Key Engineering Materials Vols. 396-398 (2009) pp 633-636
online at http://www.scientific.net

A NOVEL BONE SCAFFOLDS BASED ON HYPERBRANCHED POLYGLYCEROL FIBERS FILLED WITH HYDROXYAPATITE NANOPARTICLES: IN VITRO CELL RESPONSE

Alvaro Antonio Alencar de Queiroz[1,a], José Carlos Bressiani[2,b], Ana Helena Bressiani[2,c], Olga Zazuco Higa[3,d], Gustavo Abel Abraham[4,e]

[1]Departamento de Física e Química, Instituto de Ciências Exatas, Universidade Federal de Itajubá (UNIFEI). Av. BPS, 1303, 37,500-903, Itajubá-MG, Brasil.

[2]Centro de Ciência e Tecnologia de Materiais, Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN). Av. Prof. Lineu Prestes, 2242, 05508-000, São Paulo-SP, Brasil.

[3]Centro de Biotecnologia, Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN). Av. Prof. Lineu Prestes, 2242, 05508-000, São Paulo-SP, Brasil

[4]Instituto de Investigaciones em Ciência y Tecnologia de Materiales (INTEMA, UNMdP-CONICET). J.B. Justo 4302, B78608FDQ, Mar del Plata, Argentina.

[a]alencar@unifei.edu.br, [b]jbressia@ipen.br, [c]abressia@ipen.br, [d]ozahiga@ipen.br, [e]gabraham@fi.mdp.edu.ar

Keywords: Hyperbranched polyglycerol, Electrospun, Scaffolds, Hydroxyapatite, Tissue engineering, Hydrogels.

Abstract. A novel bone scaffolding material was successfully fabricated by electrospinning from hyperbranched polyglycerol (HPGL) solutions containing nanoparticles of hydroxyapatite (HA). The potential use of the electrospun fibrous HPGL-HA scaffolds for bone regeneration was evaluated in vitro with human osteoblasts in terms of alkaline phosphatase (ALP) activity of the cells that were cultured directly on the scaffolds. The results were compared with those on corresponding HPLG-HA solution-cast film scaffolds. It was found that all of the fibrous scaffolds promoted much better adhesion and proliferation of cells than the corresponding film scaffolds.

Introduction

The use of electrospun nanofiber structures, using natural or synthetic biodegradable polymers, has drawn increased interest for use as scaffolds in tissue engineering [1]. The three-dimensional interconnected pore networks of electrospun nanofibers generate structures that resemble native extra-cellular matrix (ECM) elements that enhance cell attachment and proliferation [2]. Due to their very similar biophysical properties to soft tissues for their high water content, hydrogels are of particular interest for such scaffolds because of their ability to absorb and retain water [3]. In this sense, composites based on hydrogels and hydroxiapatite have been previously investigated for orthopedic applications [4].

Recently, hyperbranched polyglycerol (HPGL) gels have evoked much interest of biomaterials scientists because of their topology, which can lead to molecular capsule and compartment for guest molecules or particles [5]. These polyglycerol hydrogels can potentially act as orthopedic adhesives, via their swelling ability, and could encourage bone infiltration and formation through its mesh structure. Although electrospinning is a widespread method of producing tissue-engineering scaffolds, little is known regarding the biological properties of the hyperbranched polyglycerol-Hydroxiapatite (HPGL-HA) scaffolds.

The aim of this work was to study the *in vitro* biological properties of HPLG-HA fibers obtained by electrospinning technique to produce networks of hydrogel-HA fibers with potential for use as scaffolds in tissue engineering applications.

Materials and Methods

The HPGL was prepared by the anionic ring-opening multibranching polymerization (ROMP) of glicidol [6]. The hydroxyl groups of HPGL were derivatized with methacrylate groups after reaction with glycidyl methacrylate (GMA) for the covalent crosslinking of the HPGL chains and formation of the hydrogel [7]. A HPLG with GMA degree substitution of 10% (w/w) was attained in the derivatization reaction. Hydroxyapatite nanoparticles were synthesized following a method proposed recently by Bressiani et al [8]. The mean particle size of the HA nanocristals as analyzed by a Phillips XL30 scanning electron microscopy (SEM) was 230 $\pm$ 80 nm.

