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Influence of poly lactic-co-glycolic acid scaffold with concentrated growth factor on human osteoblast cells

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ABSTRACT

The innovation of grafting materials facilitates an improved patients' care and treatment outcomes in promoting bone regeneration. A recent paradigm shift towards poly lactic-co-glycolic acid (PLGA) as an excellent scaffold for tissue engineering and autologous nature of concentrated growth factor (CGF) is starting to be in the highlight of treatment. However, the role of both materials as substitutes for bone grafting material remains unclear. In this study, we aimed to investigate the influence of both materials on the biological behavior of human osteoblast cells (HOBs). PLGA microspheres prepared using double solvent evaporation method were observed under scanning electron microscope. Blood was collected from a rabbit and centrifuged to obtain CGF. HOBs were incubated with CGF, PLGA microspheres, and CGF+PLGA for 24, 48, and 72 h. Their proliferation was assessed. Microscopic image revealed a spherical shape with interconnected pores as an architecture for cellular ingrowth. Significant differences observed in the mean proliferation of HOBs between control and CGF+PLGA group. Similar observations were observed between control and PLGA, and between CGF and PLGA, indicating the role of PLGA and CGF for bone regeneration. This result further indicated that both PLGA scaffold with CGF has the potential as alternative materials in promoting bone regeneration.

Keywords: bone graft; bone regeneration; growth factor; polymer; scaffold

1. INTRODUCTION

Over the past decades, various treatment approaches and grating materials were used in facilitating bone regeneration for periodontal defect. Bone formation in the osseous defect is facilitated by bone grafts as they provide an organisation for clot development, maturation, and remodelling (Reynolds et al., 2010). A bone graft material is considered ideal when it possesses three main features, which are osteogenesis, osteoinduction and osteoconduction (Kumar et al., 2013; Laurencin, et al., 2006; Sangeetha and Jain, 2018). Autograft is the gold standard material obtained from

the intraoral sites of the donor and it has higher osteoconductive, osteoinductive, and osteogenic properties (Reynolds et al., 2010). Nevertheless, the drawbacks in the application of autograft range from a limited volume of harvestable graft, prolonged surgical time, and morbidity of donor site (Brown et al., 2015; Hoda et al., 2016; Kumar et al., 2013). Allograft is an osteoinductive material sourced from other cadavers or donors of the same species (Jamjoom and Cohen, 2015). Allografts are available in mineralized and demineralized forms, and the osteoconductivity and mechanical properties are reduced during sterilization and storage. Although allograft is an alternative to autograft, it needs to be strictly monitored for infectious diseases to prevent cross-infection. On the other hand, a xenograft has osteoconductive property and due to its similar hydroxyapatite content to human bone, the scaffold allows for new vascularization and subsequently replaced by new bone formation (Jamjoom and Cohen, 2015). However, xenograft lacks mechanical properties and is controversial due to ethical issues regarding the use of animal tissue in the body.

Alloplastic material is a synthetic bone graft that has been recently developed to overcome the limitations of other grafting materials. It induces bone formation by providing an osteoconductive platform through their composition, morphology, and surface topography (Laurencin et al., 2006). Recently, studies have highlighted the potential application of a synthetic polymer known as poly lactic-co-glycolic acid (PLGA) in regenerative medicine and dentistry. PLGA, a copolymer consisting of lactic acid and glycolic acid, is an FDA-approved biodegradable polymer that is safe to be used in living cells due to its high strength properties and biocompatibility (Makadia and Siegel, 2011; Martins et al., 2018). Due to its high porosity and interconnected pores, the role of PLGA particles as a drug delivery system has been investigated and widely applied in various fields of medicine such as delivering chemotherapeutic agents, antimicrobials, vaccines, anti-inflammatory drugs and antihypoglycemic agents (Martins et al., 2018; Mir et al., 2017; Qi et al., 2019). These features, together with its controlled degradation, allow for the sustained release of drugs (Avgoustakis, 2005; Martins et al., 2018). PLGA has also been employed as an implant for orthopedic intervention as it can be constructed to slowly degrade and thus, accommodate the slow bone-healing process (Avgoustakis, 2005). Currently, the use of PLGA in periodontal treatment has started to gain momentum as it is commonly used as a barrier material in guided tissue regeneration procedures (Yoshimoto et al., 2018), extraction sockets scaffold material (Araujo-Pires et al., 2016; Yoshimoto et al., 2018) and suture material (Avgoustakis, 2005).

In the management of periodontal and bone injuries, growth factors need to be delivered to regulate the migration and proliferation of cells during healing. A vehicle is required to achieve the delivery of growth factors to the indicated site. The common materials employed for the vehicular transport of growth factors include bone graft, collagen and polymers. These materials are processed into various forms such as microparticles, sponge, film and sutures (Kowalczewski and Saul, 2018; Raja et al., 2009; Zhang et al., 2018). Nevertheless, the delivery of recombinant human growth factors is expensive and tends to undergo rapid dilution after application, thus limiting the half-life for prolonged action (Raja et al., 2009; Shimono et al., 2010).

