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Influence of Porosity Upon Cells Adhesion on Polyhydroxyalkanoates Films

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Abstract: The surface property of a biomaterial is known to affect its cell compatibility considerably, which is of particular importance for a medical implant, because the surface contacts directly with the host tissue. A suitable morphology on the implant's surface serves to guide cell adhesion, migration, and proliferation, and is a key to success in tissue engineering. Various medical applications of polyhydroxyalkanoates (PHAs), biodegradable and biocompatible materials have been reported, including tissue engineering scaffolds, patches for use in cardiovascular surgery, and other implants. Films used as tissue engineering scaffolds must have several important performance features, including sufficient mechanical strength, biocompatibility, biodegradability and the ability to support cell attachment and proliferation. Here, we report efforts to develop novel materials based on porous polyhydroxyalkanoates (PHAs) with biological origin as tissue engineering scaffold for living cells. The experimental conditions to obtain porous films were established. Porous polyester films were prepared by deposition from solutions with different porosity agents. The morphology and properties of the polymeric films were investigated by optical microscopy (OM), scanning electronic microscopy (SEM) and contact angle measurements (CA). The influence of the porosity upon the adherence of the living cells and the level of cytotoxicity of the polyester films were evaluated by *in vitro* assays.

Keywords: PHA, porosity, cells adhesion, cytotoxicity

1. Introduction

Along with polyisoprenoids, polypeptides, polysaccharides, and polynucleotides, Nature contains a further group of biopolymers, poly(hydroxya1kanoates). They are polyesters of hydroxyacids synthesized within the cells of microorganisms in the presence of saccharide and oil substrates [1-4]. Polyhydroxyalkanoates (PHAs) are naturally derived polyesters that accumulate as a carbon storage material in a wide variety of bacteria, usually under conditions of limiting nutrients (such as ammonium, sulphate, and phosphate) in the presence of an excess carbon source. An imbalanced nutrient supply leads to intracellular storage of excess nutrients. By polymerizing soluble intermediates into insoluble molecules, cells do not undergo alterations of their osmotic state and leakage of nutrients is prevented. Accumulated PHAs form discrete granules that can account for up to 90% of the cell's dry weight. Up to date, approximately 150 hydroxyalkanoate units with different R-pendant groups have been isolated from bacteria [4-8].

Poly(3-hydroxyalkanoates), which are synthesized by a wide variety of microorganisms, have attracted a great deal of industrial attention because of their potential applications as biodegradable and biocompatible thermoplastic polymers [9-11]. In particular, much interest has focused on the use of poly(3-hydroxybutyrate) (P3HB) and related copolymers with 3-hydroxyvalerate. Poly(3-hydroxybutyrate), P3HB, is the simplest and most common member of the group of PHAs. Discovered by Lemoigne in the 1920s, its commercial evaluation did not start until the late 1950s [5]. The potential of P3HB for biomedical applications was first suggested in a 1962 patent, which presented the ideas of biodegradable surgical sutures and of films to support tissue healing of injured arteries and blood vessels [4-6, 12-14].

PHAs are biocompatible, so they are potential scaffolds for the growth and proliferation of cells for partial or permanent replacement of living tissues.

The present paper work focuses on the obtaining of polyhydroxyalkanoates films based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with controlled porosity and the evaluation of physical and biological properties for medical uses.

2. Experimental

2.1 Materials

Natural poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV8%) copolymer was kindly provided by INCERPLAST S.A.

PHAs films were prepared by casting from chloroform solution (5% w/v) and then dried a room temperature to allow solvent evaporation. The film thickness was between 30-50 μm.

PHAs porous structures were obtained with sodium chloride (Fluka), gelatine B, sodium alginate and sugar. Gelatine B was obtained by the alkaline treatment of porcine collagen was supplied from PB Gelatines GmbH, member of Tessenderlo Group, Germany and it conforms to the requirements of Pharmacopoeia Europea. Gelatine used in this study has an isoelectric point of 4.7-5.6 and Bloom strength of 250. The viscosity of a 6.67% (w/v) solution at 60°C is 4.62 mPas and the pH at 45°C is 5.73. Low viscosity sodium alginate (SA) rich in α-L-guluronic residues (approx. 70 % of G-block content) was purchased from Medipol SA (Lausanne, Switzerland).

