacuum oven at 120°C. This process left the scaffold covered in a non-uniformly coated brownish discoloration. It is thought that the nitrogen flow was interrupted, and the prepolymer was exposed to oxygen in the atmosphere, causing it to partially oxidize. This is an educated guess and cannot yet be definitively concluded. The use of this affected batch was stopped, and the issues were investigated, and the coating process was revised.

3.3.2 Final coating protocol using PGS pre-polymer in ethanol

To develop a coating protocol to minimize strut coating non-uniformity and to minimize the occurrence of macropore occlusion, an ethanol-based solution deposition protocol was developed. The undiluted synthesized PGS prepolymer was dissolved in 99% ethanol at 2.5,

5.0, 10, 15, and 20% concentrations (G2.5–G20, Table 3.2). The sintered 45S5 scaffolds were preheated to 60°C to promote capillary forces to drive wetting and avoid premature precipitation of solute. Two 500-μL depositions were made on opposite faces of the scaffold. The scaffold was then rotated under an axial flow of filtered compressed air to clear the internal pores of excess liquid for the next deposition of solute and to drive solvent evaporation for the prior deposition. The coated scaffolds were then thermally stabilized at 60°C before being transferred to a vacuum oven for thermal curing. The final stage of thermal curing at 120°C under reduced pressure ($1.1\text{--}2.5 \times 10^{-2}$ mbar) for 96 h ensured complete solvent evaporation and ester crosslinking, resulting in elastic, transparent polysiloxane coating of the glass-ceramics.

Table 3.2 Sample group designations, PGS coating concentrations, and primary tests performed for each group.

Group code	PGS concentration (% (w/v))	Tests performed
G0 (uncoated)	0	Porosity, SEM, SBF 0-28 d, FTIR, XRD, contact angle mechanics, cell study
G2.5	2.5	SEM, contact angle, mechanics, PBS degradation, SBF 7 d, FTIR, XRD, cell study
G5	5	
G10	10	
G15	15	
G20	20	

3.4 Morphological characterization

After the first assessment, we examined scaffolds of each type and orientation using light microscopy (scale bar: 0.5 mm) to determine pore structure and surface coating. Preparation and analysis through the FE-SEM took additional steps. After loading and securing the samples onto an aluminum stub, a coating of gold was applied with a Quorum Q150T Turbo. SEM images at lower magnification (100–200 μm scale bar) were used to evaluate macroscopic structures and surface coating distribution. Higher magnification images (2–20 μm scale bar) were used to evaluate strut structures and polymer coating buildup. All structures were analyzed in triplicate per group.

3.5 Contact angle measurement

We used a drop-shape analysis (DSA30) to obtain wettability data through static sessile drop contact angle measurements. To ensure the wettability measurements were correct, pressed pellets of the 45S5 bioglass powder with the same coating concentrations for each group were used, thus decreasing potential issues with porosity and surface roughness (Paxton & Woodruff, 2022). For each group, five measurements with three microliter (3 μ L) drop volumes of deionized water were taken. The measurements were recorded 10 seconds after the drop placement. The software, DSA4 2.0, was used for the image analysis of the contact angle measurement.

3.6 Mechanical testing

A universal testing machine (Instron 3300, Instron GmbH, Germany) with a 100 N load cell was used to determine the uniaxial compressive strength of the 45S5-coated bioactive glass scaffolds using protocols established by (Arango-Ospina et al., 2021). The determined protocols and equipment were used to determine the mechanical properties of the scaffolds after they were dried to room temperature. Testing was done at a crosshead speed of 1 mm/min on the dry samples at a speed of 1 mm/min. Each group of samples had 5 replicates (n=5). Compressive strength was determined as the maximum stress prior to the first major load drop. Young's modulus was calculated from the slope of the initial stress and strain curve.

3.7 In vitro degradation in phosphate-buffered saline

PGS-coated scaffolds were placed in phosphate buffered saline (PBS, pH 7.4 at 37°C) for 1, 3, and 7 days with three scaffolds per group at each degradation period (n=3). Scaffolds were weighed before being coated ($W_{0, \text{Uncoated}}$), after being coated and cured ($W_{1, \text{Coated}}$), and after each degradation period ($W_{2, \text{after deg}}$) (W_2 was done after scaffolds were dried at 37°C to constant mass). Coating mass was calculated as $(W_1 - W_0)/W_1$. W_2 coating percentage was calculated as $(W_1 - W_2)/W_1$. The remaining mass was 100 minus the degradation percentage. The pH was also measured for the immersion medium at each time point with a calibrated pH meter (n=3). Only the scaffolds with PGS coatings were tested; the scaffolds in PBS alone were the control. Comparative data for the scaffolds without the PGS coating are cited from literature in the Results and Discussion section.

3.8 In vitro bioactivity assessment in simulated body fluid

The in vitro bioactivity of the scaffolds was analyzed by immersing them into simulated body fluid (SBF), which is commonly used to evaluate the apatite forming potential of bioactive materials, under conditions meant to resemble the ionic composition of human blood plasma (Kokubo & Takadama, 2006). A modified SBF 15 solution was made following a lab procedure supplied by Dana Rohanová. This setup included 15 mmol L⁻¹ HCO₃⁻, 2.5 mmol L⁻¹ Ca²⁺, and 137 mmol L⁻¹ Cl⁻. For the salts, NaCl (7.3638 g), NaHCO₃ (1.2601 g), KCl (0.2237 g), K₂HPO₄·3H₂O (0.2282 g), MgCl₂·6H₂O (0.3050 g), CaCl₂ (0.2775 g), and Na₂SO₄ (0.0710 g) were added one after the other, dissolved in 750 mL of demineralized water, and kept at 36.5°C. The solution was adjusted to pH 7.4, and then it was brought up to a final volume of 1000 mL using demineralized water, after which it was stored at 4°C for up to one week during use. For the uncoated scaffolds, they were immersed for 0, 7, 14, 21, and 28 days, whereas the coated scaffolds were only immersed for 0 and 7 days due to time constraints. At each time point samples were taken out, rinsed carefully with ultrapure water, and left to dry at room temperature before characterization. All SBF trials were carried out in triplicate.

3.9 FTIR and XRD characterization

FTIR spectra were collected using a Shimadzu Affinity-1S spectrometer using an ATR (attenuated total reflectance) accessory. Scans were performed with a 4 cm⁻¹ resolution across the range of 4000–400 cm⁻¹ and were the average of 32 scans at room temperature for each sample. Spectra were collected at 0, 7, 14, 21, and 28 days for uncoated scaffolds and at 0 and 7 days for PGS coated scaffolds both in SBF as the analysis environment. X-ray diffraction (XRD) was performed using a powder diffractometer operating at a scan rate suitable for phase identification and a range of 2θ between 10 and 80°. FTIR and XRD analyzes were performed using the reference FTIR spectra and XRD diffraction patterns for 45S5 bioactive glass, combeite (Na₂Ca₂Si₃O₉), PGS and hydroxyapatite given by (Arango-Ospina et al., 2021; Chen et al., 2006).