HPGL-HA fibrous scaffolds were prepared by electrospinning from neat 10% w/v HPGL solution in 50:50 v/v dichloromethane and DMF loaded with HA powder at a concentration of 1.0% w/v. In order to ensure good dispersion of the particles within the HPGL solution, the particles and were first dispersed under mechanical stirring in DMF. After a certain period of time was added. The mixture was stirred until the complete disolution and it was subsequently sonicated prior to electrospinning. The spinning solution was contained in a glass syringe, the opening end of which was connected to a gauge 20 stainless steel needle (outside diameter = 0.91 mm) used as the nozzle. Then the electrospun was made using 8-10 kV applied to the needle tip and the grounded collector was placed at 20 cm tip-to- substrate distance and a 20 mL/hr flow rate under UV radiation (200 W UV lamp) for crosslinking by photopolymerization of GMA groups presents in HPGL-HA composite fibers. A 4x4" (10.2 x 10.2 cm) sheet approximately 0.5 mm in thickness was deposited onto aluminum foil. Surface morphologies of the electrospun scaffolds were characterized using scanning electron microscopy (SEM, Phillips XL 30).

The swelling of crosslinked HPGL-HA fibrous membrane was done after immersing the scaffold in phosphate saline buffer (PBS) 0.1 M and pH 7.2 solution at 37 ºC for 24 h. The swelling ratio (Q) was calculated gravimetrically and was defined as the ratio between the weight of the gel at time t (W_t) and its initial weight (W_o).

A cytotoxicity assay was performed by adding dilutions of HPLG-HA fibers extracts to a CHO cell culture on a Petri plate (15 x 60 mm^2) [9]. The positive and negative controls were a 0.02 vol.% phenol solution and ultra-high molecular weight polyethylene (UHMWPE), respectively. The cytotoxic potential of the material was expressed by an index of cytotoxicity, IC_{50} (%), which represents the concentration of the extract that suppresses the formation of cell colonies by 50% in comparison with the control.

The potential use of the electrospun fibrous scaffolds for bone regeneration was evaluated *in vitro* with human osteoblasts (SaOS2) in terms of alkaline phosphatase (ALP) activity of the cells that were cultured directly on the scaffolds. SaOS2 were cultured on scaffold specimens for 10 days to observe the production of alkaline phosphatase (ALP) in according to methodology of Cusack et al [10].

Results and Discussion

In this work we have reported the biological evaluations of a new scaffold based on hyperbranched polyglycerol fibers filled with hydroxyapatite nanoparticles. The macromolecules are arranged in such a way to obtain a 3-D configuration and the material results elastic and flexible. The morphology of the electrospun HPGL-HA was performed using scanning electron microscopy technique (SEM) and the results is shown in Fig. 1-A. The SEM micrographs (Fig. 1-A) showed a uniform fibrous scaffold with thicker and smoother fibers.

The swelling behavior of the HPGL with HA content of 0.5 to 5% is shown in Fig. 1-B. It was observed that with increasing HA content the HPGL fibers swelled less indicating the formation of an increased contribution of a rigid network and consequently the occurrence of more intermolecular cross links. Additionally, the high swelling ratio observed for the fibrous HPGL-HA scaffold may be due to the very high surface area-to-volume and the high porosity of electrospun fibrous structure that could promote a better water interaction relatively to the casting HPGL-HA films.

