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Composite film patch for soft tissue regeneration

An Emerging Healthcare Product

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REFERENCE NO :- TS/IO/2021/133

NAME OF PRINCIPAL INVENTOR :- ASSOC. PROF. DR. SITI NOOR FAZLIAH BINTI MOHD NOOR

TITLE :- BG/PCL/CS COMPOSITE FILM PATCH

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PUBLICATIONS

Determination of Suitable Bioactive Glass-Polymer Film Conditioned Medium Extracts for Potential Applications in Tissue Regeneration: A Preliminary Study

Siti Fatimah Samsurrijal, Siti Noor Fazliah Mohd Noor, Mamun Khan Sujon, and Khirun Musa

Abstract Composite film combining bioactive particles with natural and synthetic polymer has received greater attention for enhanced cytocompatibility properties. The current study aimed to determine human mesenchymal stem cells (HMSC) responses towards different concentration of bioactive glass/poly-Ɛ-caprolactone/chitosan (BG/PCL/CS) films conditioned medium extract using Alamar Blue assay. The samples were incubated in simulated body fluid (SBF) and the pH was assessed during the 21 days of incubation. Briefly, BG/PCL/CS at optimize weight percentages in acetic acid solution were prepared using solvent casting method and left to dry under fume hood for 48 h. The BG/PCL/CS films were incubated in culture medium at 200 mg/ml for 24 h at 37 °C and was serially diluted until 0.78 mg/ml with culture medium and supplemented before exposure to HMSC. The effects of the conditioned mediums are not consistent and not in dose dependent order towards HMSC cell's viability and proliferation. Higher conditioned medium extracts concentration tends to reduce cell proliferation. The pH of the samples tends to approach equilibrium at pH 7 for 21 days duration when incubated in SBF that may be contributed by the sample's compositions. Thus, suitable concentration or dose ratio of the samples is important to reduce cytotoxicity before further biocompatibility assessment is conducted.

Keywords Bioactive glass · Poly-Ɛ-caprolactone · Chitosan

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1 Introduction

Development of three-dimensional (3D) scaffold for tissue engineering nowadays are geared towards combination of bioactive materials, natural such as chitosan (CS) and synthetic polymers such as poly-Ɛ-caprolactone (PCL) since these combinations results in superior scaffold intended for designated applications [1].

Bioactive glasses (BG) combined with polymers such as polycaprolactone and poly-lactic acid has potential in replacing common paste for skin or mucosal ulcers where BG-polymer film may provide easy applications, able to be retained and covering ulcers during healing period in acute and chronic wounds [2, 3]. Furthermore, these bioactive glasses can be tuned in its composition for soft tissue healing with addition ions such as magnesium or cobalt which promoted antibacterial properties and angiogenesis [4].

Bioactive glasses have the capability to develop a surface bonding with living tissues and stimulate a cellular response for healing and remodelling which can be used for both hard and soft tissue engineering purposes. These are biodegradable materials applicable in many clinical fields including dentistry, oral and maxillofacial and orthopaedics, osteogenesis and as means of ion delivery for antimicrobial effects [4]. These bioactive materials can degrade or being soluble in an aqueous media by ionic dissolutions of respective ions. The degradation rate can be regulated by chemical modifications into glass chemistry depending upon functional needs [5].

Ulceration is a widespread problem for certain medical conditions [6] and wound on skin following major surgery requires the need for use of patch for covering defective area. The use of synthetic and non-degradable plaster posed the need for patient to remove it and changed of plaster dressing not only creates pain but also clinical waste that sometimes are not disposed properly. Hence, there is a need to produce a suitable patch that can be used clinically, degrades over certain period and non-toxic. The incorporation of BG into the BG/PCL/CS patch will aid in bioactivity since BG is able to release bio-actives for stimulating new cell layer and promote tissue regeneration.

A wound healing material for external use must be able to maintain moisture and allowing skin to breathe while providing protection from inflammation and enhance skin fibroblast proliferation, which can be provided by CS. The addition of CS will help to enhance its process due to its antimicrobial and structural properties. For structural integrity, CS has been observed to produce crosslinking of nanofibers which are treated with heat to form water stable membranes and water absorption capabilities [7]. Addition of PCL of into the composite film patch will add strength and provide structural support for the film. PCL is FDA approved and commonly used in many biomedical field [8].

2 Materials and Methods

2.1 Chemicals and Media

Tetraethyl Orthosilicate (TEOS), Triethyl Phosphate (TEP), sodium nitrate and calcium nitrate tetrahydrate, low molecular weight chitosan (MWavg = 120 KDa) and poly-Ɛ-caprolactone (MWavg = 80,000) were purchased from Sigma-Aldrich (UK). Human mesenchymal stem cell from Lonza (Basel, Switzerland) and cell culture materials Dulbecco´s Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), antibiotic–antimycotic were purchased Gibco Invitrogen Technologies (UK). All other reagents used were of analytical or microbiological grade purchased from Merck (Darmstadt, Germany).