3.10 Cell culture and SEM-based cell morphology assessment

Early cell-scaffold interactions were checked using the MG63 human osteosarcoma cell line (ATCC CRL-1427). This is an osteoblast-like cell line that is a standard model used to study

compatibility of bioactive glass (Li et al., 2014; V. Schmidt et al., 2025). Cell cultures were kept in a humid incubator at 37°C with 5% CO₂. The culture medium also had high humidity and 10% fetal bovine serum and 1% penicillin-streptomycin. The uncoated scaffolds were preconditioned by immersing them into the medium for 24 hours before cell seeding to enhance cell attachment and protein adsorption. The PGS coated scaffolds were not preconditioned in the medium to study the impact of surface hydrophobicity (Chatterjee et al., 2012). After incubation, the scaffolds seeded with cells were fixed in 2.5% glutaraldehyde in PBS for 2 hours at 4°C and then dehydrated with an ethanol series and critical-point dried before gold sputter-coated and observed by SEM for cell attachment and morphology. Scaffolds with PGS-coating had too little cell attachment, so WST-8 metabolic activity assays were not performed.

3.11 Statistical analysis

All the quantitative data are expressed as mean plus or minus standard deviation (mean ± SD). The statistical comparisons were performed using a one-way analysis of variance (ANOVA) followed by the Tukey post-hoc testing for the multiple comparisons in OriginPro software. Statistical significance was accepted when $p < 0.05$. The significance levels are written as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ in the text.

4 Results and Discussion

4.1 Scaffold fabrication and coating optimization

The possibility of making foam replicates of 45S5 scaffolds while maintaining the original linear pore architecture was investigated. A modified protocol of PVA binder dissolved at 80°C in a water bath that gave uniform solutions was used. The results showed that foam replicates of 45S5 scaffolds could be successfully generated, and they maintained the original scaffold and pore architecture (Figures 4.2, 4.3). Preliminary studies showed that the vacuum deposition of PGS reagent on the scaffold led to inconsistent reagent coatings, presumably due to momentary exposure to oxygen during synthesis (Rosalia et al., 2024). The final method that was developed required dissolving PGS in an ethanol solvent to uniformly coat the scaffold and to study the structure, property and bioactivity relationships.

4.2 Physical characterization of sintered 45S5 scaffolds

These sintered 45S5 scaffolds showed a mean bulk density of $0.143 \pm 0.014 \text{ g/cm}^3$, a mean porosity of $94.72 \pm 0.53\%$, and a mean volumetric shrinkage of $67.77 \pm 1.80\%$ after sintering (Figure 4.1).

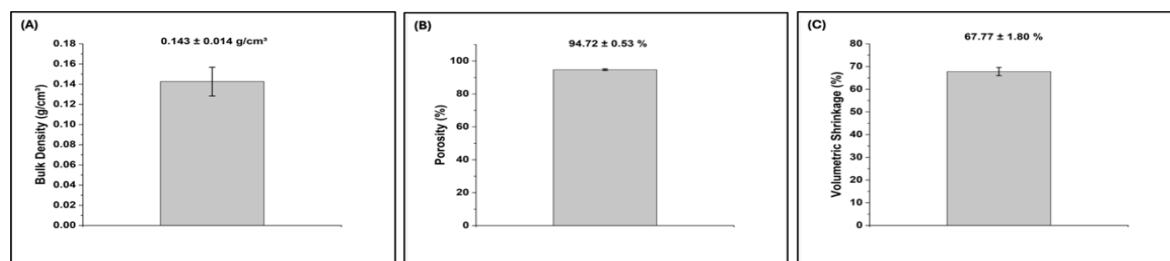


Figure 4.1. Physical characterization of sintered 45S5 bioactive glass scaffolds. Panels show (A) bulk density, (B) total porosity, and (C) volumetric shrinkage after sintering at 1050 °C. Data are presented as mean $\pm$ SD ($n = 10$).

According to (Chen et al., 2006), this foam replica method reliably produced very porous 45S5 bioactive glass networks. Our fabricated network was found to be highly porous with observed porosity greater than $\sim 90\%$, thus still remained comparable to the 95.6% porosity observed by (Arango-Ospina et al., 2021) who used similar fabrication techniques. The use of the repeated bioglass slurry impregnation technique similar to the protocols of (Arango-Ospina et al., 2021; Boffito et al., 2021) showed that the use of multiple slurry coatings can increase homogeneity and coating thickness. Volumetric shrinkage was due to template burnout and consolidation by particle rearrangement. Ultimately, the subsequent particle

neck formation rounded a chain of events as documented in foam replicated scaffolds fabricated with 45S5 by (Chen et al., 2006). The high porosity in the cutoff scaffolds confirms that strut consolidation is strong enough to support the scaffold after large alterations in structure. Some crystallization of the combeite and phosphate phases may also occur with thermal treatment of the mixtures as discussed in (Lefebvre et al., 2007) and may result in further densification and a change in final microstructure. While the characteristics of this method result in high porosity, it is actually important to control swelling and wall growth and maintain structural integrity. The high porosity ensures the cells and vessel penetrate, which is more important for the application (Karageorgiou & Kaplan, 2005). In this design system, the combination of high porosity and the control of wall expansion and compression provides a system that is reproducible and that can be modified to easily control the sequence and schedule of the PGS coating and bioactivity evaluation.

4.3 Morphology and surface coverage after PGS coating

Optical microscopy images are shown in (Figure 4.2). All original properties of the macroporous network were preserved upon coating of 45S5 scaffolds with PGS. The pores in this network had a diameter of 300–500 μm , and no pore obstruction was found when the concentration of PGS was changed. The strut thickness and smoothness increased with the concentration of PGS from 5% and above, which suggests an increase in the amount of the polymer deposited on the framework.

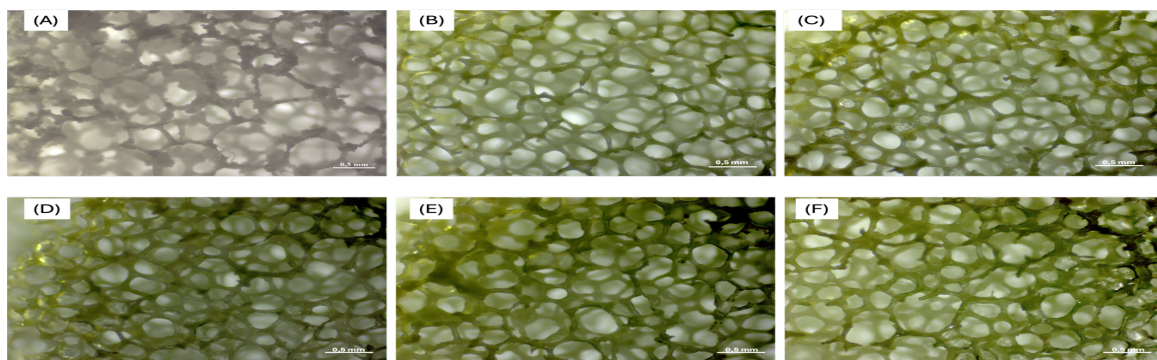


Figure 4.2. Optical microscopy and coated images panels show (A) uncoated 45S5 scaffold, (B) 2.5 % PGS, (C) 5 % PGS, (D) 10 % PGS, (E) 15 % PGS, and (F) 20% PGS-coated scaffolds. Scale bar: 0.5 mm.