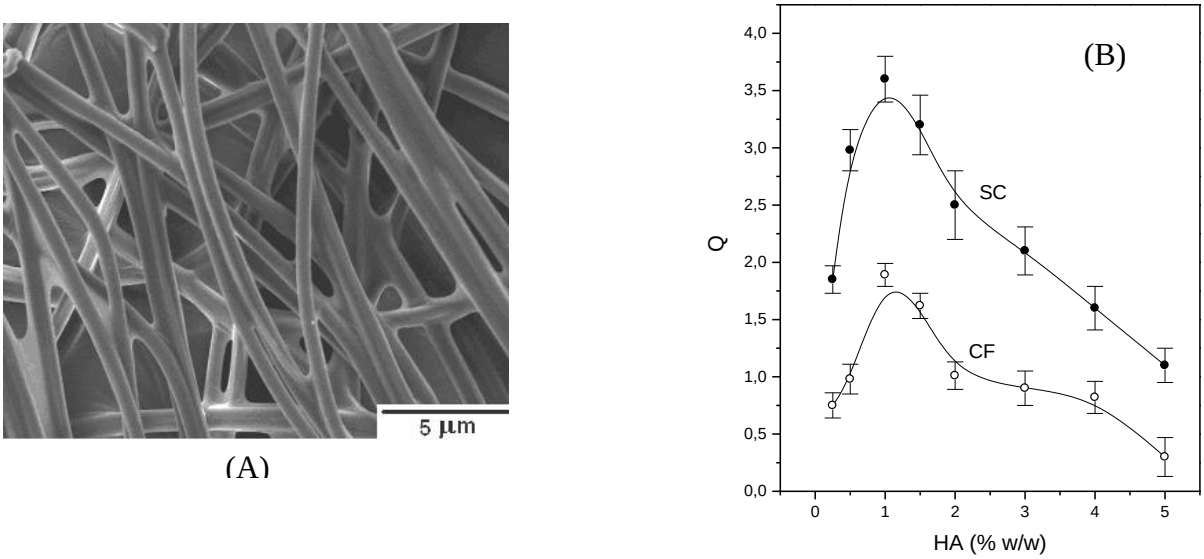

Fig. 1- SEM micrograph (A) and swelling behavior (B) of the HPLG-HA electrospun fibers. SC and CF are fibrous scaffold and casting HPLG-HA films, respectively.

The *in vitro* HPLG-HA cytotoxicity results are shown in Fig. 2-A. The biocompatibility evaluation of the HPLG-HA provided encouraging indications for the long-term safety. In fact, in the cytotoxicity study the material extracts did not induce toxic effects on the CHO cells showing high cell viability. The extracts induced neither cell viability reduction nor inhibition of cell growth resulting to have no toxic effects.