2.2 Bioactive Glass Synthesis

Sol–gel BG were synthesized according to previous published study [9] where the glass fabrication involves mixing of vital compounds such as deionized water, 2 N nitric acid, TEOS, TEP, sodium nitrate and calcium nitrate tetrahydrate. The mixture will be left stirred overnight to achieve gelling state. The semi-gelation mixture will then be further gelled at 35 °C in the oven for 3 days before the sealed container containing the gelled mixture is then aged at 60 °C for 2 days. The mixture is then subjected to 110 °C for 2 days to ensure it is fully dried before the samples will then be sintered at 700 °C for 1 h. The sol gel BG powder is then will be grounded and sieved to achieve powder with particle size less than 50 μm.

2.3 BG/PCL/CS Patch Synthesis

The patch was synthesized using solvent casting method. Briefly, BG (10 weight percent, wt%), CS (10 wt%) and PCL (80 wt%) were dissolved in 100.0% (w/v) acetic acid, stir overnight for 16 h at 500 rpm and then poured inside a PTFE mold and left to dry for 48 h under fume hood. Once dried, the patch was removed from the mold and kept inside a desiccator until further tests.

2.4 PH Analysis in Simulated Body Fluids

The simulated body fluid (SBF) was prepared based on procedures described by Kokubo and Takadama [10]. The patch was incubated in SBF at a liquid/solid ratio

of 50 mg/ml and placed inside an incubator shaker at 37 °C and the solution pH was recorded at designated time points (Days 1, 4, 7, 14 and 21).

2.5 Determination of Suitable Dosage for BG/PCL/CS Extracts Using Alamar Blue Assay

The patch was sterilized by UV exposure for 40 min on each side and then incubated with DMEM (200 mg/ml) inside an incubator shaker for 24 h at 37 °C [11] followed by sterile filtration using 0.22 μm syringe filter and kept at −20 °C. Prior to exposure to HMSC, the BG/PCL/CS conditioned medium extracts were serially diluted until 0.78 mg/ml and supplemented with 10% FBS and 1% A/A inside a vented T25 cm^2 flask and placed inside a CO_2 incubator at 37 °C overnight for pH stabilization prior to use on cells.

The HMSC was seeded with a seeding density of 5×10^3 cells/cm^2 inside a 96-well plate for 24 h and the next day was exposed to varying concentration of BG/PCL/CS extracts for another 24 h and the cells responses were assessed using Alamar Blue (Life Technologies) assays. Briefly, old medium was removed, and cells were wash using 100 μl of DPBS and then 150 μl of 10% (v/v) AB in DMEM with no phenol red (Gibco) was added per well (including one with no cells to be used as blank) and the well plates were further incubated for 2 h at 37 °C and then, 100 μl of the reaction product was transferred to a black Costar 96-well plate. The fluorescence of AB was read at an excitation wavelength of 544 nm and emission of 590 nm using a microplate reader (FLUOstar Omega, BMG Labtech).

2.6 Statistical Analysis

The experiment was conducted at least twice with four ($N = 4$) replicates and data was reported as mean ± standard error of mean (Mean ± SE). The data were analyzed using SPSS version 26.0 (IBM, Armonk, USA) using one-way analysis of variance (ANOVA) with subsequent *Bonferroni* post-hoc test with P value less than 0.05 as statistically significant.

3 Results and Discussion

3.1 pH Changes of BG/PCL/CS Patch in SBF

The pH profiles of PCL samples inside the SBF show almost comparable trend with the control SBF showing a steady profile from initial samples incubation until day

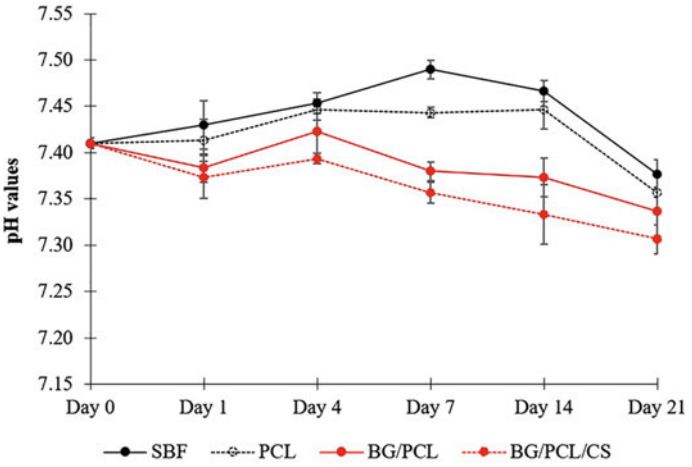

Fig. 1 The pH profiles of samples incubated in SBF throughout the 21 days of incubation