In place of recombinant human growth factors, the usage of autologous platelet concentrates has gained attention as an alternative in delivering a high concentration of growth factors to indicated sites, and as a scaffolding for cells (Chen and Jiang, 2020). The invention of platelet concentrate using autologous sources of blood began with the development of platelet rich plasma (PRP) and was followed by platelet rich fibrin (PRF) in 2001 (Toffler et al., 2009). The concentrated growth factor (CGF) is the current generation of platelet concentrate products, which was first introduced by Sacco in 2006 (Borsani et al., 2015; Takeda et al., 2015). It was found that CGF contained a higher amount of growth factors as compared to PRP and PRF (Masuki et al., 2016). The CGF is produced using single centrifugation with an alternate speed that allows for the isolation of larger and denser fibrin matrix as compared to PRP and PRF. It also results in clots with increased stability, strength, and protection against plasmin degradation. Therefore, it can prolong the release of growth factors and action (Rodella et al., 2011).

Due to the unique features of PLGA and CGF, the effects of both materials on bone regeneration were evaluated in this study, particularly on the osteoblast cells. To the knowledge of the authors, this present study is the first attempt to evaluate the combinatory action of PLGA and CGF in bone regeneration. PLGA microspheres in this study were mainly aimed to substitute the bone graft materials currently used in clinical practice. Apart from that, it also acted as a vehicle for delivery of growth factors when the CGF was combined in bone regeneration. This combination effect between PLGA and CGF in the bone generation study, however, is relatively unclear. These events prompted the hypothesis that a combination of PLGA and CGF will significantly affect the proliferation of human osteoblast cells (HOBs). Therefore, the objective of this *in vitro* study was to investigate the influence of PLGA scaffold with CGF on the biological behavior of HOBs as a model for bone regeneration.

2. MATERIALS AND METHODS

2.1 Fabrication of PLGA microspheres

PLGA microspheres were firstly prepared using the double solvent evaporation method. Approximately 1 g of PLGA was mixed with 5 mL dichloromethane (DCM) and 250 µL of phosphate buffer saline was added to the mixture prior to homogenization at 9000 rpm to form a primary emulsion. Subsequently, 200 mL of 0.3% w/v polyvinyl alcohol (PVA) solution was supplemented into the mixture and homogenized again at 4000 rpm to form a secondary emulsion. The secondary emulsion mixture was stirred for 4 h at 300 rpm to allow DCM to evaporate and the microspheres to precipitate. The microspheres were then washed with distilled water by centrifugation and suspended in 10 mL of ethanolic-sodium hydroxide solution to enhance the porosity of the microspheres. The suspension was vortex-mixed at 1400 rpm at different durations, sieved with a pore size of 40 µm, and washed again using distilled water. Finally, the particles were freeze-dried for 72 h to obtain the final product of PLGA microspheres that will act as a scaffold for CGF (Qutachi et al., 2013).

2.2 Observation of PLGA microspheres under scanning electron microscopy (SEM)

Freeze-dried PLGA microspheres that were previously diluted and vortex-mixed in distilled water were directly

dropped onto its platform, air-dried, and covered with a 15-20 nm layer of gold. The microspheres were then examined by scanning electron microscope (Tabletop TM3000, Hitachi, Tokyo, Japan) for its pore characteristics under 400x magnification (Qutachi et al., 2013; Vyslouzil et al., 2016).

2.3 Blood collection for fabrication of CGF

This study was reviewed and approved by the Committee on Animal Research and Ethics (UiTM CARE) with the approval number 256/2018 and was conducted according to animal research: reporting *in vivo* experiments (ARRIVE) guidelines 2.0. An eight-month old healthy male New Zealand white rabbit was quarantined for one week at a quarantine room and allowed for acclimatization for two weeks in a procedure room prior to the research procedure. The rabbit was housed in a cage with temperature controlled rooms, fed with standardized diet and free access to tap water, and was inspected daily for their general health, feeding and the cage condition. Approximately 6 mL of venous blood was drawn from the marginal ear vein and collected into serum vacutainers. The vacutainers were immediately centrifuged (Tabletop Centrifuge Kubota 2420, Tokyo, Japan) using alternate speed to form CGF (Kim et al., 2014; Takeda et al., 2015).

2.4 Combination of CGF with PLGA microspheres by centrifugation

For the incorporation of both PLGA microspheres and CGF, both materials were centrifuged at 1000 rpm for two minutes (Tabletop Micro Refrigerated Centrifuge Model 3500, Tokyo, Japan) at room temperature (Lee et al., 2015).