Results of SEM analysis (Figures 4.3 and 4.4) agree with the previously mentioned findings and demonstrate that interconnected porosity was preserved in all groups. The control

uncoated 45S5 scaffolds (0% PGS concentration) exhibited a rough granular ceramic surface with clear microporosity. At the 2.5% PGS concentration, the ceramic produced a very thin, patchy layer, leaving many sections of the ceramic exposed at different depths. At high (5%-10%) PGS concentration, however, the ceramic produced a more uniform and continuous thin layer across the scaffold and improved the surface roughness. Increasing the concentration of PGS (15% and 20%) further helped cover more ceramic, but even more discontinuities became present. There were also large patches of very thick polymer and uneven ceramic covering. Similarly, a trend of gradually applying a polymer layer to cover a ceramic has also been found for a zein/Manuka honey composite and a PDLLA polymer (Arango-Ospina et al., 2021) and (Chen & Boccaccini, 2006).

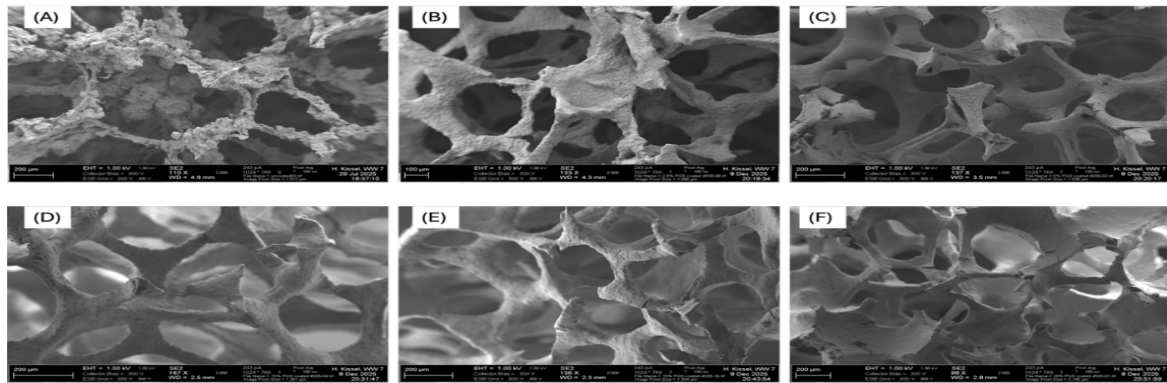


Figure 4.3. Low-magnification SEM micrographs of uncoated and coated 45S5 scaffolds. Panels show (A) uncoated, (B) 2.5%, (C) 5%, (D) 10%, (E) 15%, and (F) 20% PGS-coated 45S5 scaffolds. Scale bars: 200 µm (A, C-F) and 100 µm (B).

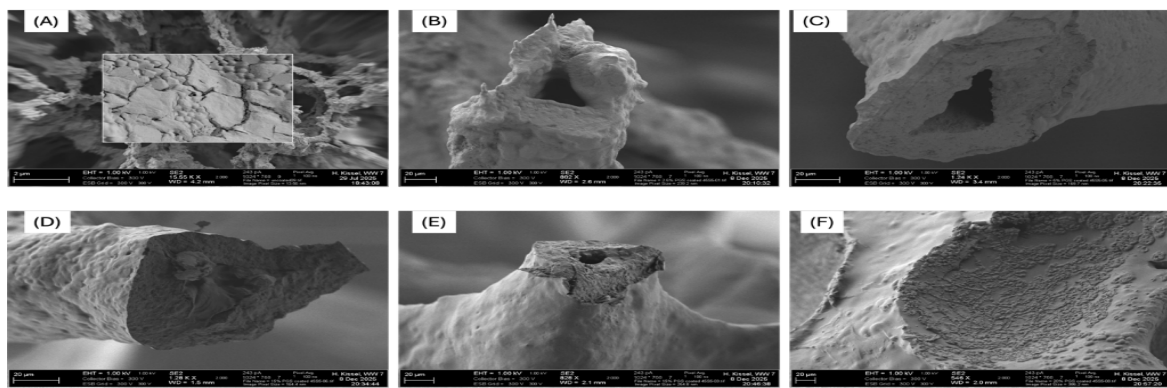


Figure 4.4. High magnification SEM micrographs of uncoated and coated 45S5 scaffold. Panels show A) uncoated, (B) 2.5%, (C) 5%, (D) 10%, (E) 15%, and (F) 20% PGS. Scale bars: 20 µm (B-F); panel (A) includes a 2 µm inset.

4.4 Surface wettability

The contact angle measurements (Figure 4.5) showed that the contact angle for the uncoated 45S5 scaffold was $41.53 \pm 3.2^\circ$, indicating a hydrophilic surface which is in agreement with the presence of hydroxyl groups and silicate species released after sintering.

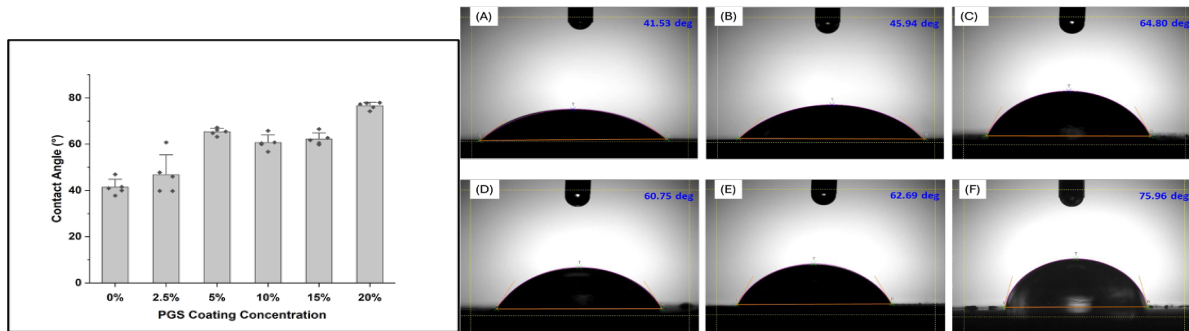


Figure 4.5. Surface wettability of uncoated and PGS coated pellets. The bar graphs show quantitative contact angle values, while representative sessile-drop images show (i) uncoated 41.53° , (ii) 2.5% 45.94° , (iii) 5% 64.80° , (iv) 10% 60.75° , (v) 15% 62.69° , and (vi) 20% 75.96° . Data are mean $\pm$ SD ($n=5$)

The contact angles increased gradually when coated with PGS, 45.94° (2.5%), 64.80° (5%), 60.75° (10%), 62.69° (15%), and 75.96° (20%). This is indicative of the hydrophobic nature of PGS, which is due to the aliphatic backbone that comes from sebacic acid, thus decreasing the number of available hydrophilic groups and restricting the spreading of water. The slight variations that were seen at 10% and 15% PGS was actually most likely due to differences in surface roughness and coating uniformity, not just the polymer content alone. And usually for porous biomaterials, the chemistry and topography both influence wettability (Menzies & Jones, 2010). Hydrophilicity was retained across all samples as evident from the contact angles below 90° . For osteoblast adhesion and proliferation surfaces of moderate wettability tend to be the most favorable, while surfaces of extremely high or low hydrophilicity are likely to be inhibitory (Bacakova et al., 2011; Rosales-Leal et al., 2010). Coating the scaffolds with 15% PGS was observed to maintain a level of wettability favorable for cell attachment while the 20% PGS coating resulted in the formation of a hydrophobic surface.