14 which tend to decrease to below pH 7.4 at day 21 (Fig. 1). Nonetheless, the pH of the samples containing BG (BG/PCL and BG/PCL/CS) show a decreasing trend from pH 7.4 towards pH 7.3 in which the pH values for BG/PCL/CS were lower compared to BG/PCL only. The addition of BG within the samples may provide buffering effect leading to lower pH towards stabilization at pH 7. A study reported that scaffolds containing 45S5 BG (PCL/50-45S5) and strontium (PCL/50-SrBG) showed higher pH at 7.75 and 7.82 after initial incubation which then reduced and fluctuated between pH 7.65 and 7.35 throughout 10 weeks immersion in α-MEM and this is contributed by the BG dissolutions into the immersion media, and the initial pH increase aided the apatite formation while the subsequent reduction in pH over time is likely caused by the gradual apatite formation [12]. The authors believed that the use of medium from a controlled source is more reliable and reflective towards cell culture studies using the same culture media [13].

Furthermore, the presence of CS also reduces the sample's pH towards pH 7.0 compared to BG/PCL only since time is needed for balancing the pH values which tend to be lower at the final when equilibrium is achieved [1]. The solubility, biological activity and ion exchange ability of samples containing CS is influenced by CS degree of deacetylation [14]. BG scaffolds impregnated with CS and PCL were incubated in SBF for up to 8 weeks where the results showed that CS influenced the bioactivity of the BG by enhancing mineralization [1].

In the current study, the SBF solution was not replaced during the 21 days incubation which may have resulted in lower pH for BG/PCL and BG/PCL/CS. A study replenished the SBF once every four weeks since the pH of the incubation media is influenced by the polymer coating degradation and BG dissolution [1] since the CS and PCL may release different organic degradation product that affects the pH of the SBF.

3.2 *HMSC Responses Towards BG/PCL/CS Extracts Conditioned Medium*

This preliminary study is to determine the toxic dose that reduces HMSC viability. Based on suggestion by ISO 10993-part 5, the material is incubated for 24 h at 37 °C with dose of 200 mg/ml [11] to prepare the material extracts usually known as the conditioned medium. Since no previous data is available for the current sample, serial dilution of the dose was carried out to ensure that future preparation of the film for direct cell seeding will not create toxic effects to the cells using AB assay. Serial dilution of 200 mg/ml extracts until 0.78 mg/ml were performed and the results showed the 200 mg/ml for BG/PCL and BG/PCL/Cs conditioned medium extracts were toxic to the cells (Fig. 2). At high dose (200 mg/ml), the BG concentration inside the medium is higher contributed by the increased in the weight percentages of the BG components; the sol–gel BG was fabricated based on a 45S5 system containing SiO_2–CaO–NaO–P_2O_5 and earlier study showed that higher NaO content is toxic to cells [15]. The gold standard 45S5 system modifications are performed through substitution of CaO and NaO with other components such as cobalt oxide [16], strontium oxide [17], and lithium oxide [18] are geared towards developing BG with enhanced angiogenesis, hard tissue repair and regeneration. BG conditioned medium in α-MEM (45S5/LiO, Li25, Li50 and Li100) at 6, 60 and 300 mg/ml incubated at specific time frame were exposed to mouse fibroblast MC3TC-E1 for 24 h and the results showed that cells exposed to the BG conditioned media at 300 mg/ml had significantly lower metabolic activity suggesting that the higher dose were toxic to cells [18].

Overall, HMSC seeded on BG/PCL/CS patch showed acceptable cell viability despite the fluctuation of the dose with cells exposed to 3.12 mg/ml had the highest

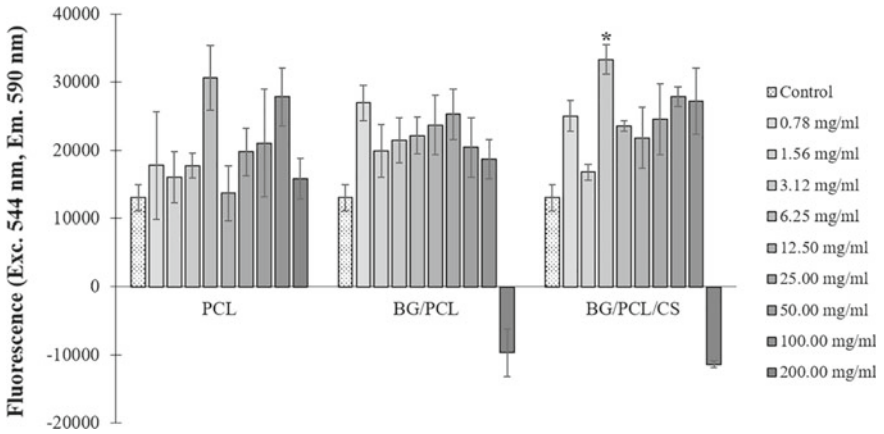

Fig. 2 The BG/PCL and BG/PCL/CS extracts at 200 mg/ml are toxic to the cells. ($P < 0.05$ when compared to PCL and BG/PCL)