2.5 Treatment of HOBs with CGF, PLGA and combination of CGF and PLGA

The extraction media was prepared by immersing the specimens in Dulbecco's modified Eagle's medium (DMEM). The HOBs (HFOB1.19.AddexBioT0004005) were seeded at a density of 1×10^3 cells/well containing 10% heat-inactivated fetal bovine saline and 5% penicillin-streptomycin solution prior to incubation at 37°C. The cell culture media were removed with an aspirator after one day, and the cells were extracted and transferred into 96 well plates containing the following treatments: i) 1 g of CGF, ii) 10 mg of PLGA, and iii) CGF + PLGA (containing a mixture of 1 g of CGF + 10 mg of PLGA) (Borsani et al., 2015; Lee et al., 2015; Takeda et al., 2015). All experiments were performed in triplicate and subjected to a 24, 48, and 72 h incubation.

2.6 Evaluation of cell proliferation by MTS assay

The CellTiter 96 aqueous one solution cell proliferation assay system (Promega, Madison, USA) was used to determine the HOBs proliferation according to the manufacturer's instructions (Takeda et al., 2015). After each incubation period (24, 48, or 72 h), approximately 20 µL of CellTiter 96 aqueous one solution reagent (Promega, Madison, USA) was added into each well and incubated for one hour in a humidified atmosphere containing 5% CO_2. The absorbance was measured at 490 nm using an enzyme-linked immunosorbent assay plate reader (Multilabel Plate Reader Victor™ X5, PerkinElmer, USA).

2.7 Statistical analysis

A sample from a rabbit was used for blood taking as source of CGF to avoid bias from multiple sources of blood of different rabbits and examined in triplicate for statistical analysis. The data were then exported to Statistical Package for Social Science (SPSS, Version 26.0, IBM) software for data analysis by comparing cellular proliferation rate in between treatment groups at three time points. A repeated-measure ANOVA was applied to evaluate the time and treatment effects. Pairwise comparison with confidence interval adjustment together with Bonferroni correction was performed for time-effect analysis. *Post-hoc* multiple comparisons were executed when a significant treatment between groups was found. A $p<0.05$ was considered for significant effects and statistical difference between the treatments. Assumption of normality, homogeneity of variances and compound symmetry were checked and fulfilled.

3. RESULTS AND DISCUSSION

3.1 Characterization of PLGA microspheres under SEM

The surface morphology of the PLGA microspheres observed by SEM revealed a spherical shape with the presence of interconnected pores on its surface. To stabilize the emulsion formed between the polymer and drug solution with water, PVA was used as an emulsifier. Following the evaporation of the organic solvent from the surface of droplets, the concentration of the polymer increased until it reached a critical point at which the polymer concentration exceeded its solubility in the organic phase and subsequently precipitated to produce microspheres (Iqbal et al., 2015). Figure 1 shows the features of the microspheres under SEM with 400x magnification. The size of the microspheres was analyzed using the Image J software and ranged between 53.7 µm to 120 µm.

In this study, microspheres were fabricated using PLGA showed interconnected pores within the microspheres. This characteristics of microspheres, which have high porosity, are important in this study. Upon centrifuged, it is postulated that the growth factors (CGF) were trapped in between the interconnected pores and were gradually released during the HOBs treatment. The extraordinary characteristics of PLGA microspheres of high porosity and interconnected pores also help to facilitate cell behavior and performance as they provide a convenient substrate surface area for cellular adhesion and biodegradable cell carriers (Martins et al., 2018). It is also important to emphasize the pore size dimensions in the formation of scaffolds from microspheres as the scaffolds should not only have the mechanical properties, but also the porosity and appropriate pore diameter to support the formation of new tissue (Kirby et al., 2016; Liu et al., 2010). Porosity is an important factor characterized by the presence of pores which influences the diffusion of drugs to the external environment, cellular invasion into the scaffold structure, and cellular attachment (Abbasi et al., 2020; Xu et al., 2016). In addition, adequate diameter of the pore size is required to allow the sustainable release of drugs (Han et al., 2016; Lemperlee et al., 2004). In this study, porous PLGA microspheres were fabricated with different sizes, with some pore sizes above 30 µm. This size is considered to be acceptable for use in scaffold preparation to favor scaffold vascularization and the growth of osteoblasts (Abbasi et al., 2020; Sicchieri et al., 2012).

3.2 The effect of CGF, PLGA and CGF + PLGA on osteoblastic proliferation

Table 1 and Figure 2 show the descriptive statistics of mean percentage of HOBs proliferation, reported as mean and standard deviation. There was a gradual increase in the mean percentage of cells upon incubating the HOBs with CGF. The rate of cell multiplication was 79.74%, 107.65% and 127.86% at 24, 48 and 72 h, respectively. Meanwhile, cells treated with PLGA and CGF + PLGA exhibited a similar pattern of increased cell proliferation from 24 to 48 h but decreased from 48 to 72 h. Compared with the cells treated with CGF and PLGA alone, HOBs treated with CGF + PLGA showed an overall higher mean percentage of cell proliferation estimated as 147.97%, 468.43%, and 260.25% at 24, 48, and 72 h, respectively.