4.5 Mechanical properties under compression

Compressive strength and Young's modulus results for all groups are presented in Figure 4.6. Uncoated scaffolds showed a mean compressive strength of 0.0043 ± 0.0010 MPa, which matches the already low mechanical integrity expected in foam replicated bioactive glass

scaffolds due to that combination of high porosity, thin struts, and a brittle glass ceramic composition. After PGS coating, the compressive strength increased, with PGS coating levels rising to 10% PGS, where it reached the highest value of 0.0541 ± 0.0202 MPa ($p < 0.001$ vs. 0% PGS); then it started dropping again when the coating concentrations increased further. Compressive strength increased in a non-linear manner: 0.0133 ± 0.0022 MPa at 2.5% (not statistically different from G0, $p > 0.05$), 0.0370 ± 0.0192 MPa at 5% ($p < 0.05$), 0.0541 ± 0.0202 MPa at 10% ($p < 0.001$), 0.0364 ± 0.0212 MPa at 15% ($p < 0.05$), and 0.0284 ± 0.0165 MPa at 20% ($p > 0.05$). Also, a one-way ANOVA showed an overall statistically meaningful effect of coating concentration on compressive strength ($F = 6.40$, $p < 0.001$). The maximum mechanical performance was shown at 10% PGS, followed by a decrease at 15% and 20%, which implies an optimal range for coating concentration. At low concentrations (2.5%), the polymer layer is too thin, and it doesn't fully span the surface defects and the microcrack networks. For the intermediate concentrations (5-10%), the PGS layer forms a more continuous film over the strut surface, and this promotes the load transfer better; thus, crack growth is delayed. The elastomeric character of PGS, together with its capacity to absorb and dissipate energy via viscoelastic deformation, fits this reinforcing function. At intermediate concentrations (5-10%), the PGS layer turns into a more continuous film over the strut surface, and it helps the load get transferred better, so crack growth is also delayed. The elastomeric nature of PGS, together with its ability to soak up and dissipate energy through viscoelastic deformation, fits the reinforcing role. At higher concentrations (15-20%), excessive polymer accumulation can cause coating heterogeneity; the surface becomes uneven, which leads to non-uniform stress distribution with weaker strengthening efficiency.

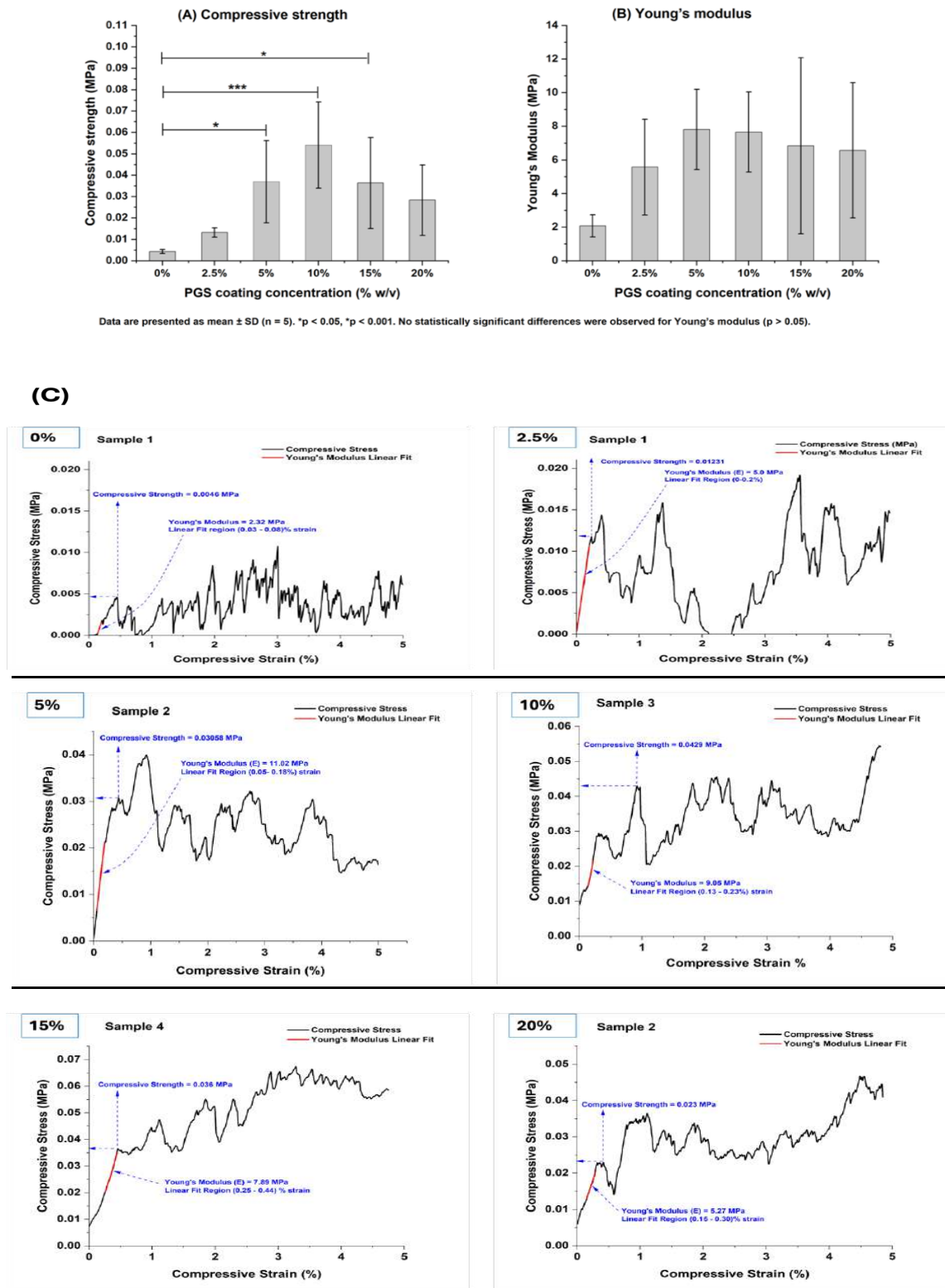


Figure 4.6. Compressive strength and Young's Modulus of 45S5 Scaffolds: Panels show (A) Compressive strength and (B) Young's modulus of uncoated (G0) and PGS-coated 45S5 scaffolds (G2.5-G20). The data mean $\pm$ SD (n = 5). *p < 0.05, ***p < 0.001 versus G0 (one-way ANOVA, Tukey post hoc). Below (C) is representative compressive stress-strain curves with Young's modulus linear fits for uncoated and PGS-coated 45S5 scaffolds (0–20% w/v PGS).

