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Porous and dense poly(L-lactic acid) membranes: *in vitro* degradation

S. H. Barbanti ; C. A. C. Zavaglia and E. A. R. Duek
Faculty of Mechanical Engineering, Department of Materials Engineering,
State University of Campinas, P.O. Box 6122, CEP 13083-970 Campinas, SP, Brazil,
E-mail: samuel@fem.unicamp.br.

Abstract

Absorbable polyesters implants, such as poly(L-lactic acid), have been used in various fields of medicine. The understanding of morphologic characteristics of the material and the interaction material-tissue is basic for the success of the implant. In this research the degradation *in vitro*, in phosphate-buffered solution, of dense and porous membranes of PLLA prepared by casting-solvent, was evaluated. Porous membranes were prepared by adding sodium citrate (range: less than 45µm, 180-250µm and 250-350µm) into the polymeric solution. Samples were immersed in a bath of buffer phosphate 0.13 mol/L (pH=7.4), at temperature of 37 °C. After the samples were submitted for 30, 60 and 90 days to the degradation process, they were observed by Scanning Electron Microscopy (SEM). Dense membranes presented superior degradation in relation to the porous ones due to the process of autocatalysis of PLLA that generates the concentration of acids inside the material.

Introduction

Synthetic biodegradable polymers have been used as scaffolds in tissue engineering and orthopaedic implants. Applications of biodegradable implants suggest specific morphologic characteristics: the degradation time of the material should be enough for tissue recuperation and its morphologic characteristics cannot cause any noxious effect to the patient. The study focus of this research is directed to poly(α-hydroxy acids), specifically to the lactic poly(L-lactic acid) (PLLA) belonging to a class of bioabsorbable synthetic polyesters, which satisfy the conditions of a biomaterial (5).

In applications such as the support for cells culture (7), membranes of bioabsorbable polymers have received especial attention due to the facility of their preparation and for the control of the characteristics and properties of the material during the process of preparation (4, 6). The main preparation techniques include the injection of the polymer melted in the mold, the phase separation process and the method used in this work: solvent-casting, particulate leaching technique.

The control of the morphology of membranes of bioabsorbable polymers is fundamental for the success of the implant to be biocompatible and biofunctional, and it is directly related to the degradation time of the structures of PLLA. In the case of the material degrading at higher speed, its mechanical properties are committed; on the other hand if the degradation time is excessively long it can induce inflammatory reactions (2).

The rate of degradation is influenced by several factors, such as the size of the implant, structure of the phase (crystalline or amorphous), degradation mechanism (enzymatic or hydrolytic), presence of addictive and mainly the morphology associated to the porosity of the membranes (1). Apart from the autocatalytic effects of poly(α-hydroxy acids), the size and the distribution of the size of the pores influences induction and cellular growth, participating actively in the regeneration of the tissues (4). For the analysis of the morphology of PLLA in function of degradation time the study *in vitro* represents a practical way simulating conditions of cells culture. Understand how the morphology of the bioabsorbable polymers change during the culture process represents a progress in the understanding of the interaction between synthetic material and growth and cellular differentiation.

Materials and Methods

Poly(L-lactic acid) (PLLA) was supplied by Medisorb (Du Pont), Mw = 300,000. Dense membranes were prepared by casting, dissolving the polymer in chloroform (H_2CCl_2) (MERCK) in the 10% (w/v) concentration. Porous membranes were prepared in the same way with addition of sodium citrate ($C_6H_5Na_3O_7.2H_2O$) (MERCK), in the 40% (w/v) concentration with a size particle of salt established in the range of 250-350μm, 180-250μm and particles smaller than 45μm. After complete dissolution by stirring, the solutions were carefully deposited on a glass plate, thus avoiding bubbles formation. After the evaporation of the solvent, membranes containing salt were washed, under agitation, in distilled water for approximately 24 hours, followed by washing in ethanol for 2 hours. The ethanol was dried at room temperature and the membranes submitted to vacuum.

The degradation process was accomplished by the immersion of samples in a phosphate-buffered solution (PBS) 0.13 mol/L, pH = 7.4, at 37 °C for 30, 60 and 90 days.

The degradation process was observed by SEM, (JEOL JXA 840A). Fragments of membranes surfaces and fractures of N_2 liquid were fastened onto a metallic support, metallized with Au-Pd (Sputer Coater BAL-TEC SCD 050) using 10 kV of tension and a current of 40 mA.

Results and Discussion

Figures 1 and 2 shows the micrographs of dense and porous membranes, respectively, before degradation time and after 30, 60 and 90 days of immersion in buffer phosphate solution.