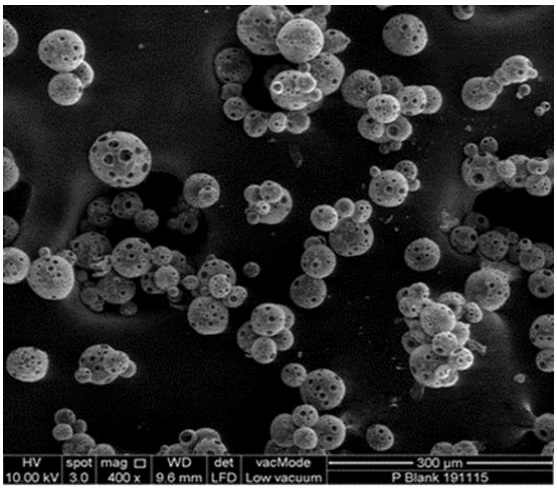

Figure 1. SEM image of porous PLGA microspheres (400x magnification)

Table 1. Mean percentage of HOBs proliferation (%)

Time (h)	Treatment groups (mean (SD))			
	Control	CGF	PLGA	CGF+PLGA
24	100 (2.13)	79.74 (0.82)	121.13 (7.10)	147.97 (0.00)
48	100 (5.10)	107.65 (9.02)	444.8 (161.92)	468.43 (126.36)
72	100 (2.65)	127.86 (4.62)	240.96 (28.23)	260.25 (52.63)

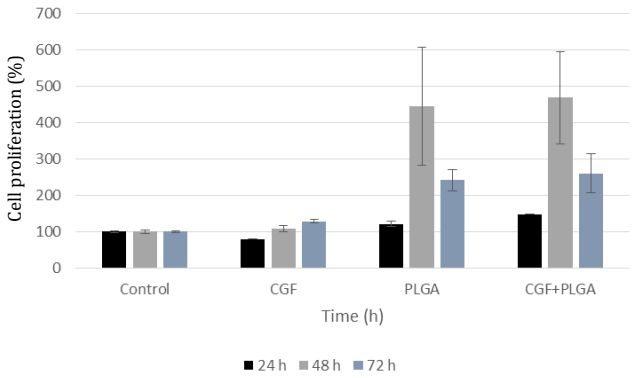

Figure 2. Mean percentage of HOBs proliferation at three different time points

The mean percentage of cell proliferation increased gradually when treated with CGF and a significant difference was observed between 24 and 72 h upon treatment. These findings corroborated findings from previous studies as treatment with CGF, resulting in a gradual increased proliferation of stem cells, human periosteal cells, bone stromal cells, and osteoblast cells (Borsani et al., 2015; Sahin et al., 2018; Takeda et al., 2015). The high content and prolonged release of growth factors from CGF were also influenced by its fabrication process, whereby a single centrifugation step with alternate speed allowed for the isolation of larger and denser fibrin matrix. Since an alternate speed is employed in the preparation of CGF, this further enhanced the release of growth factor which is facilitated by the increased of platelet rupture occurring during the centrifugation (Chen and Jiang, 2020). This centrifugation

leads to the formation of a regular, cross-linked fibrin matrix with increased stability, strength and does not dissolve rapidly upon application (Rodella et al., 2011). CGF is the current generation of platelet concentrate products used in clinical practice to enhance the healing process. It also has the additional benefit of reduced costs as the patient's own blood is drawn for its fabrication, thus minimizing the risk of cross-reaction.

PLGA is widely recognized as an excellent scaffold material for drug delivery and tissue engineering procedures. In addition, PLGA has also becoming a popular choice for its use in periodontal regeneration (Araujo-Pires et al., 2016; Avgoustakis, 2005; Yoshimoto et al., 2018; Zhao et al., 2019). In a recent study by Cai and colleagues, PLGA microspheres were used as a vehicle to test the efficacy of the P24 component, which is a synthetic derivative of bone morphogenetic protein (BMP)-2 on the osteogenic potential. It was observed that PLGA loaded with P24 resulted in the continuous release of P24 for over 60 days as well as enhanced the proliferation of MC3T3-E1 cells and secretion of alkaline phosphatase (Cai et al., 2019).

In the present study, since the PLGA microspheres were porous with adequate pore size, osteoconductivity was facilitated for the migration and attachment of osteoblast cells on the PLGA scaffold, thus promoting a greater proliferation of osteoblast cells as compared to CGF (Abbasi et al., 2020; Ebrahimi, 2017; Hosseinpour et al., 2017). Based on Figure 2, the proliferation of HOBs cultured with CGF + PLGA was higher than those cultured with the control group, CGF, and PLGA alone at three different time points. The higher HOBs proliferation observed when treated with CGF + PLGA is possibly due to the combinatory effects of both the unique features of CGF and PLGA.

The time effect comparison showed a significant difference of mean percentage of HOBs proliferation within each treatment group based on time (p=0.007). There were significant differences of mean percentage of HOBs proliferation among four treatment groups (p<0.001) regardless of time. The *post-hoc* multiple comparison results (Table 2) showed that there were significant differences in the mean percentage of cell proliferation between control and PLGA but not significant between PLGA and CGF + PLGA. For bone regeneration to take place, a suitable environment for cellular attachment and proliferation, adequate supply of blood and nutrients, and provisions for complete removal of by-products are required. These events are promoted when the surface materials employed as scaffold possess interconnected pores and adequate porosity (Abbasi et al., 2020).