viability compared to the other doses. This may highlight that a certain BG dose maybe suitable for cells [14, 15]. HMSC exposed to BG/PCL extract is not dose dependent and beginning to show reduced cell viability at 50 mg/ml. The cell viability differences when exposed to BG/PCL and BG/PCL/CS extracts at higher dose is contributed by the CS presence in which cells showed higher viability in BG/PCL/CS at 50 and 100 mg/ml. CS addition in the BG/PCL/CS samples may have slowed down the release of BG into the surrounding and reduces mechanical degradation of the film while enhancing its bioactivity [1], hence the better cell viability observed at doses lower than 100 mg/ml. However, this needs further exploration towards several types of cells and future works should address this issue since the material is targeted for wound healing use, human skin fibroblasts should be the next evaluation target. Besides, different cells will provide different responses and the cells chosen must reflect the intended application of the film. Furthermore, other parameters should be included in biocompatibility assessments such as scratch assay, functional assay for skin fibroblast and gene expression study.

4 Conclusion

The study implies the effects of different BG/PCL/CS conditioned medium extracts on HMSC where concentration at 200 mg/ml is toxic to the cells. Despite fluctuation of certain dose from 0.78 mg/ml to 100 mg/ml, the 3.12 mg/ml of BG/PCL/CS conditioned medium extract showed highest HMSC cell viability when compared to other concentration which may highlights certain dose may be suitable for certain types of cells.

The pH of the samples overall showed a decreasing trend towards pH 7.0 at final equilibrium and longer duration is needed with medium replenishment at certain time points to avoid saturation of samples dissolution into the SBF which may contribute to plateau pH value.

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Development of bioactive glass-poly-ε-caprolactone polymer composite film for soft tissue regeneration

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Development of Bioactive Glass-Poly-Ɛ-caprolactone Polymer Composite Film for Soft Tissue Regeneration

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Abstract. Biomaterials known as bioactive glasses (BG) have been introduced for application in medicine and dentistry since 1969. Bioactive glasses are biodegradable and compatible towards human used and enhanced properties can be achieved by adding compatible polymers such as Poly-Ɛ-caprolactone (PCL) that is Food and Drug Administration (FDA) approved. Aim of the study was to develop composite films fabricated by adding PCL reinforced with BG at 5 and 10 weight percent (wt.%), characterized using Field Emission Scanning Electron Microscope (FESEM) with Energy dispersive spectroscopy (EDS), and subjected to biocompatibility assay using AlamarBlue towards Human Dermal Fibroblast (HDF) and Human Umbilical Vein Endothelial Cells (HUVEC). Results showed that pristine PCL morphology is smooth. The addition of BG in the composite film leads to increase in surface roughness with agglomeration and formation of pits with certain pores in between when the BG weight increases from 5 to 10 wt.%. Carbon and oxygen were the predominant elements of PCL. As for PCL/BG, existence of Si, Ca, Na and P were expected confirming the inorganic phase incorporation into the film showing certain homogeneity. AlamarBlue cell viability assay demonstrated the produced composite film is biocompatible towards HDF and HUVEC as observed by the increase percentage of viable cells upon exposure at Days 1, 4 and 7. These results are promising for further studies on PCL/BG film and development of wound patch to facilitate wound healing and the potential for guided tissue regeneration.

Key words: Bioactive glass (BG), Poly-Ɛ-caprolactone (PCL), Biocompatibility

INTRODUCTION

Bioactive glasses (BG) have been introduced in medicine and dentistry since the success of bioactive glass cones as middle ear prosthesis in human [1]. Bioactive glass has been widely applied in bone tissue engineering applications for its properties in being biodegradable aiding many treatment modalities. Various types of bioactive glass are available ranging from silicate-based, borate-based and phosphate-based. It is well known that bioactive glasses are biocompatible, whose biocompatibility can be enhanced by adding polymers to them [2]. Polymers are known to interact with cells of the tissues creating a regenerative environment, a process widely known as tissue engineering.

Polymers are currently being used in a wide range of biomedical applications especially the biodegradable polymeric materials. Biodegradable polymers provide significant advantages of being able to be broken down after its intended function and slowly removed thereafter. Numerous polymers are available for use in biomedical applications such as polyurethanes, polyethylene, polymethyl methacrylate, poly-Ɛ-caprolactone, chitosan, polylactide-co-glycolide, poly (D,L-lactide), polylactic acid and many more [3,4]. Biocompatible polymers such as polycaprolactone, poly-lactic acid showed promising as films for oral ulcer and can be fine-tuned with bioactive glass to enhance BG properties [5]. To accomplish that, bioactive glass combined with polymers can be brought into use after assessing their mechanism of actions. If the study proves to be a success, these bioactive glass polymers composite film can be commercially introduced and prescribed to and subsequently applied in soft tissue healing and regeneration.