2.2 Methods

The morphology of the specimens was assessed by SEM and optical microscopy. The SEM device was a Zeiss Evo 50 XVP Scanning Electron Microscope. Prior to imaging the samples were sputtered with a thin layer of gold. The optical images were achieved with an Olympus BX41 Microscope equipped with Live view digital SLR camera E-330 (7.5 Mpxl) and special software Quick Photo Micro 2.3.

KSV CAM 200 apparatus was used for static contact angle measurements performed on dried films. Ultrapure water droplets were used with a drop volume of 20 µl. The measurement of each contact angle was made within 10 s after each drop to ensure that the droplet did not soak into the compact. The contact angles reported were the mean of 10 determinations. Smaller contact angles correspond to increased wettability.

2.3. Biocompatibility tests

The biocompatibility of materials was assessed by using L929 mouse fibroblast cells monolayers grown on polymer films covering approximately 90% of the well surfaces in 24-well cell culture plates. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotics (penicillin and streptomycin) at 37ºC in a humidified incubator with 5% CO_2. After 24 h incubation, polymer films were removed and the number of cells grown both on their surface and adjacent well bottom were measured using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]-based cell viability test. This assay is based on the ability of dehydrogenase enzymes from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form insoluble dark blue formazan crystals. Cell lysis and crystals solubilization was performed according to described method [15]. The absorbance at 540 nm is directly proportional to the number of viable cells. Finally, the cells were microscopically examined for detecting cytotoxicity visible signs, cellular lysis or cellular components dimensions and conformation (optical microscopy, ZEISS - Axiovert 135 Microscope). A cellular viability test (MTT) was also employed and the number of cells grown on polymer films was calculated relative to an equivalent area of cells culture plastic.

3. Results and Discussion

The non-porous PHBHV films have variable thickness (30-50 µm). Fig. 1 shows SEM and optical images of these polyester films with clear non-porous morphology.

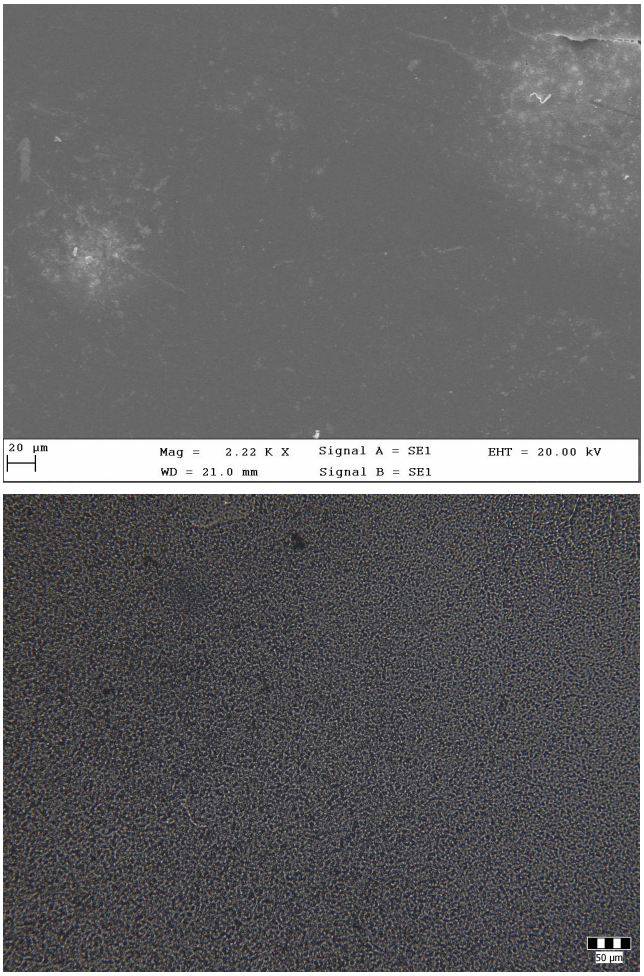

Figure 1. SEM microphotograph and optical microphotographs OM (20x objective) image of PHBHV film

The high values of the contact angles of the specimens (~95°) prove the hydrophobic character of these materials.

