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Research Article

Human PDL Fibroblasts Proliferation in Scaffolds on Bioactive Glass Modified Ceramics

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Abstract Scaffolds are commonly used as cell's vehicles in order to promote cell development. The aim of this study was to investigate the proliferation of human periodontal ligament fibroblasts (PDLF) in composite ceramic scaffolds. Chitin (CHN), chitosan (CHS) and chitosangelatin (CH-G) scaffolds were fabricated on the surface of ceramic disks, coated with a mixture of a bioactive glass modified ceramic. Cell proliferation was evaluated with the MTS method. All composite ceramic scaffolds were successfully loaded with PDLF cells. However, only in CH-G composite ceramic-scaffolds cell population progressively increased until day 7 and presented a sudden drop at day 10 before being stabilized for the rest of the testing period. In the case of CHN and CHS the cell number was progressively decreasing. The successful observed cellular response in CH-G composite ceramic scaffolds could support the development of a protocol for tissue engineering on the materials used.

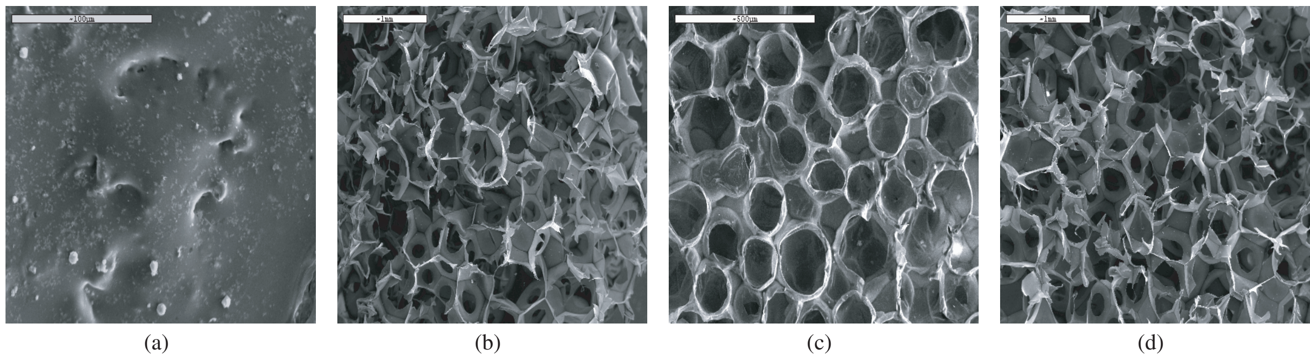
Keywords human PDL fibroblasts; scaffolds; chitin; chitosan; chitosan-gelatin; dental ceramics; cell proliferation

1 Introduction

Bioactive glass-modified dental ceramics can develop biological apatite that resembles cementum structure and enhance PDLF proliferation [9]. A cementum-like surface on dental ceramics where cells could be organized in a 3D order could lead to the establishment of a bond with surrounding tissues and a sealing of the marginal gap between the tooth structure and the restoration, thus improving the restoration's long-term prognosis. Scaffolds are commonly used in tissue engineering in order to provide a friendly micro-environment for 3D cell development [1,4]. Although synthetic scaffolds are widely used, biocompatible and biodegradable natural biopolymers (such as chitin and chitosan) or natural scaffolds (such as gelatin (collagen bio-product)) have several advantages as they contain sites for cellular adhesion and also-due to their origin-can provide guidance for nature-like tissue repair [6,7,8, 13]. Bio-polymeric scaffolds attached on bioactive glass modified dental ceramics could act as vehicles in order to guide the PDLF proliferation to an organized 3D structure. The aim of this study was to investigate the proliferation of human periodontal ligament fibroblasts on composite scaffolds (chitin/chitosan/chitosan-gelatin), attached on bioactive glass modified dental ceramics.