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The incorporation of the polymers into the bioactive glass has led to the immergence of the term nanocomposites also known as bio-nanocomposites which result in the formation of scaffolds. In this study, focused are directed towards the use of bioactive glass and polymer composite film in wound healing. The basic requirement of a wound healing material is its ability to maintain the moisture and oxygen levels within the wound gap and skin exterior for preventing inflammation and promoting fibroblast proliferation for skin regeneration [6]. One of the polymers being used for wound healing purposes is poly-Ɛ-caprolactone (PCL) which is biocompatible, linear, biodegradable and have received the Food and Drug Administration (FDA) approval for human use [7]. However, PCL is inactive and to overcome this problem, it is combined with bioactive glass to speed up the degradation process [8]. In this study, combination of bioactive glass and poly-Ɛ-caprolactone composites films fabricated through solvent casting method and their characteristics were evaluated. Therefore, the current project aim was to investigate the effect of bioactive glass combined with poly-Ɛ-caprolactone (PCLBG) and assessed the *in vitro* biological responses towards human cell lines for potential application of the PCLBG composites films that can play a major role in wound healing for soft tissue regeneration.

MATERIALS AND METHODS

Materials

Materials utilized for bioactive glass powder synthesis are tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP), calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$), sodium nitrate ($NaNO_3$) and nitric acid (HNO_3) and were used as-received. Chloroform ($CHCl_3$) and poly-Ɛ-caprolactone (PCL) were used in fabrication of the composite film.

Synthesis of bioactive glass powder

The 45S5 sol-gel bioactive glass powder is prepared based on the compositions shown in Table 1. Briefly, precursors materials were prepared using deionized water, 2N nitric acid, tetraethyl orthosilicate, triethyl phosphate, sodium nitrate and calcium nitrate as reported previously [9]. The mixtures were poured into a sealed container and dried for 2 days in oven at temperature 60 °C and 110 °C for the next two days. Then, the samples were subjected to sintering process at 600 °C for 1 hour producing sol-gel frits. The resulting frit was milled by using planetary micro mill (Pulverisette, Germany) to obtain a fine powder. The sol-gel BG powder was sieved to obtained particle size less than 50 μm.

TABLE 1: The 45S5 BG composition in weight percentages (wt. %) and mole percentages (mol. %).

	Oxide	**SiO_2**	**CaO**	**Na_2O**	**P_2O_5**
Weight %	45S5	45.00	24.50	24.50	6.00
Mole %	45S5	46.10	26.90	24.40	2.50

Fabrication of bioactive glass polymer films

Bioactive glass powder in 5 and 10 weight percentages (wt.%) was added into poly-Ɛ-caprolactone (PCL) and chloroform slurry. The composite films or the blending of PCL and BG were prepared using solvent casting method. Briefly, PCL was homogenously dissolved in 5% chloroform (w/v) and kept under stirring for 4 hours. Sol-gel BG (particle size less than 50 μm) with 5 and 10 wt.% were added separately to the polymer blend and kept under stirring for further 24 hours. Once it is completely dissolved, the obtained mixture was poured onto an aluminum plate to produce thin films and left to harden by allowing evaporation of the chloroform in a fume hood at room temperature (25 ± 2 °C). Eventually, the end films were air-dried for further 24 hours. The obtained PCL (control) and PCLBG films (PCLBG 5% and PCLBG 10%) were placed inside desiccators to avoid material degradation and subjected to characterization and *in vitro* biocompatibility studies.

Characterization of the PCL and PCLBG composite films

The films were subjected to scanning electron microcopy (SEM) with energy dispersive X-Ray spectroscopy (EDS) analyses for morphological characterization and quantifying elemental compositions within the films using a Field Emission Scanning Electron Microscope (Carl Zeiss, Oberkochen, Germany). The fabricated films were mounted on copper stubs, introduced into a sputter coater and coated with gold and observed with a FESEM with a 5 to 15 kV accelerating voltage for taking high-resolution pictures.

In vitro biocompatibility assays

Biocompatibility assessment were performed to study the effect of fabricated composite film towards human cell lines. Human Dermal Fibroblast cell line (HDF) was purchased from Lonza (USA) and Human Umbilical Vein Endothelial cell (HUVEC) from Invitrogen (UK). Briefly, HDF were cultured and expanded in Medium 106 with additional supplements, LSGS Kit (Gibco Invitrogen, UK) while HUVEC was grown in Endothelial Cell Growth medium (PromoCell, UK). Initially the cells were seeded and grown in a 75 cm^2 flask in a humidified atmosphere of 95 % air with 5 % CO^2 at 37 °C. The growth medium was changed every 2-3 days.