At the same time, the larger standard deviations seen at 15% and 20% confirm this interpretation because they show more variability in mechanical behavior across the different specimens. The absolute compressive strength values, even at the optimum, remain below the reported range for human trabecular bone (2-12 MPa). Two processing related factors might help explain the relatively low baseline compressive strength. First, the scaffolds in this study were made using two slurry impregnation cycles. With repeated slurry coating, green-body coating thickness can increase, and glass coverage over the polyurethane template becomes more complete, but in this study the limited slurry deposition may have resulted in thinner or less consolidated glass struts after template burnout. Overall you get a more open architecture and lower strength (Boffito et al., 2021; Chen et al., 2006). This is in agreement with (Arango-Ospina et al., 2021) who reported that very high porosity in foam-replicated 45S5 scaffolds is favorable for bone tissue engineering, but it also lowers the compression strength. Second, the replacement of the sintering oven due to equipment failure in the middle of the study may have introduced extra batch variability because the thermal treatment affects crystallization, densification, and even strut consolidation in 45S5 scaffolds (Chen et al., 2006; Lefebvre et al., 2007). Hence, the low absolute strength values should be interpreted in relation to scaffold porosity and processing conditions. Moreover, the comparisons within the study still suggest that the PGS coating improved compressive strength compared to the uncoated control. Thus, the important comparison lies in this present dataset, relative to the uncoated control rather than native bone. In this study, the 10% PGS coating produced about a 12.5-fold increase in compressive strength versus the uncoated scaffold, which represents a great improvement in scaffold stability and its resistance to fracture when it's being handled. Young's modulus seemed to increase after the PGS coating, especially in the 5–10% groups, but the differences were not statistically significant across groups (one-way ANOVA $p = 0.096$). This implies that the PGS coating enhanced compressive strength more clearly than stiffness. The initial elastic response of these very porous scaffolds is probably still driven by the sintered 45S5 strut network, whereas the elastomeric PGS phase seems to contribute more to crack-bridging, deformation tolerance, and post-yield energy dissipation than to the initial stiffness part (Rai et al., 2012). The representative stress-strain curves (Figure 4.6 combined, Appendix A1-A6) show the usual, nonlinear behavior of highly porous foam-replicated scaffolds, with an initial quasi

linear stretch, then progressive strut fracture, pore collapse, and stress fluctuations (Arango-Ospina et al., 2021). The large standard deviation values in Young's modulus probably indicate the high level of variability that appears when you test highly porous scaffold structures, where even tiny differences in overall geometry, pore arrangement, strut thickness, or the loading alignment can strongly influence what you finally measure in the elastic region (Miri et al., 2024). In general, 10% PGS seemed to provide the best mechanical reinforcement, improving compressive strength significantly while still maintaining the characteristic deformation behavior seen in porous 45S5 scaffolds.

4.6 In vitro degradation behavior in PBS

The degradation of PGS-coated 45S5 scaffolds in PBS over 7 days was assessed in a series of tests (Figures 4.7, 4.8). To better understand how the concentration of PGS coatings affect scaffold stability and interactions with the bioglass of the scaffold, tests were performed on weight loss, the mass remaining, and the pH changes. In this PBS (phosphate buffered saline) degradation experiment, no uncoated scaffold samples were included. The raw uncoated data given in the pH plots is PBS buffer only and indicates the stability of the buffer standard to which this experiment was done. The absence of uncoated data is a limitation, but prior studies have shown that uncoated 45S5 bioactive glass degrades rapidly in aqueous environment and causes an increase in the pH as well as the release of Na^+ and Ca^{2+} ions leading to surface layer transformations (Cerruti et al., 2005; Jones, 2013). (Boffito et al., 2021) showed that uncoated 45S5 bioglass degrades rapidly in an PBS environment, and the decrease in weight is accompanied by an increase in the pH. Our current result has shown that PGS-coated samples degrade more slowly compared uncoated samples. In all the PGS-coated groups, the higher PGS coated systems degraded slower and lost less weight. The weight loss after day 1 was moderate, around 8-15%, with the lowest degradation by G15 at 7-8% loss. Higher PGS concentrations experienced greater weight loss after 1 day (G5 at 13%). At day 3, weight loss nearly doubled for the lower PGS groups (G2.5, G5, G10) to as much as (21–23%) in comparison to the higher coated ones (G20: 12%, G15:13%). This showed the PGS coating protected the bioglass layer for some time from PBS penetration.

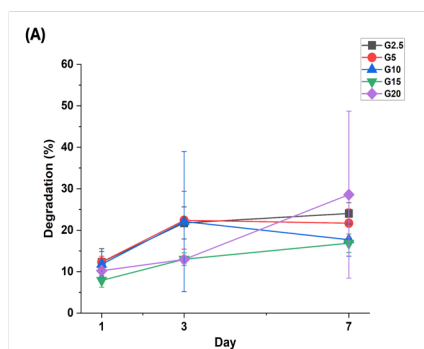


Figure 4.7. (A) Degradation of PGS coated 45S5 scaffolds in PBS over 7 days. Data are mean $\pm$ SD ($n=3$)

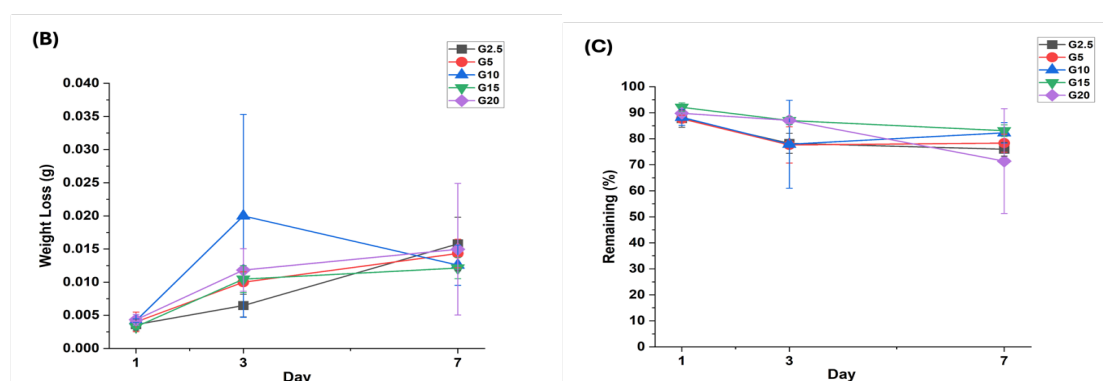


Figure 4.8. Weight loss and remaining mass of PGS coated 45S5 scaffolds in PBS.

The panel shows (B) absolute weight loss (g) and (C) remaining mass (%) after 7 days of in vitro degradation in PBS. Data are mean $\pm$ SD ($n = 3$).