2 Materials and methods

A conventional feldspathic ceramic used in metal ceramic restorations was used (IPS In-Line Margin-Ivoclar, Schaan, Liechtenstein) and disk shaped specimens were fabricated using a procedure described previously, following the manufacturers' instructions [8]. Bioactive glass (SiO_2 Na_2O CaO P_2O_5) was prepared as described in the literature [6]. Ceramic disks with a diameter of 5 mm and a thickness of 2 mm were fabricated and then coated with a mixture composed of equal quantities of bioactive glass and ceramic. Also, bioactive glass powder was mixed with ceramic powder with 1:1 weight ratio, then sieved in a ball milling machine and finally mixed with modeling liquid. The mixture was transferred and spread on the surface of the prefabricated ceramic disks with simultaneous vibration for uniform distribution of the two materials. The coated disks were exposed to a second thermal cycle as recommended for dental ceramics (final temperature 920 °C, temperature increase 80 °C min), according to the manufacturer's instructions. Three types of



(a) (b) (c) (d)

Figure 1: SEM microphotographs of (a) control ceramic specimen, (b) ceramic composite specimen (CHN) with chitin scaffold, (c) ceramic composite specimen (CHS) with chitosan scaffold (d) and ceramic composite specimen (CH-G) with chitosan-gelatin scaffold.

scaffolds were fabricated—through the method of particular leaching with non water soluble particles—on the surface of ceramic disks, made of chitin (CHN), chitosan (CHS) and chitosan-gelatin (CH-G), with pore size 70–200 μm. Chitin was dissolved in n,n dimthylacetamide (DMA) + 6% LiCl mixture. Chitosan and chitosan/gelatin were dissolved in 2% acetic acid aqueous solution. The solutions were mixed up with PMMA particles. The chitin solution was left 1 day at room temperature for gelation. The chitosan and chitosan/gelatin solutions were cross-linked with glutaraldehyde vapor. The obtained gels were washed with water and methanol and dried. Afterwards, they were immersed in dichloromethane to leach PMMA particles. After drying, the polymer scaffolds were obtained. One specimen of each group was carbon-coated for Scanning Electron Microscopy (SEM) observations with a Jeol microscope (J.S.M. 840A, Tokyo, Japan). The pore size distributions were obtained after image analysis of the SEM microphotographs with the appropriate software (ImageJ 1.32j). Four composite ceramic-scaffolds (CCSs) of each type were constructed. One CCS from each group served as a control, while ceramic disks were used as negative controls. A total of 12 specimens (CCS) and 4 negative controls were constructed and the experiments were performed in triplicate. All specimens were incubated at 37 °C up to 15 days on well plates, with culture medium (DMEM supplemented with 10% FCS) in the presence of 10^5 cells PDLF. Cells used in all in vitro experiments were from a primary culture developed from pieces of periodontal ligament obtained from extracted human third molars [13]. Informed consent was obtained from all patients under a protocol approved by the Ethical Committee of the Aristotle University of Thessaloniki, Greece. Cells were obtained as described by Somerman et al. [13]. In all experiments, cells from passages 5th–11th were used. To evaluate the cell proliferation, the MTS technique (Aqueous Non-Radioactive Cell Proliferation Assay, Promega) was used. A tetrazolium compound MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) in the presence of phenazine metosulfate (PMS) is reduced by living cells to yield a formazan product at 37 °C, that can be assayed colometrically [10]. This product is observed at cell surface or at the level of the plasma membrane via trans-plasma membrane electron transport [3]. It is reported that this method allows the investigation of cells' proliferation rate in one specimen at different times [2,3]. Cell proliferation was evaluated at 1, 3, 7, 10 and 15 days. Finally, optical density (OD) was measured at a test wavelength of 490 nm and a reference wavelength of 630 nm.

3 Results and discussion

Observation of SEM microphotographs revealed that control ceramic exhibited a relatively smooth surface with some elevations and depressions (Figure 1(a)). Although bioactive glass particles are not distinguishable, analysis of elemental composition (EDS analysis) revealed the existence of Al, Si and P on the surface of modified ceramic discs. SEM microphotographs were also used to observe the surface morphology and the pore structure of the composite scaffolds. For the fabrication of the scaffolds, water-insoluble particles were used instead of salt (water-soluble) particles and solvent removal was made by bad-solvent exchange [14].

As a result, the method of leaching water non-soluble particulates resulted in pore structures with high surface porosity as shown in Figures 1(b)–(d). They appeared to be macro porous with high interconnectivity. Distribution of pores seemed to be more uniform in case of CHS composite ceramic-scaffolds (Figure 1(c)).

The optical density values of the PDLF cultured on the surfaces of all specimens (control specimens and composite ceramic-scaffolds) are presented in Figure 2.