Once HDF and HUVEC reached 80 to 90% confluent, they were trypsinized, counted using hemocytometer and seeded onto the films in 48-well plates with a seeding density of $5x10^3$ cells/cm^2 per well. The composite films were cut according to the size of 6.25 mm x 1 mm and each sample were prepared in quadruplicate (n=4). The films were pre-wet for 30 minutes with 500 µl of complete medium. The cells were seeded onto the film and incubated again in a CO_2 incubator at 37°C with 5% CO_2 for 30 minutes. Then, 500 µl of medium were added into each well and further incubated in an incubator. At designated time points (1, 4 and 7 days), the old medium was aspirated from the well plates followed by washing steps using 500 µl of DPBS. Approximately, 500 µl of 10% AB (v/v) in DMEM with no phenol red (Gibco, UK) was added per well (including one with no cells to be used as blank) and the well plates were further incubated for 2 hours at 37 ºC. Following the 2 hours incubation period, 200 µl of the reaction product was transferred to a black Costar 96-well plate in duplicate. The fluorescence of AB was read at an excitation wavelength of 544 nm and emission of 590 nm using a microplate reader (FLUOstar Omega, BMG Labtech).

RESULTS AND DISCUSSION

Morphological characterization of the composite film

The surface morphology of the prepared pristine PCL, PCLBG5 and PCLBG10 (wt.%) composites film is shown in Fig.1. Based on SEM images, the surfaces of the solvent casted PCL film exhibited smooth and dense surface texture and no porosity was visible [10]. In contrast, PCLBG composites containing BG at 5 and 10 wt.% show changes in the surface morphology observable as rough surfaces and as the BG amount was increased to 10 wt.% the surface roughness also gradually increase with visible pores compared to the PCL film. Therefore, the roughness observed in the PCLBG samples is due to the presence of BG particles that have been combined with PCL. Meanwhile, from these figures, clearly it can be seen somehow PCLBG with 5 wt.% BG powder film surface appeared less porous and smoother than the PCLBG 10 wt.%. This is due to increment of BG weight percentages (wt.%) in the PCLBG10. This morphological observation is in agreement with previous study which proved that the incorporation of BG particles into PCL film showed the increase of surface roughness that plays an important role in cell attachment [11], and also porosity on the film surface had a contributing effect on the bioactivity, water uptake and cytocompatibility of the composites film and significantly improved the bioactivity of the fabricated composites [12]. BG particles also contributed to altering the degradation rate of PCL as evident can be seen in the roughness appearance on both PCLBG morphology surface as observed. Faster degradation properties that was achieved and improved with the increase of BG content is conducive to the guidance of new bone formation.