Observing dense membranes of PLLA (Figure 1), before degradation (Figures 1A, 1A' and 1A") it is possible to verify that the bottom surface of the material is smooth, regular and without pores (Figure 1A"). Figure 1A indicates that the upper surface is irregular, with globular portions of the material, agglomerated in all its extension, with concavities suggesting a porous material. The analysis of the cross sections of the samples confirms a dense and compact material internally, without any indication of pores in its interior (Figure 1 A'). During the degradation process the samples modify their morphology in function of degradation time. The bottom surface presents progressive fragility (Figures 1B", 1C", 1D") with erosion points in its extension. After 90 days the material continues compact but the morphology changes from smooth and regular to rough and irregular (Figure 1D"). The superior surface presents two degradation stages. The first is related to the evidence of the globular areas of the material, observed after 30 days of immersion in phosphate-buffered solution (Figure 1B). This process is more advanced after 60 (Figure 1C) and 90 days (Figure 1D). The second stage is related to the degradation of the globules themselves on the surface of the material. With 60 days of degradation this area presents a larger and clearer mass loss in the central portion (Figure 1C), at 90 days (Figure 1D). The surface of fracture of the samples of PLLA during degradation shows fragility in the internals regions of the membranes. Comparing the material after 30 days (Figure 1B') with the material before degradation (Figure 1A'), it is possible to verify that its continues compact, without any porosity. After 60 days (Figure 1C'), fragility through its cracks can be noted. After 90 days of degradation (Figure 1D') the material presents voids spaces and agglomerates visualized by the spherical areas.

The differentiated degradation of the areas of dense membranes of PLLA is discussed in literature (3). The hydrolysis process starts in amorphous regions because there is larger susceptibility to diffusion of water. In a second stage, the crystalline areas begin degradation process. At the beginning of the process, it is probable that the degradation occurs principally on the surface because of the absorption gradient of water, but as the concentration of carbonyl groups increases in the center, these serve as catalysts for the process. This self-catalyzing behavior was shows to occur in general during the degradation process of aliphatic polyesters. However, the process depends on the chemical structure and configuration of the polymeric chains, as well as the morphology of the device involved (3). Lam *et al.* (4) confirmed such self-catalysis when they showed that non-porous membranes undergo degradation more rapidly than porous ones, because the alter facilitate dissolving the degradation products throughout the aqueous medium, thus discouraging self-catalysis.

The autocatalysis effect can be observed through the central area of the globules on the surface of the membrane.

Figure 2 shows the results obtained by SEM for porous membranes of PLLA with salt particle sizes in the range of 250-350μm, 180-250μm and particles smaller than 45μm, studied for different periods of time.

All the porous membranes presented similar morphologic aspect regardless of salt particle size (Figures 2A, 2E and 2I). Bottom surface of porous membranes are similar to bottom surface of dense membranes, due to the contact with the glass plate, and the upper surface is rough and porous. The size of pores, and the distribution of the size of pores on the upper surface is differentiated for each type of membrane, however they are homogeneous in relation to their distribution. The cross sections reaffirm the presence of pores in all the thickness of the material, indicating that they are interconnected, and that there are no residues of salt crystals in the material. It can be verified that the distribution of the pores is homogeneous, but heterogeneous in relation to their format.

During the degradation process, no significant change in the pore morphology was observed. All of the samples maintained their macroscopic aspect of porosity compared to membranes before degradation process, however it is possible to verify the presence of erosion points on the surface of the material (Figures 2B, 2C, 2D, 2F, 2G, 2H, 2J, 2K, 2L). It is not possible to observe significant changes in the structure of the material, such as alterations in the size and in the distribution of the size of pores. The analysis of the cross sections confirms the maintenance of the porosity throughout the thickness of the membranes.

Analyzing the size of the pores during degradation process it is possible to verify that it does not significantly influence the morphologic alterations of the material. In studies of samples of PLLA prepared by casting of solvent submitted to a larger period of degradation showed a similar behavior for porous membranes (6).

CONCLUSIONS

Comparing dense and porous membranes of PLLA, considering the period of degradation, in relation to the morphologic aspect, it is possible to conclude that dense membranes degrade faster than porous ones. This was confirmed by cracks and fragility shown by dense membranes during the degradation as a result of self-catalytic process. On the other hand porous membranes did not present significantly morphologic alterations during the period studied.

The results suggest that the porosity is a determining factor of PLLA membranes degradation rate.

Captions of Figures:

Figure 1: Scanning electron micrographs of dense membranes of PLLA - 0 days (A, A', A"); after 30 days (B, B', B"); 60 days (C, C', C") and 90 days (D, D', D") of degradation. All scale bars represents 10μm.