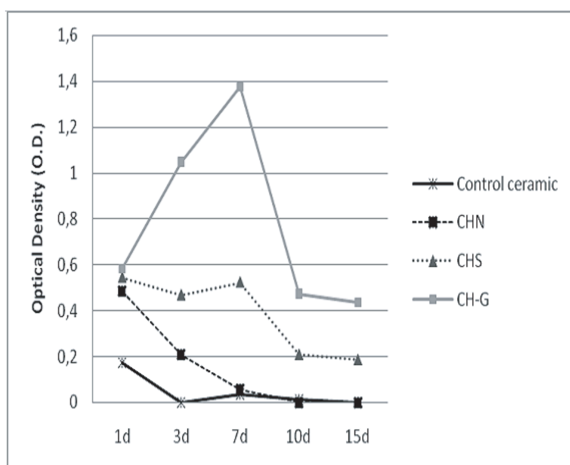


Figure 2: Optical Density values of control ceramic specimens, ceramic specimen (CHN) with chitin scaffold, ceramic specimen (CHS) with chitosan scaffold and ceramic specimen (CH-G) with chitosan-gelatin scaffold.

MTS assay OD values of day 1 confirm the presence of cells at all scaffolds suggesting a successful loading of cells. OD values were higher, in case of the examined ceramic composite scaffolds compared to the negative controls. Although the initial loading was almost equal for the three tested groups, variation of OD values confirmed differences of cell proliferation among the examined specimens. More specific the inability of chitin scaffolds to enhance cell proliferation agrees with literature evidences, without organic modification with substances such as collagen or gelatin [15].

Cell death was observed in case of CHN. More specific, the number of attached cells seemed to be progressively decreasing, during the whole experiment. On the contrary, cell population was almost stable in case of CHS, until day 7 and then a decreasing of cell population began. Finally, the proliferation rate on CH-G presented an increase initially and a peak at day 7. A rapid decrease of cell population was observed, until day 10. Then cell population seemed to attend a constant rate. At negative controls, cells were progressively decreased. OD values confirmed the inability of dental ceramics to support the cell proliferation.

4 Conclusions

The produced scaffolds exhibited high interconnectivity. OD values indicate that all 3 types of the examined composites ceramic-scaffolds were successfully loaded with PDLF cells. However, only in CH-G composite ceramic-scaffolds the human PDLF cells proliferated, then decreased and finally remained stable, while in case of CHN and CHS the number of cells was progressively decreasing. They seem to benefit compared with the other examined specimens. Besides, scaffolds of chitosan-gelatin are suggested for tissue engineering [16], while chitosancollagen scaffolds had already been used for periodontal tissue engineering, with quite positive results [11]. However, SEM microphotographs as well as, cross section analysis should follow to investigate cell development, especially in case of the examined chitosan gelatin composite ceramic scaffolds. It is supported that the degradation of such scaffolds is based on the alteration of the chemical structure of the polymer [5, 12]. Such alterations may lead to changes in other properties of scaffolds, such as the ability to promote cell adhesion [12]. Sedimentation of DMEM's ingredients could affect surface's porosity and consequently cell proliferation and development. Besides, it is reported that cell's function products affect scaffolds morphology and eventually furtherer cell proliferation and development [5]. Furthermore, it is supported that bioreactors and growth factors have an important role in tissue engineering. It is already established that the promotion of cell proliferation as well as the expression of type I collagen is achieved through reinforcement of scaffolds with growth factors, such as Ad-TGF –b1 [17]. Such substances were not used in this experimental model. So, scaffold degradation products, as well as the absence of bioreactors could have probably restricted the cell proliferation in this experiment. Under the limitations of this in vitro study it can be concluded that the successful observed cellular response at chitosan-gelatin scaffolds attached on dental ceramics could support the development of a protocol for tissue engineering on the materials used.

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References

[1] I. F. Amaral, P. Sampaio, and M. A. Barbosa, *Three-dimensional culture of human osteoblastic cells in chitosan sponges: the effect of the degree of acetylation*, J Biomed Mater Res A, 76 (2006), pp. 335–46.

[2] J. A. Barltrop, T. C. Owen, A. H. Cory, and J. G. Cory, *5-(3-carboxylmethoxyphenyl)-2-(4,5-dimethylthiazolyl)-3-(4-sulfophenyl) tetrazolium, inner salt (mts) and related analogs of 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (mtt) reducing to purple water-soluble formazans as cell-viability indicators*, Bioorg. Med. Chem. Lett, 1 (1991), pp. 611–614.