Since the PLGA microspheres were fabricated with features previously described, this shows that the application of PLGA scaffold alone was able to induce osteoblast cell proliferation. Owing to the porosity, greater surface area and lower mass density of the fabricated PLGA particles, the cell attachment and proliferation were improved (Cai et al., 2013). In addition, a review paper by Zhao and colleagues (2021) has emphasized the role of PLGA in promoting bone formation due to its advantages such as adequate mechanical strength, excellent biocompatibility and processability. This facilitates the manufacturing of PLGA scaffold with various pore sizes and the ability to adjust the ratio of lactic and glycolic acid allows a controlled degradation time to match with turnover rate of bone formation. Additionally, various bioactive materials can be incorporated with the PLGA in promoting bone regeneration (Zhao et al., 2021). This is applied in this present study as CGF was incorporated with the PLGA and a significant difference was observed between control and CGF + PLGA. This shows that the combination of PLGA with CGF might have enhanced the sustained release of growth factors from CGF and protected the CGF from early degradation (Yu et al., 2019). Based on the observation of the proliferation of HOB cells in this study, the growth factors from CGF were postulated to be released in a sustained manner for the growth of cells.

A significant difference was also observed between CGF and PLGA, indicating that, in addition to source of growth factors, CGF also act as a scaffold for cell migration as it is associated with an interwoven cross-linked structures and networks. Overall, the networks provide an attachment for cytokine and recruitment of cells towards the CGF scaffold, enabling its role in promoting bone formation (Qiao et al., 2016; Fang et al., 2020). However, no significant difference was observed when the treated cells were compared between control and CGF. Our result contradicts with the previous findings as the culture of osteogenic cells with CGF reported on significant differences in cellular proliferation (Chen et al., 2019; Masuki et al., 2016; Sahin et al., 2018; Zhang and Ai, 2019). This could be contributed by one of the limitations that only one density of CGF (1 g) was assessed. Multiple density of CGF is suggested for future studies. Even though there was no significant difference observed between control and CGF, mean proliferation of HOBs increased gradually from 24 to 72 h.

Table 2. Mean differences in HOBs proliferation among treatment groups

Comparison	Mean difference (95% confidence interval)	*p*-value
Control and CGF	-5.09 (-88.40, 78.23)	0.997
Control and PLGA	-168.96 (-252.27, -85.65)	0.001*
Control and CGF+PLGA	-192.22 (-275.53, -108.91)	<0.001*
CGF and PLGA	-163.88 (-247.19, -80.56)	0.001*
CGF and CGF+PLGA	-187.13 (-270.44, -103.82)	<0.001*

Note: Statistically significant (p<0.05)

There was a significant difference of mean percentage of HOBs proliferation among four treatment groups based on time (p < 0.001) (Table 3). From the study, it was found that there was significant difference in mean percentage of HOBs proliferation at 24 h between control and CGF + PLGA group and in the mean percentage of HOBs proliferation at 48 and 72 h between control and PLGA group. In addition, a significant difference at 48 and 72 h was also observed between control and CGF + PLGA group.

There is data paucity on the use of PLGA microspheres as a depot for CGF, nevertheless, a few studies have reported on the delivery of growth factors from other generations of platelet concentrate products such as PRP. For instance, it was observed that the combination of PLGA and PRP showed improved treatment outcomes following the use of heparin-conjugated PLGA nanospheres treated with PRP and fibrin gel as compared to PRP and fibrin gel alone in promoting skin wound healing in mice. Improved treatment outcomes were measured based on the release of PDGF-BB and the rate of regeneration of epidermal and dermal tissue (La and Yang, 2015). Besides, Lee and colleagues found that the centrifugation technique showed a significant increase in cell proliferation of human bone marrow stromal cells, compared to a simple dripping and dynamic oscillation technique (Lee et al., 2015). The authors selected PRP as a coating material to improve the surface characteristics of the PLGA as a scaffolding structure. These observations indicate the feasibility of PLGA as a depot for enhanced release of growth factors.

Table 3. Comparison of mean percentage of HOBs proliferation among four different treatment groups based on time

Time (h)	Treatment	Mean	95% Confidence interval
24	Control	100	95.04,104.96
	CGF	79.74	74.78, 84.70
	PLGA	121.13	116.16, 126.09
	CGF + PLGA	147.97	143.01, 152.93
48	Control	100	-36.90, 236.90
	CGF	107.65	-29.25, 244.56
	PLGA	444.8	307.90, 581.70
	CGF + PLGA	468.43	331.53, 605.33
72	Control	100	60.08, 139.92
	CGF	127.86	87.95, 167.78
	PLGA	240.96	201.04, 280.88
	CGF + PLGA	260.25	220.33, 300.17