By day 7, G20 lost up to 28% of its original and had the highest degradation and variability likely because of non uniform coating. G15 showed the most stability at about 16-17% loss. Degradation was strongly inversely linear with coating content ($R^2 \approx 0.95$ for G2.5-G15), confirming that increased PGS concentration generally led to less early mass loss. G20 however showed a different trend, perhaps due to uneven polymer distribution, leading to more unpredictable degradation. Results for remaining mass supported this: G15 had the most residual mass (~ 82 - 83% at Day 7) whilst G20 had the most mass loss (~ 70 - 72%). Intermediate concentrations of PGS provide a useful tradeoff between level of control of degradation and scaffold structure (Gerhardt & Boccaccini, 2010; Jones, 2013). pH monitoring (Figure 4.10) revealed steady levels for PBS alone controls (G0, ~ 7.3 - 7.4). All coated groups showed a quick increase in pH of Day 1 to Day 3 which is consistent with the characteristic ion exchange of the 45S5 glass wherein Na^+ and Ca^{2+} are released and exchanged for H^+

increasing the alkalinity of the medium (Jones, 2013; Kokubo & Takadama, 2006). With the PGS coating, this process was diminished but not eliminated. Coatings with a thinner thickness showed much larger increases in pH with time (up to 11.65 ± 0.41 after 7 days). In contrast, thicker coatings showed a lesser increase in pH than thinner coating, suggesting a less direct contact with the bioglass surface. These results showed that PGS behaves as a physical barrier. To the extent that it slows, but does not eliminate, both ion exchange and dissolution. The combination of ion exchange and mass loss observations relates well to prior work on surface modified bioactive glasses, showing that the biomaterial reduces but does not eliminate the characteristic bioactivity related degradation responses (Gerhardt & Boccaccini, 2010; Jones, 2013). A secondary observation is made in the study of (Boffito et al., 2021) in that a rapid rise is seen in the pH to approximately 9.7-10.2 in one day for uncoated 45S5. Thus, the pH changes observed after PGS coating confirm the slow controlled degrading action of the biomaterial.

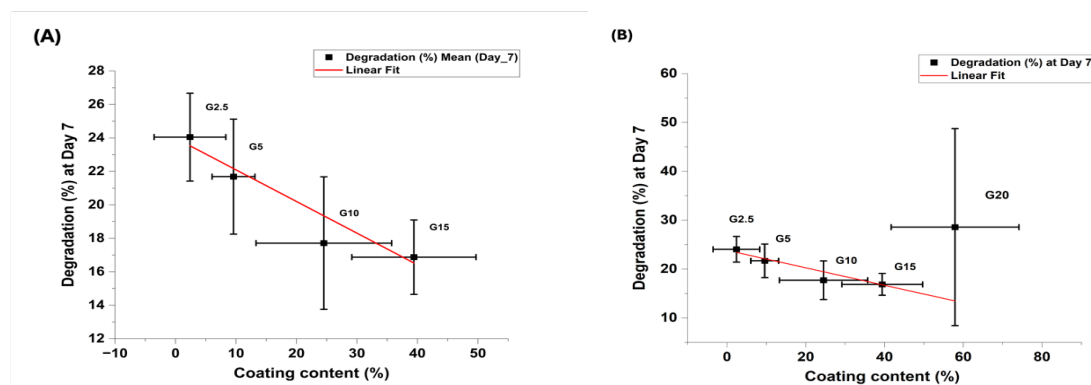


Figure 4.9. Structural property.

The panel shows (A) the relationship between PGS coating content (%) and degradation (%) at Day 7. (B) Linear regression fitted to G2.5-G15 (R^2 approximately 0.95). G20 was excluded from the fit due to deviation consistent with coating heterogeneity.

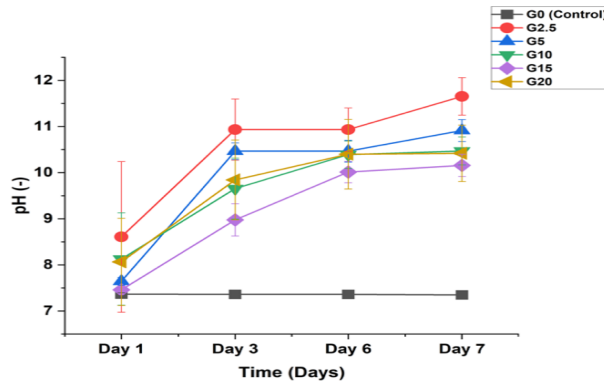


Figure 4.10. pH evolution during in vitro degradation of PGS coated 45S5 scaffolds

The panels show pH evolution during in vitro degradation of PGS coated 45S5 scaffolds with varying PGS coating concentrations (G0-G20) in PBS over 7 days. Data are mean $\pm$ SD ($n = 3$).

4.7 In vitro bioactivity in simulated body fluid

FTIR spectra of the uncoated 45S5 scaffolds, before and after being immersed in SBF (see Figure 4.11A), and of the PGS coated scaffolds after 7 days in SBF (Figure 4.11B) are shown together with the XRD patterns (Figure 4.12).

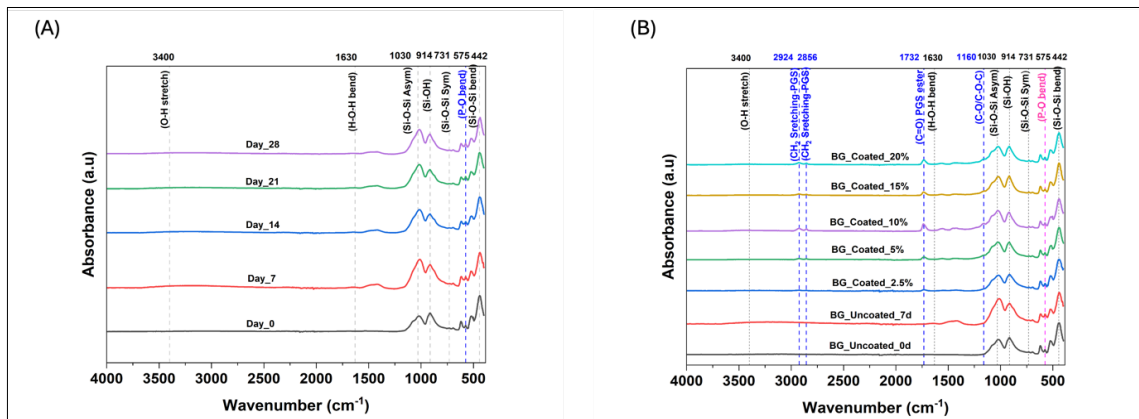


Figure 4.11. FTIR spectra of uncoated and Coated 45S5 scaffolds immersed in SBF.

Panels show (A) uncoated 45S5 scaffolds immersed in SBF for 0, 7, 14, 21, and 28 days, and (B) PGS-coated scaffolds (G0, G2.5-G20) after 7 days of SBF immersion.

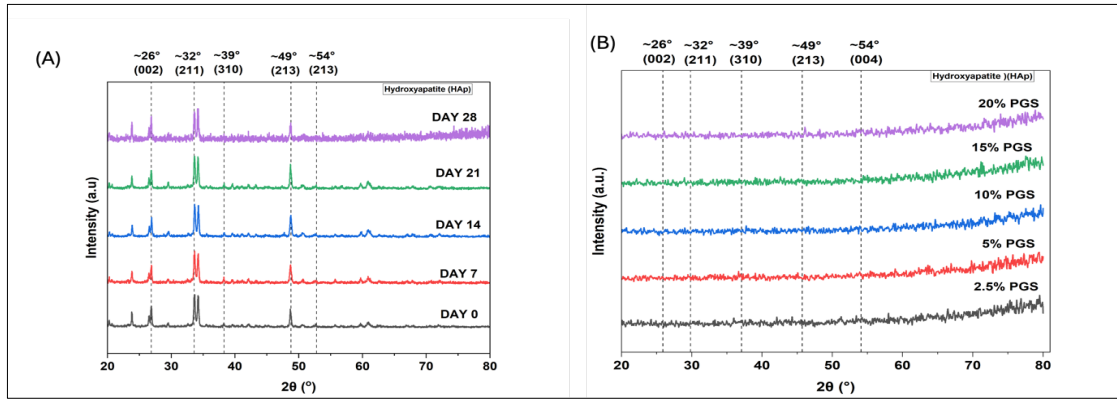


Figure 4.12. XRD patterns of uncoated and coated 45S5 scaffolds immersed in SBF. Panels show (A) uncoated 45S5 scaffolds immersed in SBF for 0-28 days and (B) PGS-coated scaffolds after 7 days of SBF immersion. Characteristic HAp peaks are marked.