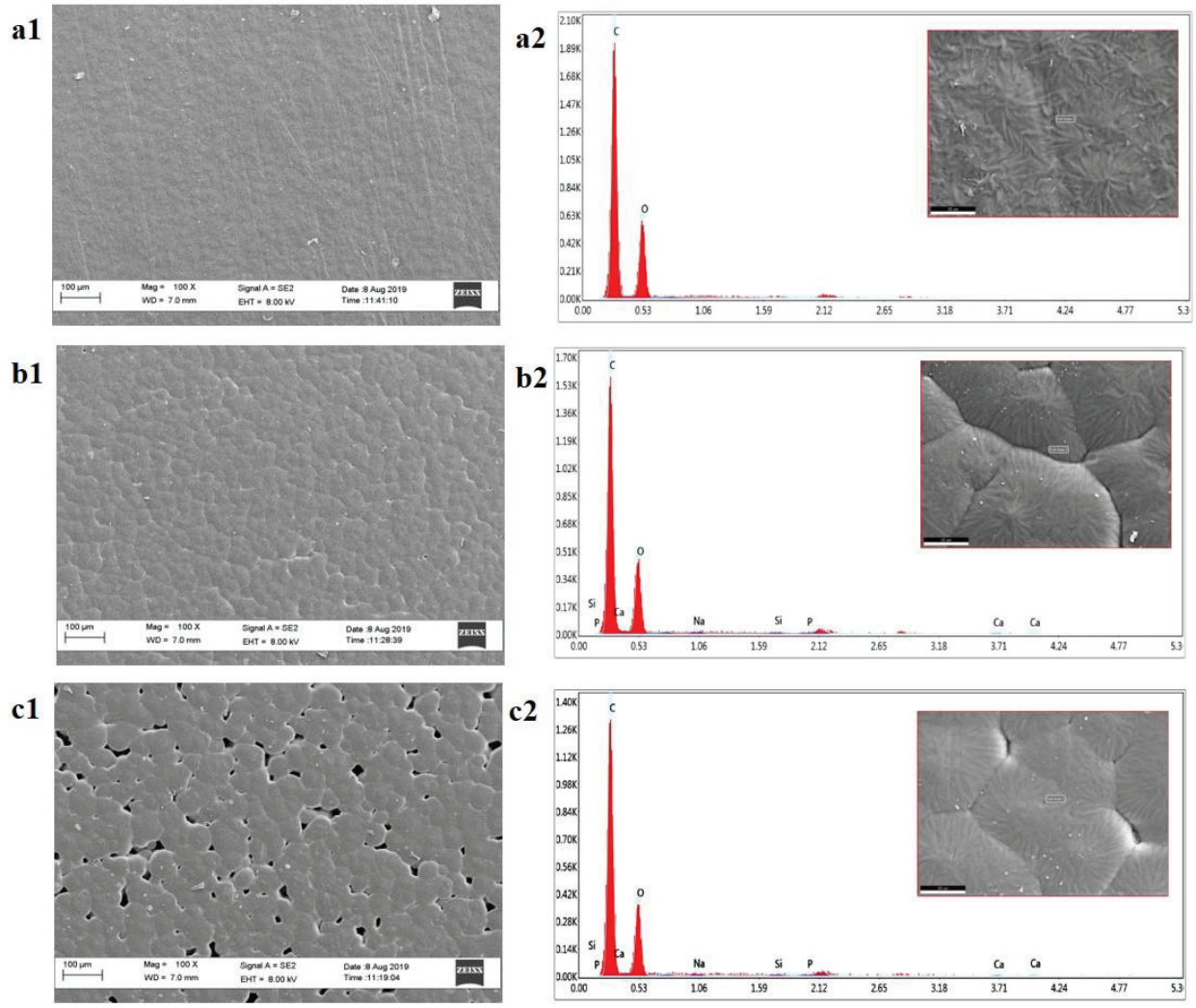

FIGURE 1: The morphological changes based on FESEM showing smooth surface characteristics on PCL pristine film (a1) when compared to PCLBG5 which shows streak (b1) and these streaks became prominent with pits in PCLBG10 (c1). The EDX profiles is shown for PCL (a2), PCLBG5 (b2) and PCLBG10 (c2 with corresponding scan areas).

Based on the energy dispersive spectroscopy (EDS), the chemical composition of the fabricated films and the presence of BG particles can be detected as shown in Fig. 1. For the fabricated film of pure PCL (Fig. 1a2), existence of oxygen and carbon elements were expected. The resulting spectrum and tabulated results revealed that carbon and oxygen were the predominant components that were present with carbon being the most abundant from the results obtained. On the other hand, for both PCLBG5 and PCLBG10 composites films investigated, the result of EDS analysis from the surface of PCL combined with BG indicates that the main elements in bioactive glass particles are Si, Ca, Na and P and that can be seen as the peaks of Si, P, Na and Ca belong to components of bioactive glass particles, therefore confirming the inorganic phase incorporation into the hybrid films [13]. The comparison by EDS analysis between the polymeric film (Fig. 1a2) and the biocomposites film (Fig. 1b2 and 1c2) indicates the presence of Si, Ca, C, P and Na. It seems clear that C are the most abundant elements for both PCLBG and PCL films. The EDS results obtained are in concordance as reported previously [14] that suggesting all of the elements observed reflect the combined contribution of both composition of PCL and BG to the new fabricated composites films in this study.

Biocompatibility of PCLBG composite film towards HDF and HUVEC

A preliminary biological evaluation of PCL, PCLBG5 and PCLBG10 composite films were performed using HDF and HUVEC. HDF cells are cells present on the skin harvested from adult while HUVEC is endothelial cells from the lining of umbilical cord vein, hence the function of both these cells are different due to their source of origin. However, both HDF and HUVEC lines the skin cells and vessels to ensure proper cells renewal in designated areas when needed. In the present study, the effects of fabricated composites films on the proliferation rate of both cells were evaluated. The Alamar Blue was therefore used to demonstrate that there was a considerable effect on the growth of HDF and HUVEC seeded on the fabricated films. A quantitative evaluation of both cells responses towards fabricated films were performed for Days 1, 4 and 7. Viability results of HDF and HUVEC cells seeded on the PCL, PCLBG5, and PCLBG10 along with the cells only (HDF and HUVEC seeded on the 48-well plate) after 1 to 7 days of incubation at 37 °C are shown in Fig. 2. According to the results, the numbers of viable cells on both PCLBG films were higher compared to PCL and control well. Results obtained for HUVEC demonstrated that higher cell proliferation was observed on the film containing PCLBG10 compared to film consisted of only polymer in all day evaluated but PCLBG5 showed the highest cell viability on the last day of the treatment. As for HDF, it was noted on Day 7, that an increase in terms of per cent reduction of Alamar Blue for both PCLBG film has been observed and it showed higher cell viability compared with the control well and PCL film. The results demonstrate that all the fabricated PCL and PCLBG composites were not toxic to the HDF and HUVEC cells during the investigated time period. Furthermore, the results suggested that PCLBG5 and PCLBG10 composite films had more pronounced in promoting and enhancing effect on the cell attachment and proliferation of both cell lines as compared with PCL film and control group. Moreover, after 7 days of evaluation, the best results are related to PCLBG fabricated composites films. The results proved that the cells were successfully attached to the surfaces of films and suggested there is a positive effect of PCLBG compared to PCL alone in terms of cell proliferation activity of the cells.