Based on the findings of this study, the use of CGF + PLGA resulted in a significant difference in the comparison of the mean percentage of proliferation of HOBs between treatment groups and at different time points. However, the comparison between the various time periods used in this study contribute to one of the limitations in this study as previous studies observed cell proliferation on alternate days such as at day 1, 3, 5, and 7 (Borsani et al., 2015; Cai et al., 2019; Chen et al., 2019; Sahin et al., 2018; Takeda et al., 2015). Nevertheless, the mean proliferation rate in the CGF + PLGA group increased from 24 to 48 h and reduced from 48 to 72 h. We postulated that high density of cells may affect the reading at 72 h and contribute to the limitation of the present outcome as the culture of cells with CGF and PLGA only did showed increased in cellular proliferation from 24 to 48 h. However, this pattern of mean cell proliferation rate percentage corroborates the findings from other studies as there was an increased cellular proliferation from 24 to 72 h (Sahin et al., 2018; Zhang et al., 2019). For the group treated with only PLGA, there was no difference in the mean HOB cells proliferation compared to CGF + PLGA group but greater cell proliferation was observed when compared with CGF alone. This shows that PLGA itself has an osteogenic effect in inducing the proliferation of HOB cells. Furthermore, the result highlights that PLGA microspheres may act as a depot. The incorporation of CGF into the scaffold allows a sustained release of growth factors for enhanced healing and regenerative effect.

Notwithstanding these limitations, the unique features of PLGA microspheres facilitated cell behavior and performance by acting as a scaffold and allowing the sustained release of growth factors from CGF. The use of PLGA as a depot for CGF delivery and as a scaffold material could be applied clinically to the designated area, thus increase the probability of an improved treatment outcome and augment periodontal bone regeneration. As this study serves as a preliminary study on the influence of CGF and PLGA scaffold on proliferation of osteoblast, it is suggested to increase the time interval for cellular proliferation with various densities of CGF and PLGA, to study the release profile of CGF from PLGA and observation of cellular adhesion in future studies.

4. CONCLUSION

In a nutshell, it can be concluded that the use of PLGA scaffold and CGF has the potential to induce greater human osteoblast cell proliferation and regenerative activity to facilitate improved bone regeneration. In addition, this study serves as an ideal standard and benchmark for future investigations on the beneficial role of CGF + PLGA for the enhancement of bone regeneration.

REFERENCES

Abbasi, N., Hamlet, S., Love, R. M., and Nguyen, N.-T. (2020). Porous scaffolds for bone regeneration. *Journal of Science: Advanced Materials and Devices*, 5(1), 1-9.

Araujo-Pires, A. C., Mendes, V. C., Ferreira-Junior, O., Carvalho, P. S. P., Guan, L., and Davies, J. E. (2016). Investigation of a novel PLGA/CaP scaffold in the healing of tooth extraction sockets to alveolar bone preservation in humans. *Clinical Implant Dentistry and Related Research,* 18(3), 559-570.

Avgoustakis, K. (2005). Polylactic-co-glycolic acid (PLGA). In *Encyclopedia of Biomaterials and Biomedical Engineering* (Wnek, G. E., and Bowlin, G. L, eds.), 2nd , Boca Raton, FL: CRC Press.

Borsani, E., Bonazza, V., Buffoli, B., Cocchi, M. A., Castrezzati, S., Scarì, G., Baldi, F., Pandini, S., Licenziati, S., Parolini, S., Rezzani, R., and Rodella, L. F. (2015). Biological characterization and *in vitro* effects of human

concentrated growth factor preparation: an innovative approach to tissue regeneration. *Biology and Medicine*, 7(5), 1000256.

Brown, A., Zaky, S., Ray, H., and Sfeir, C. (2015). Porous magnesium/PLGA composite scaffolds for enhanced bone regeneration following tooth extraction. *Acta Biomaterialia*, 11, 543-553.

Cai, Q., Qiao, C., Ning, J., Ding, X., Wang, H., and Zhou, Y. (2019). A polysaccharide-based hydrogel and PLGA microspheres for sustained P24 peptide delivery: an *in vitro* and *in vivo* study based on osteogenic capability. *Chemical Research in Chinese Universities*, 35, 908-915.

Cai, Y., Chen, Y., Hong, X., Liu, Z., and Yuan, W. (2013). Porous microsphere and its applications. *International Journal of Nanomedicine,* 8, 1111-1120.

Chen, J., and Jiang, H. (2020). A comprehensive review of concentrated growth factors and their novel applications in facial reconstructive and regenerative medicine. *Aesthetic Plastic Surgery*, 44, 1047-1057.

Chen, X., Chen, Y., Hou, Y., Song, P., Zhou, M., Nie, M., and Liu, X. (2019). Modulation of proliferation and differentiation of gingiva-derived mesenchymal stem cells by concentrated growth factors: Potential implications in tissue engineering for dental regeneration and repair. *International Journal of Molecular Medicine*, 44, 37-46.

Ebrahimi, M. (2017). Bone grafting substitutes in dentistry : general criteria for proper selection and successful application. *Journal of Dental and Medical Sciences*, 16(4), 75-79.