The uncoated 45S5 scaffolds showed the expected FTIR bands for a sintered bioactive glass, including broad Si-O-Si stretching ($1150\text{--}960\text{ cm}^{-1}$; $960\text{--}850\text{ cm}^{-1}$), Si-O-Si rocking (445 cm^{-1}), and silicate peaks at 730 and 696 cm^{-1} , in addition to phosphate bands at 620 , 575 , and 524 cm^{-1} (Arango-Ospina et al., 2021; Chen et al., 2006). In the FTIR spectra of uncoated samples additional characteristics were observed when immersed most notably a doublet at $600\text{--}560\text{ cm}^{-1}$ and increased PO₄ stretching at $1130\text{--}975\text{ cm}^{-1}$ indicating the beginning and the progression of hydroxyapatite (HAp) formation. These alterations were more pronounced by days 14–28 consistent with continued apatite maturation. A peak at approximately 800 cm^{-1} showed the formation of a silica-rich surface layer, in agreement with the known pathways of mineralization in SBF (Kokubo & Takadama, 2006). These trends were confirmed by XRD, where low intensity crystalline reflections of the uncoated scaffolds were observed at Day 0, which were pronounced for HAp signatures (26° , 32° , 39° , 49° , and 54° 2θ) with prolonged immersion, similar to classic bioactive glass behavior (Boccardi et al., 2017; Chen et al., 2006; Lefebvre et al., 2007). Conversely, PGS-coated scaffolds showed a concentration dependent attenuation of these mineralization signals after 7 days in SBF. G2.5 still showed weak HAp characteristics, however coatings of 10% and above were essentially amorphous in profile with little evidence for crystalline apatite. This is in line with the barrier effect as discussed above, where the thicker PGS coating decreases the ion exchange and prevent the generation of silanol groups needed for apatite nucleation (Kokubo & Takadama, 2006; Li et al., 2015). Similar results have been observed for various polymer-modified bioactive glass systems (Gómez-Cerezo et al., 2020). Interestingly, bioactivity was sustained to a certain degree in the

lighter coatings (G2.5, G5) suggesting that partial glass exposure is adequate for delayed, but eventual mineralization, which could be a beneficial attribute for phased tissue regeneration. However, the short 7 days immersion time for coated samples is a restriction and extended SBF exposure is probably required (14-28 days) to evaluate long-term mineralization completely, especially when PGS degrades gradually (Gómez-Cerezo et al., 2020). Further SEM imaging showed more and more apatite progressed to the surfaces of the uncoated scaffold samples. Coated samples had progressively less apatite with increased PGS thickness. This coating delays the ability of the scaffold to rapidly integrate with tissue but does not completely prevent scaffold bioactivity depending on coat thickness. However, the final outcome and tissue integration, is determined by the thickness of the barrier and engineering trade-off. Coatings that are initially too thin do not prevent the scaffold from further degradation and integration with the surrounding tissue.

Table 4.1. FTIR band assignments for 45S5 bioactive glass, PGS, and hydroxyapatite features relevant to this study.

Wavenumber (cm ⁻¹)	Assignment	Phase/compound
1150-960	Si-O-Si asymmetric stretching	45S5 glass network
960-850	Si-O stretching (silica-rich layer)	45S5 glass network
800	symmetric stretching (silica layer)	Intermediate silica layer
730, 696	Si-O-Si symmetric stretching (crystalline)	Combeite / crystalline phase
620, 575, 524	P-O bending (crystalline / amorphous phosphate)	Rhenanite / amorphous CaP
600, 560 (doublet)	P-O bending (orthophosphate lattice)	Hydroxyapatite
1130-975 (broad)	PO ₄ stretching (crystalline HAp)	Hydroxyapatite
960 (shoulder)	P-O stretching (crystalline HAp)	Hydroxyapatite
1734	C=O ester stretching	PGS
2928, 2855	C-H stretching (alkane groups)	PGS
1645	Amide I / C=O	PGS / protein contaminant

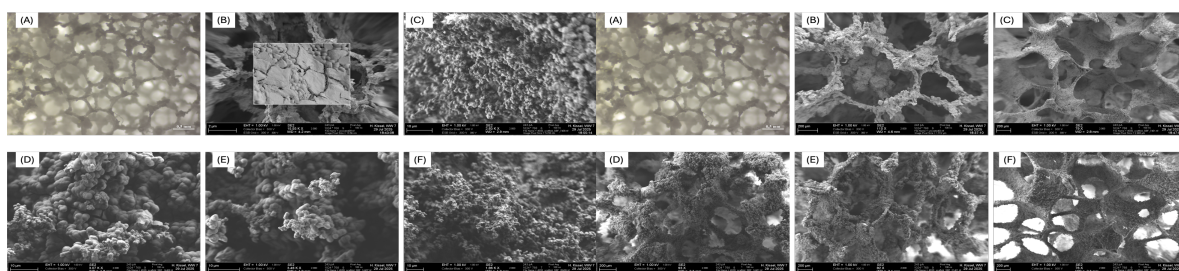


Figure 4.13. Apatite formation on uncoated 45S5 scaffolds before & after SBF immersion.

Panels show optical microscopy images and SEM micrographs of uncoated 45S5 scaffolds before and after SBF immersion: (A) optical before SBF, (B) SEM at Day 0, and SEM at (C) Day 7, (D) Day 14, (E) Day 21, (F) Day 28. Progressive apatite deposition and surface mineralization are evident. Scale bars as labelled.

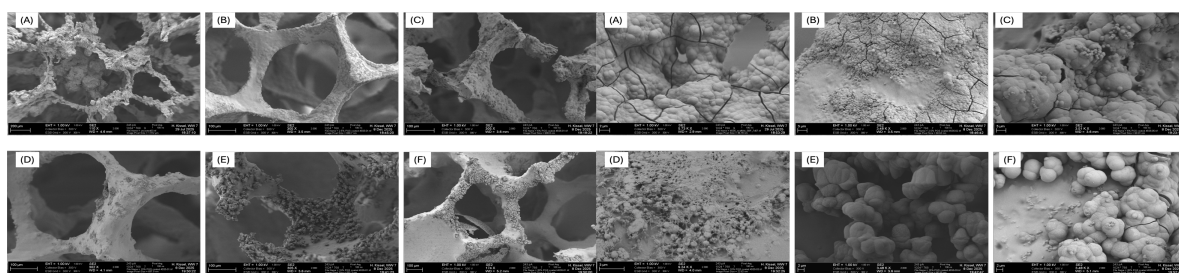


Figure 4.14. Apatite formation on uncoated & PGS-coated 45S5 scaffolds after SBF immersion (7days).