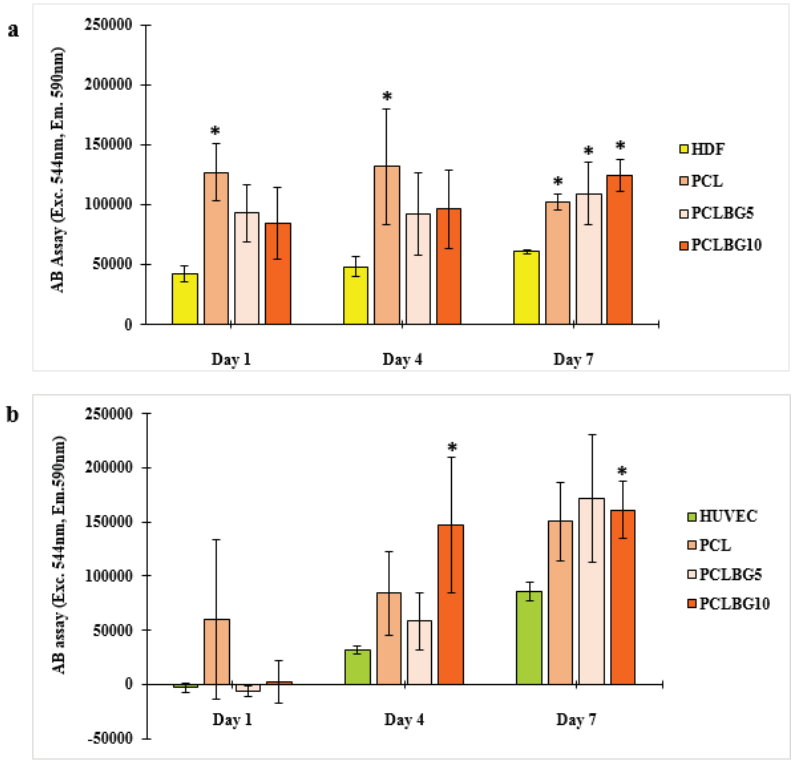

FIGURE 2: Cell proliferation of seeded HDF (a) and HUVEC (b) using Alamar Blue assay that were performed at Day 1, 4 and 7 on PCL, PCLBG 5% and PCLBG 10%.

These findings are consistent with results obtained in previous works that the addition of BG particles to the PCL film induced hydrophilicity of composites blend film and the specific area, which enhances cell attachment and proliferation of the cells [15]. It should be noted that pure PCL is a well-known biocompatible and hydrophobic polymer but it shows very poor cell adhesion characteristics and lack of biological activity. [16]. Therefore, fabricated composites films in this study indicated the suitability that could have supported and showed positive effect on the growth of HDF and HUVEC to allow for higher cell proliferation [17]. Also as reported from previous study using HDF cells, similar result demonstrating that fibroblast cells prefer samples with the finest pore structure [10]. This finding from previous work explains the similar result obtained this time that PCLBG10 films with increased surface area and porosity are able to provide physical support to cells that resulting as the most favourable for the attachment and proliferation of HDF. Previous study also proved that inorganic constituent of BG when combined with PCL improves the biocompatibility of degradable polymers and enhance biological performance of PCL [16]. This *in vitro* evaluation also suggested that both fabricated PCLBG were non-toxic towards cells and is biocompatible with human fibroblast and endothelial cells since there was no significant decrease in cell viability compared to cells in control well.

CONCLUSION

Based on the current research, there were two types of composite films namely PCLBG5 and PCLBG10 that were successfully fabricated using solvent casting method. The composites films were found to have a suitable characteristic for cell attachment and cell proliferation as shown in *in vitro* study. The addition of bioactive glass particle to PCL polymer provided a good surface textures and porosity that enhanced biocompatibility and bioactivity. The result also proved that PCLBG composites have better bioactivity compared to PCL only by improving cells viability and growth on the surface of the fabricated films. It was positively proven that the composites films were non-toxic to human when tested on human fibroblast and endothelial cells. This finding is in good agreement to the previous studies of other authors suggesting that combination of BG with PCL can function as the suitable filler for degradable polymer that served as a good potential for bone regenerative material or as a skin patch for skin dehiscence which is able to facilitate wound healing.

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