Fang, D., Long, Z., and Hou, J. (2020). Clinical Application of concentrated growth factor fibrin combined with bone repair materials in jaw defects. *Journal of Oral Maxillofacial Surgery*, 78(6), 882-892.

Han, F. Y., Thurecht, K. J., Whittaker, A. K., and Smith, M. T. (2016). Bioerodable PLGA-based microparticles for producing sustained-release drug formulations and strategies for improving drug loading. *Frontiers in Pharmacology*, 7, 185.

Hoda, N., Saifi, A. M., and Giraddi, G. B. (2016). Clinical use of the resorbable bioscaffold poly lactic co-glycolic acid (PLGA) in post-extraction socket for maintaining the alveolar height: A prospective study. *Journal of Oral Biology and Craniofacial Research*, 6(3), 173-178.

Hosseinpour, S., Ahsaie, M. G., Rad, M. R., Baghani, M. T., Motamedian, S. R., and Khojasteh, A. (2017). Application of selected scaffolds for bone tissue engineering: a systematic review. *Oral and Maxillofacial Surgery*, 21(2), 109-129.

Iqbal, M., Zafar, N., Fessi, H., and Elaissari, A. (2015). Double emulsion solvent evaporation techniques used for drug encapsulation. *International Journal of Pharmaceutics,* 496(2), 173-190.

Jamjoom, A., and Cohen, R. E. (2015). Grafts for ridge preservation. Journal of Functional *Biomaterials,* 6(3), 833-848.

Kim, T. H., Kim, S. H., Sádor, G. K., and Kim, Y. D. (2014). Comparison of platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and concentrated growth factor (CGF) in rabbit-skull defect healing. *Archives of Oral Biology*, 59(5), 550-558.

Kirby, G. T. S., White, L. J., Steck, R., Berner, A., Bogoevski, K., Qutachi, O., Jones, B., Saifzadeh, S., Hutmacher, D. W., Shakesheff, K. M., and Woodruff, M. A. (2016). Microparticles for sustained growth factor delivery in the regeneration of critically-sized segmental tibial bone defects. *Materials,* 9(4), 259-278.

Kowalczewski, C. J., and Saul, J. M. (2018). Biomaterials for the delivery of growth factors and other therapeutic agents in tissue engineering approaches to bone regeneration. *Frontiers in Pharmacology,* 9, 513.

Kumar, P., Fathima, G., and Vinitha, B. (2013). Bone grafts in dentistry. *Journal of Pharmacy and Bioallied Sciences,* 5(5), 125-127.

La, W. G., and Yang, H. S. (2015). Heparin-conjugated poly (lactic-co-glycolic acid) nanospheres enhance large-wound healing by delivering growth factors in platelet-rich plasma. *Artificial Organs,* 39(4), 388-394.

Laurencin, C., Kan, Y., and El-Amin, S. (2006). Bone graft subtistutes. *Expert Review of Medical Devices,* 3(1), 49-57.

Lee, J. H., Nam, J., Kim, H. J., and Yoo, J. J. (2015). Comparison of three different methods for effective introduction of platelet-rich plasma on PLGA woven mesh. *Biomedical Materials,* 10(2), 025002.

Lemperlee, G., Morhenn, V. B., Pestojamasp, V., and Gallo, R. L. (2004). Experimental migration studies and histology of injectable microspheres of different sizes in mice. *Plastic and Reconsructive Surgery*, 113(5), 1380-1390.

Liu, F., Guo, R., Shen, M., Cao, X., Mo, X., Wang, S., and Shi, X. (2010). Effect of the porous microstructures of poly (lactic-co-glycolic acid)/ carbon nanotube composites on the growth of fibroblast cells. *Soft Materials*, 3, 239-253.

Makadia, H. K., and Siegel, S. J. (2011). Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers,* 3(3), 1377-1397.

Martins, C., Sousa, F., Araújo, F., and Sarmento, B. (2018). Functionalizing PLGA and PLGA derivatives for drug delivery and tissue regeneration applications. *Advanced Healthcare Materials*, 7(1), 1701035.

Masuki, H., Okudera, T., Watanebe, T., Suzuki, M., Nishiyama, K., Okudera, H., Nakata, K., Uematsu, K., Su, C. Y., and Kawase, T. (2016). Growth factor and pro-inflammatory cytokine contents in platelet-rich plasma (PRP), plasma rich in growth factors (PRGF), advanced platelet-rich fibrin (A-PRF), and concentrated growth factors (CGF). *International Journal of Implant Dentistry*, 2(1), 19.

Mir, M., Ahmed, N., and Rehman, A. (2017). Recent applications of PLGA based nanostructures in drug delivery. *Colloids and Surfaces B: Biointerfaces,* 159, 217-231.

Qi, F., Wu, J., Li, H., and Ma, G. (2019). Recent research and development of PLGA/PLA microspheres/nanoparticles : A review in scientific and industrial aspects. *Frontiers of Chemical Science and Engineering,* 13(1), 14-27.