[3] M. V. Beridge, P. M. Herst, and A. S. Tan, *Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction*, Biotechnol Ann Rev, 11 (2005), pp. 127–152.

[4] D. Z. Cai, C. Zeng, D. P. Quan, L. S. Bu, K. Wang, H. D. Lu, and X. F. Li, *Biodegradable chitosan scaffolds containing microspheres as carriers for controlled transforming growth factor-beta1 delivery for cartilage tissue engineering*, Chin Med J (Engl), 120 (2007), pp. 197–203.

[5] C. Cunha-Reis, K. TuzlaKoglu, E. Baas, and Y. Yang, *Influence of porosity and fibre diameter on the degradation of chitosan fibre-mesh scaffolds and cell adhesion*, J Mater Sci Mater Med, 18 (2007), pp. 195–200.

[6] A. El-Ghannam, P. Ducheyne, and I. M. Shapiro, *Formation of surface reaction products on bioactive glass and their effects on the expression of the osteoblastic phenotype and the deposition of mineralized extracellular matrix*, Biomaterials, 18 (1997), pp. 295–303.

[7] A. Fakhryl, G. Schneider, R. Zaharias, and S. Senel, *Chitosan supports the initial attachment and spreading of osteoblasts preferentially over fibroblasts*, Biomaterials, 25 (2004), pp. 2075–2079.

[8] E. Kontonasaki, L. Papadopoulou, T. Zorba, E. Pavlidou, K. Paraskevopoulos, and P. Koidis, *Apatite formation on dental ceramics modified by a bioactive glass*, J Oral Rehabil, 30 (2003), pp. 893–902.

[9] E. Kontonasaki, A. Sivropoulou, L. Papadopoulou, P. Garefis, K. Paraskevopoulos, and P. Koidis, *Attachment and proliferation of human periodontal ligament fibroblasts on bioactive glass modified ceramics*, J Oral Rehabil, 34 (2007), pp. 57–67.

[10] G. Malich, B. Markovic, and C. Winder, *The sensitivity and specificity of the MTS tetrazolium assay for detecting the in vitro cytotoxicity of 20 chemicals using human cell lines*, Toxicology, 124 (1997), pp. 179–192.

[11] L. Peng and R.-X. Zhuo, *Biological evaluation of porous chitosan/collagen scaffolds for periodontal tissue engineering*, in 2nd International Conference on Bioinformatics and Biomedical Engineering, Shanghai, 2008, pp. 897–900.

[12] H. Shin, K. Zygourakis, M. Farach-Carson, M. Yaszemski, and A. G. Mikos, *Attachment, proliferation, and migration of marrow stromal osteoblasts cultured on biomimetic hydrogels modified with an osteopontin-derived peptide*, Biomaterials, 25 (2004), pp. 895–906.

[13] M. J. Somerman, S. Y. Archer, G. R. Imm, and R. A. Foster, *A comparative study of human periodontal ligament cells and gingival fibroblasts in vitro*, J Dent Res, 67 (1988), pp. 66–70.

[14] C. Tsioptsias, I. Tsivintzelis, L. Papadopoulou, and C. Panayotou, *A novel method for producing tissue engineering scaffolds from chitin, chitin-hydroxyapatite, and cellulose*, Mater Sci Eng C, 29 (2009), pp. 159–164.

[15] M. Wang, L. J. Chen, J. Ni, J. Weng, and C. Y. Yue, *Manufacture and evaluation of bioactive and biodegredable materials and scaffolds for tissue engineering*, J Mater Sci Mater Med, 12 (2001), pp. 855–860.

[16] A. Xiaoyan, Y. Jun, W. Min, H. Zhang, C. Li, Y. Kangde, and Y. Fanglian, *Preparation of chitosangelatin scaffold containing tetrandrine-loaded nano-aggregates and its controlled release behavior*, Int J Pharm, 350 (2008), pp. 257–264.

[17] Y. Zhang, X. Cheng, J. Wang, B. Wang, Y. Shi, C. Huang, et al., *Novel chitosan/collagen scaffold containing transforming growth factor-β1 DNA for periodontal tissue engineering*, Biochem Biophys Res Commun, 344 (2006), pp. 362–369.