The porosity was induced by various porosity agents for a higher cell adherence of the polyester films according to their nature and granulometry.

The SEM and OM images of some porous structures are presented in figure 2.

Pore dimensions lie between 1-50 µm for most of the porogen agents, except gelatine, which induces higher pores (200-300 µm). The pore size distribution is presented in table 1. Lower pore size is obtained with NaCl and alginate.

TABLE 1. Pore size measurements

	Distilled water	Sugar	Sodium chloride	Gelatine	Alginate
Pore size, µm	< 1	2-50	5-25	200-300	3-20

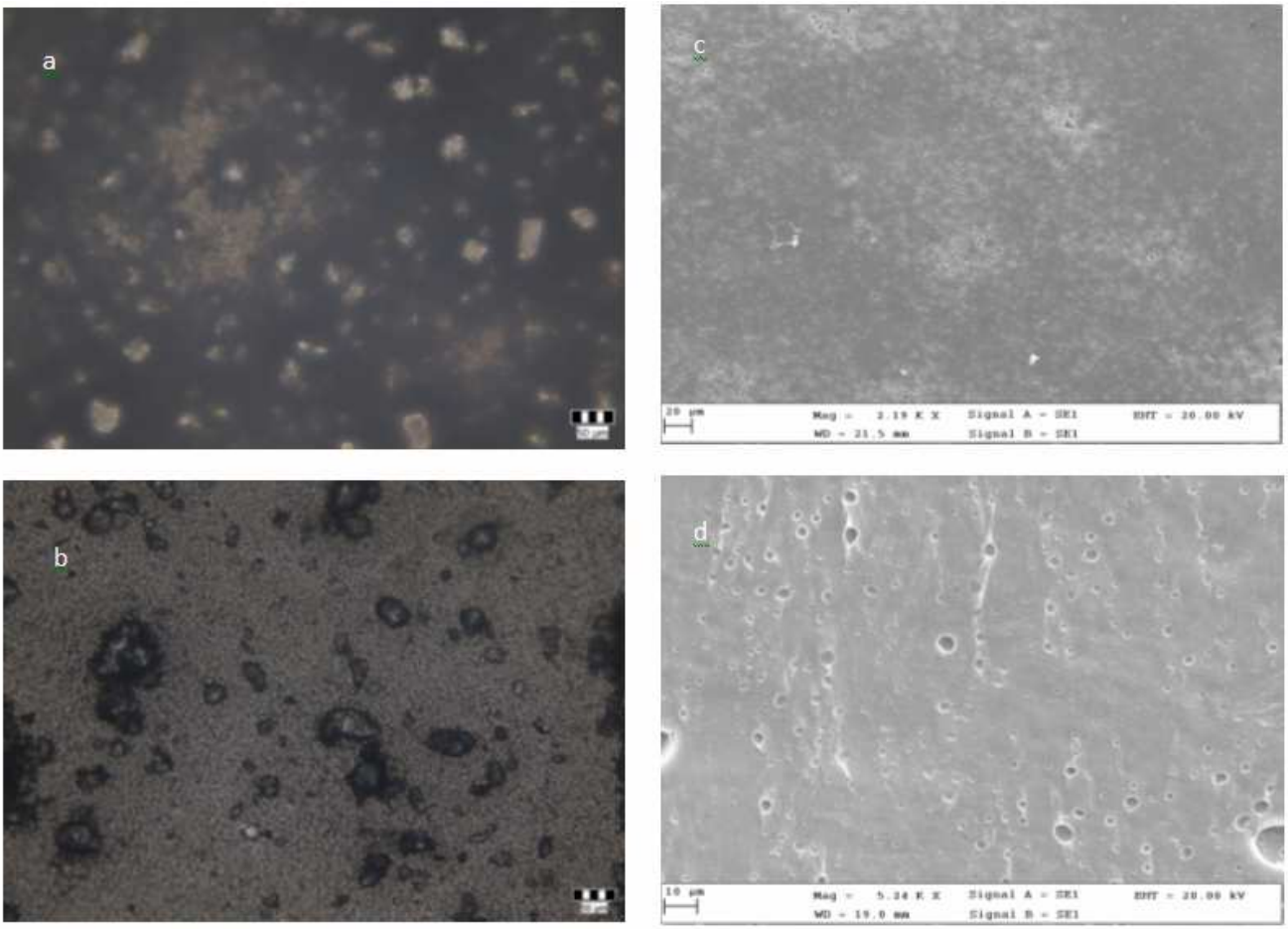

Figure 2. Optical microphotographs OM and SEM microphotograph image of PHBHV porous films: a, c – sodium chloride; b, d – alginate

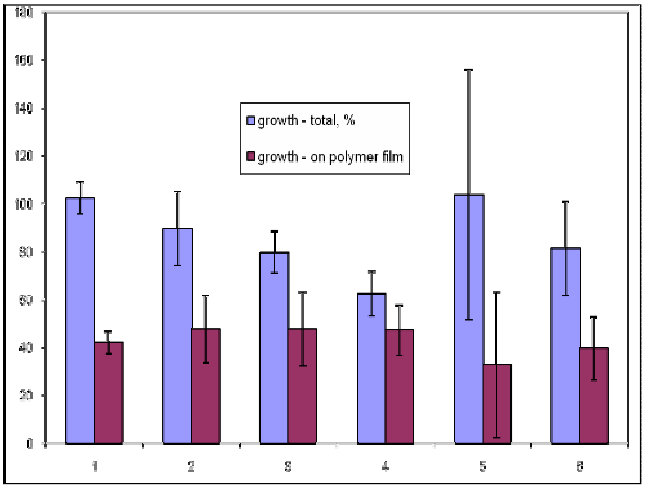