SEM micrographs of uncoated (200 µm scale bar) and PGS-coated scaffolds after 7 days of SBF immersion, shown at low (100 µm scale bar) and high (2-10 µm scale bar) magnification for each group (A-F corresponding to G0, G2.5, G5, G10, G15, G20).

4.8 Cell morphology and cell-scaffold interaction

SEM micrographs of scaffolds seeded with MG63 cells are shown in Figure 4.15.

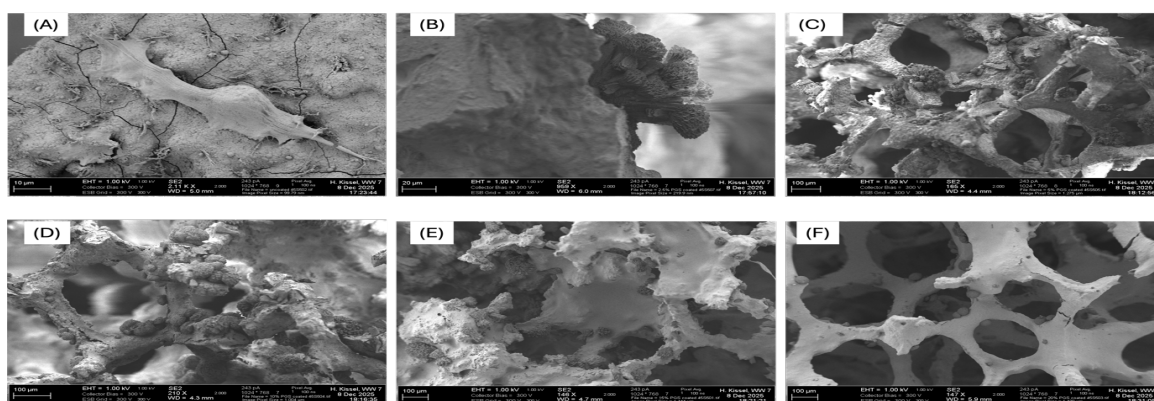


Figure 4.15. SEM micrographs showing cell morphology on uncoated & PGS coated 45S5 scaffolds

Panels show (A) uncoated 45S5 scaffold, (B) 2.5% PGS, (C) 5% PGS, (D) 10% PGS, (E) 15% PGS, and (F) 20% PGS-coated scaffolds after cell culture. Scale bars: (A) 10 µm, (B) 20 µm, (C-F) 100 µm.

Qualitative observations of SEM imaging of MG63 cell osteoblast-like behavior on 45S5 scaffolds indicated that healthy osteoblast cells should preferentially attach and spread on the scaffold. Observations of SEM imaging also appropriately supported the osteoconductive properties of the 45S5 scaffold known to promote early cell attachment and up to the point that the scaffold was saturated with cell (Gerhardt & Boccaccini, 2010). PGS scaffolds were observed using identical testing conditions and there was no evidence to suggest that MG63 cells attached to the PGS scaffolds. It is reasonable to suggest that this could be due to multiple factors. Firstly, the serving conditions used for the 45S5 scaffolds preconditioned the matrix to allow the adsorption of cell adhesive proteins. In the absence of cells, adsorbed fibronectin and/or vitronectin can drive integrin mediated cell adhesion (Chatterjee et al., 2012). The PGS-coated samples which were not preconditioned for protein adsorption, may have impacted the initial cell attachment. The reduced surface wettability likely contributed to the reduced cell attachment. This was especially evident as the hydrophobic character of the PGS coatings was reflected by the increased contact angles. Osteoblast cell attachment is enhanced in a moderately hydrophilic environment but PGS coatings being hydrophobic can lead to a decrease of early cell attachment (Bacakova et al., 2011; Wei et al., 2009). The PGS-coated scaffolds had a much less favorable cell attachment after 7 days in SBF as a consequence of the delayed mineralization. Thus, combined effects of the absence of medium preconditioning, increased surface hydrophobicity, and delayed mineralization resulted in the observed lack of MG63 cell attachment on the PGS-coated scaffolds. This is a qualitative observation at best because it is based on SEM imaging. Future studies should include consistent medium preconditioning, extended SBF incubation and quantification of cell viability using assays such as live/dead staining or WST-8, and evaluation of osteogenic markers to determine the extent of cell, scaffold interactions.

5 Conclusions and Outlook

5.1 Conclusions

The present study examined the effect of different concentrations of poly (glycerol sebacate) (PGS) on the architecture and mechanical properties of foam replicated 45S5 bioactive glass scaffolds (0 - 20% w/v).

- **Scaffolding:** The scaffolds displayed high structural porosity (approximately 95%) and interconnected macropores with a diameter range suitable for bone regeneration (300-500 μm). However, uncoated samples displayed significant structural weaknesses due to strut hollow nature.
- **Coating:** In solvating PGS prepolymer with ethanol, it became possible to apply PGS coatings with uniformity up to 15%. However, at 20%, the resulting PGS solution exhibited substantial viscosity, negatively affecting structural strength and decreased macropore connectivity.
- **Surface wetting:** Wettability of the scaffold decreased with an increase of the contact angle, from 41.5° to 76.0°, but did not reach 90°, thus retaining wettability.
- **Mechanical properties:** The compressive strength of the scaffold was maximum at a 10% coating of PGS, resulting in improved compressive strength, but Young's modulus was mostly unchanged, indicating a higher toughness and no change in stiffness.
- **Degradation:** Increased concentration of PGS (from 10-15%) reduced mass loss and alkalinization in PBS ($R^2 \approx 0.95$), creating a controlled and staged degradation profile.
- **Bioactivity and Cell Response:** In SBF, PGS promoted a delay of hydroxyapatite formation that depended on the extent of coating. On the PGS coated samples, MG63 cell attachment was not observed; however, attachment was only promoted on uncoated, preconditioned scaffolds pointing to the increased hydrophobicity and decreased early mineralization by the coating.

From the results the 10-15% PGS was optimal as it provided the best combination of balanced mechanical properties, wettability, and degradation control with the surface chemistry to provide prolonged degradation, although early cell attachment was poor.

5.2 Limitations and future work

- **Duration of SBF Studies:** The bioactivity study for PGS coated scaffolds was only investigated for 7 days. It would be important to determine if the formation of apatite matches uncoated glass after the PGS coatings degrade by doing longer studies (14-28 days).
- **Method for Determining Contact Angle:** The contact angle was measured on pellets of 45S5 bioglass powder. A more meaningful characterization of the surface wettability would be to measure contact angles on the scaffold to better reflect the wetting of the surface.
- **The Range of Mechanical Properties:** Although 10% PGS showed improvement in compressive strength of 12.5 times, the values still remain below the loadbearing bone, but these scaffolds are suited for non load bearing bone.

Future work should:

- **Use Nanoparticles:** The addition of bioactive or mesoporous glass nanoparticles in the PGS coatings is expected to improve the early apatite nucleation and the mechanical strength as published by
- **Compare with Protein Coatings:** New studies should compare PGS, gelatin and composite coatings. Previous studies have shown that gelatin coatings have favorable mechanical and bioactive results.
- **Preconditioning:** Preincubation with a serum or protein could overcome the increased hydrophobicity of the PGS-coated scaffolds and could provide better attachment of osteoblasts.

These changes will make scaffold design for clinical use easier.

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