Qiao, J., Duan, J., Zhang, Y., Chu, Y., and Sun, C. (2016). The effect of concentrated growth factors in the treatment of periodontal intrabony defects. *Future Science OA*, 2(4), FS136.

Qutachi, O., Shakesheff, K. M., and Buttery, L. D. K. (2013). Delivery of definable number of drug or growth factor loaded poly(dl-lactic acid-co-glycolic acid) microparticles within human embryonic stem cell derived aggregates. *Journal of Controlled Release*, 168(1), 18-27.

Raja, S., Byakod, G., and Pudakalkatti, P. (2009). Growth factors in periodontal regeneration. *International Journal of Dental Hygiene*, 7(2), 82-89.

Reynolds, M. A., Aichelmann-Reidy, M. E., and Branch-Mays, G. (2010). Regeneration of periodontal tissue: bone replacement grafts. *Dental Clinics of North America*, 54(1), 55-71.

Rodella, L. F., Favero, G., Boninsegna, R., Buffoli, B., Labanca, M., Scarì, G., Sacco, L., Batani, T., and Rezzani, R. (2011). Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. *Microscopy Research and Technique*, 74(8), 772-777.

Sahin, O., Gokmenoglu, C., and Kara, C. (2018). Effect of concentrated growth factor on osteoblast cell response. *Journal of Stomatology Oral & Maxillofacial Surgery,* 119(6), 477-481.

Sangeetha, S., and Jain, A. R. (2018). Current bone substitutes for implant dentistry. *Journal of Pharmacy Research,* 10(8), 1583-1589.

Shimono, K., Oshima, M., Arakawa, H., Kimura, A., Nawachi, K., and Kuboki, T. (2010). The effect of growth factors for bone augmentation to enable dental implant placement: A systematic review. *Japanese Dental Science Review*, 46(1), 43-53.

Sicchieri, L. G., Crippa, G. E., de Oliveira, P. T., Beloti, M. M., and Rosa, A. L. (2012). Pore size regulates cell and tissue interactions with PLGA – CaP scaffolds used for bone engineering. *Journal of Tissue Engineering and Regenerative Medicine*, 6(2), 155-162.

Takeda, Y., Katsutoshi, K., Matsuzaka, K., and Inoue, T. (2015). The effect of concentrated growth factor on rat bone marrow cells *in vitro* and on calvarial bone healing *in vivo.* The International *Journal of Oral & Maxillofacial Implants*, 30(5), 1187-1196.

Toffler, M., Toscano, N., Holtzclaw, D., Corso, M. D., and David, D. E. (2009). Introducing Choukroun's platelet rich fibrin (PRF) to the reconstructive surgery milieu. *Journal of Implant & Advanced Clinical Dentistry*, 1(6), 21-31.

Vyslouzil, J., Dolezel, P., Kejdusova, M., Kostal, V., Benes, L., and Dvorackova, K. (2016). Long-term controlled release of PLGA microparticles containing antidepressant mirtazapine. *Pharmacetutical Development and Technology*, 21(2), 214-221.

Xu, Y., Kim, C., Saylor, D. M., and Koo, D. (2016). Polymer degradation and drug delivery in PLGA-based drug – polymer applications: A review of experiments and theories. *Journal of Biomedical Materials Research - Part B*, 105(6), 1692-1716.

Yoshimoto, I., Sasaki, J. I., Tsuboi, R., Yamaguchi, S., Kitagawa, H., and Imazato, S. (2018). Development of layered PLGA membranes for periodontal tissue regeneration. *Dental Materials,* 34(3), 538-550.

Yu, M., Wang, X., Liu, Y., and Qiao, J. (2019). Cytokine release kinetics of concentrated growth factors in different scaffolds. *Clinical Oral Investigations*, 23(4), 1663-1671.

Zhang, L., and Ai, H. (2019). Concentrated growth factor promotes proliferation, osteogenic differentiation, and angiogenic potential of rabbit periosteum-derived cells *in vitro*. *Journal of Orthopaedic Surgery and Research*, 14(1), 146.

Zhang, Y., Sun, T., and Jiang, C. (2018). Biomacromolecules as carriers in drug delivery and tissue engineering. *Acta Pharmaceutica Sinica B*, 8(1), 34-50.

Zhang, Z., Li, X., Zhao, J., Jia, W., and Wang, Z. (2019). The effect of autogenous growth factors released from platelet concentrates on the osteogenic differentiation of periodontal ligament fibroblast: a comparative study. *PeerJ,* 7, e7984.

Zhao, D., Zhu, T., Li, J., Cui, L., Zhang, Z., Zhuang, X., and Ding, J. (2021). Poly (lactic-co-glycolic acid)-based composite bone-substitute materials. *Bioactive Materials,* 6(2): 346-360.

Zhao, X., Tay, F. R., Fang, Y., and Meng, L. (2019). Topical application of phenytoin or nifedipine-loaded PLGA microspheres promotes periodontal regeneration *in vivo. Archives of Oral Biology*, 97, 42-51.