Figure 3. Cells growth on polymer porous and nonporous films: 1 – gelatine; 2 – sugar; 3 – PHBHV without porogen agent; 4 – sodium chloride; 5 - distilled water; 6 – alginate

As we have already mentioned the cytotoxicity of the PHBHV films was assessed on L929 murine cell line. It is known that porous structures lead to a better cell adhesion of the polymeric materials. Figure 3 shows the adherence of the cells onto the PHBHV films and control sample (polystyrene - cells culture plastic) and the image of cells growth on a blank test, respectively on polymer porous film

The porous PHBHV films do not induce a higher cell adhesion as compared to non-porous films. In most of the cases the cytotoxicity was a little bit increased. The porous materials obtained with NaCl do not have significant cytotoxicity effects.

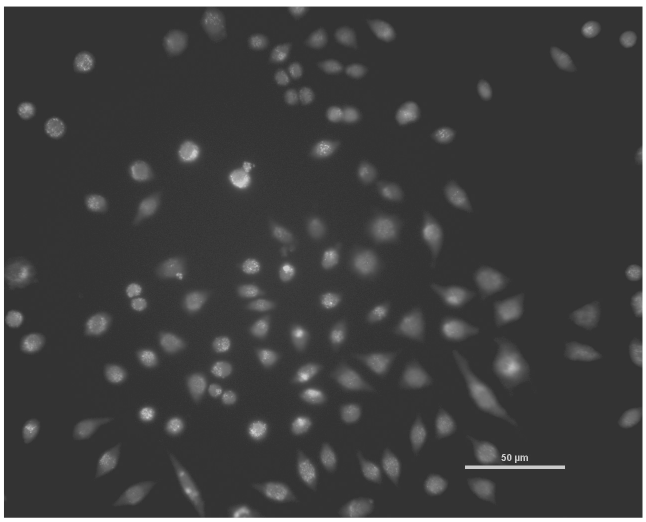

Figure 4a. Optical microphotographs OM cells cultured grown on blank test;