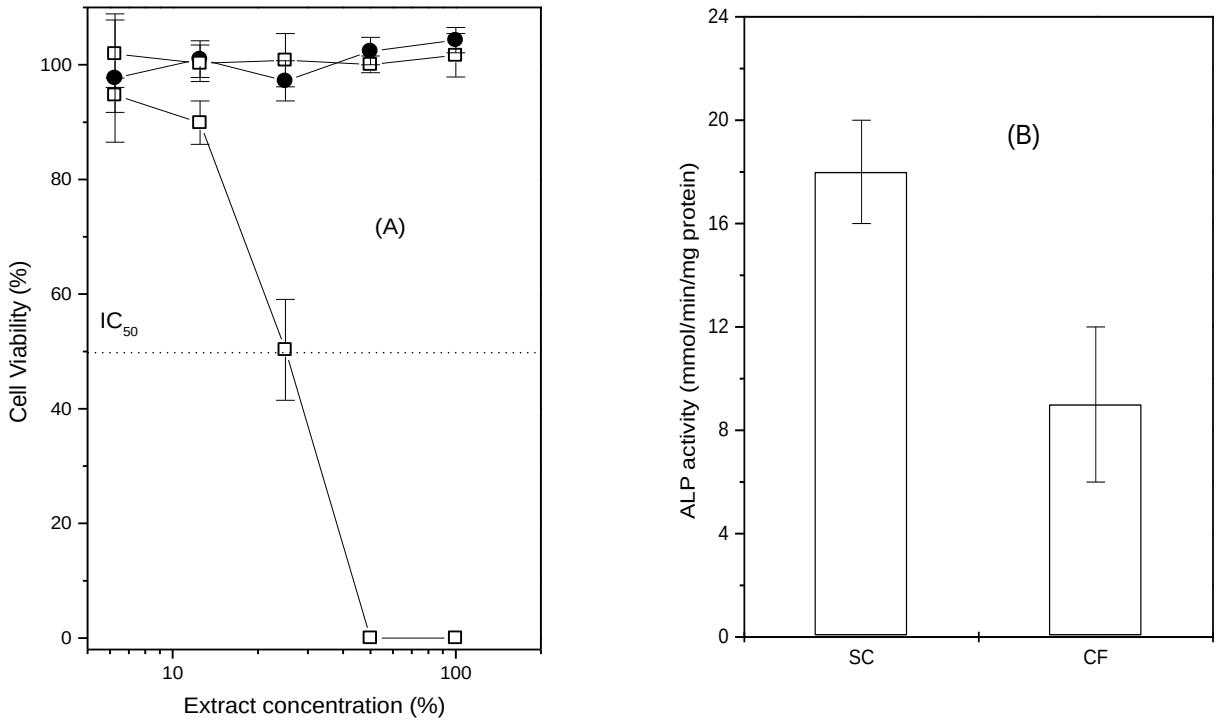

Fig. 2- Cytotoxicity (A) and ALP activity (B) of the HPGL-HA electrospun fibers. Negative control (•), and positive control (□) against Chinese hamster ovary (CHO) cells. SC and CF are fibrous scaffold and casting HPLG-HA films, respectively.

The cellular differentiation is an important property of a synthetic material candidate to scaffold. In this sense the secretion of alkaline phosphatase (ALP) is an important indicator of the cellular activity on a scaffold [11]. The potential use of the HPGL-HA composite fibers as scaffolding materials for bone regeneration was evaluated in vitro with human osteoblasts (SaOS2), in which the ALP activity on HPGL-HA scaffold was monitored at 10 days in culture (Fig. 2-B). Figure 2-B shows that HPGL-HA electrospun fibers exhibit highest ALP activity and appears to promote a better both proliferation and differentiation of SaSO2 relatively to the casting HPLG-HA films.

Conclusions

The *in vitro* citotoxicity and alkaline phosphatase (ALP) activity were performed for the biocompatibility evaluation of HPLG-HA hydrogel nanofibers. None of the tests examined indicated HPLG-HA toxicity. The biocompatibility of HPLG-HA could make this material suitable for tissue engineering. Further studies are in progress to verify the physicochemical and mechanical properties of the HPLG-HA and implant tolerance for more prolonged periods and additional experiments to clarify the effect of HPGL-HA scaffolds on the differentiation of osteoblasts are currently underway and will be published in the near future.

Acknowledgments

The authors gratefully acknowledge to Finep, CNPq and Fapemig for the financial support.

References

[1] H. Yashimoto, Y.M. Shin, H. Terai and J.P. Vacanti: Biomaterials Vol. 24 (2003), p. 2077.
[2] C.Y. Xu, R. Inai, M. Kotaki, S. Ramakrishna: Biomaterials Vol. 25 (2004), p. 877.
[3] S. Dunitriu: *Polymeric Biomaterials: Second Edition* (Marcel Deckker, New York 2002).
[4] F.Y. Hsu, S.C. Chueh and Y. J. Wang: Biomaterials Vol. 20 (1999), p. 1931.
[5] A. Sunder, M. Kramer, R. Hanselmann, R. Mulhaupt and H. Frey: Angew. Chem. Int. Ed. Engl. Vol. 38 (1999), p. 2928.
[6] S.E. Stiriba, H. Kautz and H. Frey: J.Am.Chem.Soc. Vol. 122 (2000), p. 2954.
[7] W.N.E.van Dijk-Wolthuis, J.J.K. van den Bosch, A. van der Kerk-van Hoof and W.E. Hennink: Macromolecules Vol. 30 (1997), p. 3411.
[8] D.S. Gouveia, A.C.S. Coutinho, A.H.A. Bressiani and J.C. Bressiani: Key Materials Eng. Vol. 361 (2008), p. 203.
[9] ISO 10993-5. Biological evaluation for medical devices tests for cytotoxicity in vitro methods international standards organization. Geneva: ISO; 1999.
[10] S. Cusack, C. Jewell and K.D. Cashman: Prostaglandins Leukot. Essent. Fatty Acids Vol. 72 (2005), p. 29.