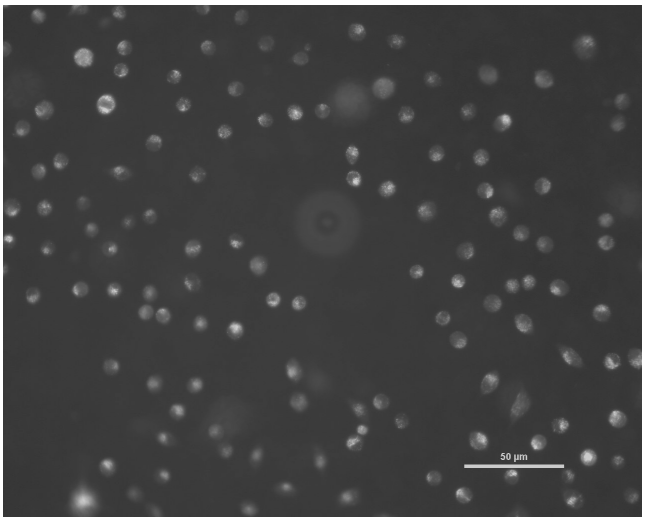

Figure 4b. Optical microphotographs OM cells cultured grown onPHBHV porous films

4. Conclusions

The PHBHV8% films have a hydrophobic character resulting from the high value of the contact angle.

The best porosity was achieved with sodium chloride, but this porous film has an increased cytotoxicity. The idea that the porous structures favour a higher cell adhesion is not sustained by our results with porous polyester films. The optimum cell adhesion was obtained with porous films with gelatine as porogen.

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REFERENCES

1. Müller H. M. and Seebach D., *Angew. Chem. Int. Ed. English*, 32 (4), **1993**, 477-502.
2. Pompe. T., Keller K., Mothes G., Nitschke M., Teese M., Zimmermann R. and Werner C., *Biomaterials*, 28, **2007**, 28–37.
3. Kim J.S., Lee B.H. and Kim B.S., *Biochem. Eng. J*., 23 (2), **2005**, 169-174
4. Freiera T., Kunzea C., Nischana C., Kramera S., Saß K.M., Hoptb U.T. and Schmitza K.P., *Biomaterials*, 23, **2002**, 2649–2657.
5. Freier T., *Adv. Polym. Sci*., 203, **2006**, 1-61.
6. Williams S. F., and Martin D. P., Applications of Polyhydroxyalkanoates (PHA) in Medicine and Pharmacy, Wiley-VCH Verlag GmbH & Co. KGaAm, **2005**.
7. Peschel G., Dahse H.-M., Konrad A., Wieland G. D., Mueller P.-J., Martin D. P. and Roth M., *J. Biomed Mater. Res. A*., 85(4), **2008**, 1072-1081.
8. Sun M., Zhou P., Pan L.F., Lui S. and Yang, H.X., *J. Mater. Sci.: Mater. Med*, 20, **2009**, 1743-1751.
9. Shishatskaya E.I., Voinova O.N., Goreva A.V., Mogilnaya O.A. and Volova T.G., *J. of Mat. Sci.: Mat. in Medicine*, 19(6), **2008**, 2493-2502.
10. Hu S.G., Jou C.H. and Yang M.C., *Biomaterials*, 23, **2003**, 2685-2693
11. Hua Y.J., Zhao X., W., Liu Y.S. and Chen G.Q., *Acta Biomaterialia*, 5, **2009**, 1115–1125.
12. Federov M.B., Vikhoreva G.A., Kildeeva N.R., Borisova Y.K., Vinokurova T.I., Bonartseva G.A. and Galbraikh L.S., *Fibre Chemistry*, 38 (6), **2006**, 471-475.
13. Rezwana K., Chena Q.Z., Blakera J.J. and Boccaccini A.R., *Biomaterials*, 27, **2006**, 3413–3431.
14. Kroeze R.J., Helder M.N., Govaert L.E. and Smit T.H., *Materials*, 2, **2009**, 833-856.
15. Zecheru T., Zaharia C., Salageanu A., Tucureanu C., Rusen E., Marculescu B., Rotariu T. and Cincu C., *J. Optoelectr. Adv. Mat.*, 9(9), **2007**, 2917-2920.

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