Figure 2: Scanning electron micrographs of porous membranes of PLLA - Salt particles: smaller than 45μm, A, B, C and D after 0, 30, 60 and 90 days, respectively; 180-250μm, E, F, G and H after 0, 30, 60 and 90 days, respectively; 250-350μm, I, J, K and L after 0, 30, 60 and 90 days, respectively. A, E and I represent the cross sections and B, C, D, F, G, H, J, K, L represent the upper surface. All scale bars represents 100μm, except K and L.

Acknowledgments

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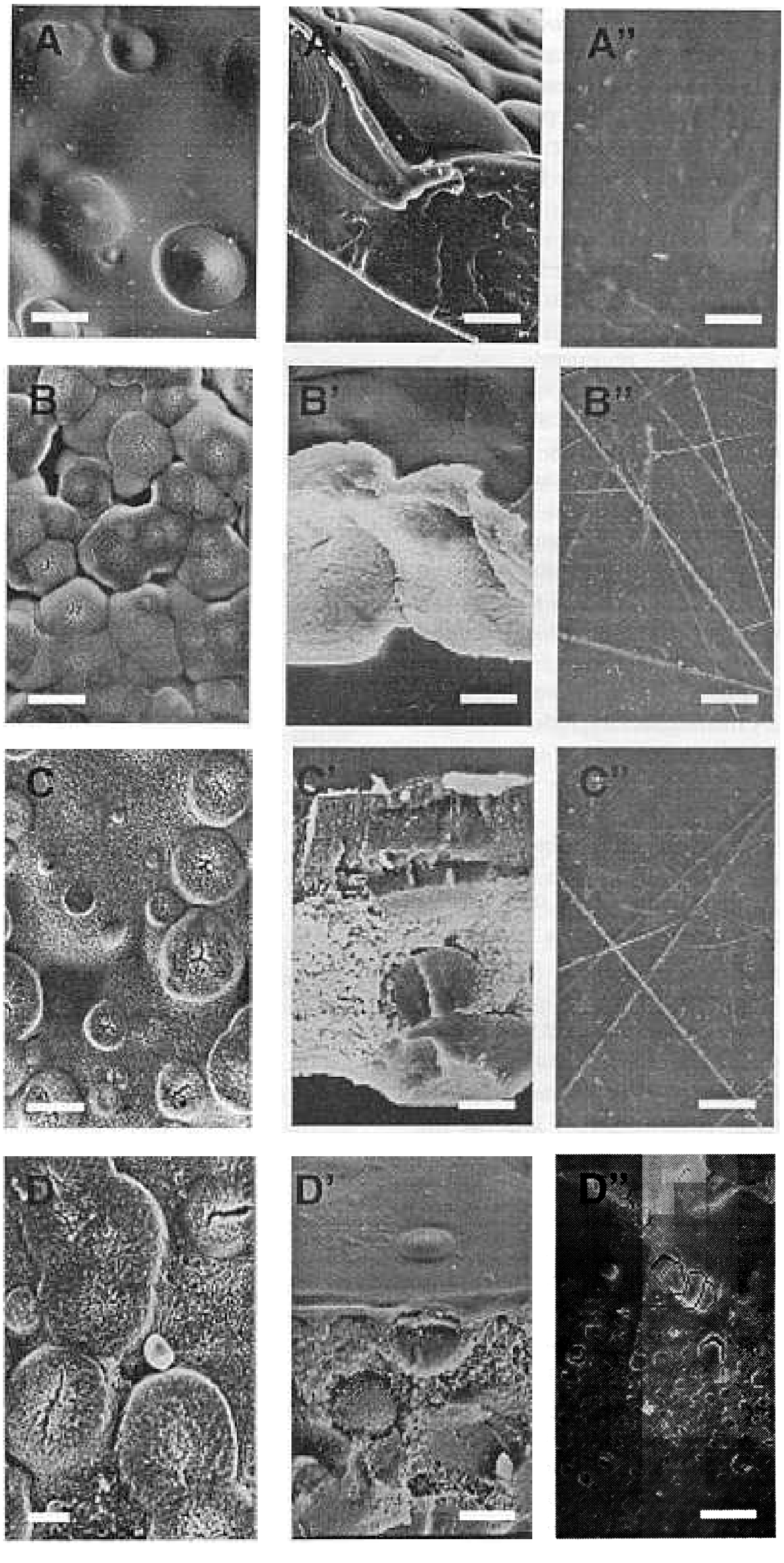
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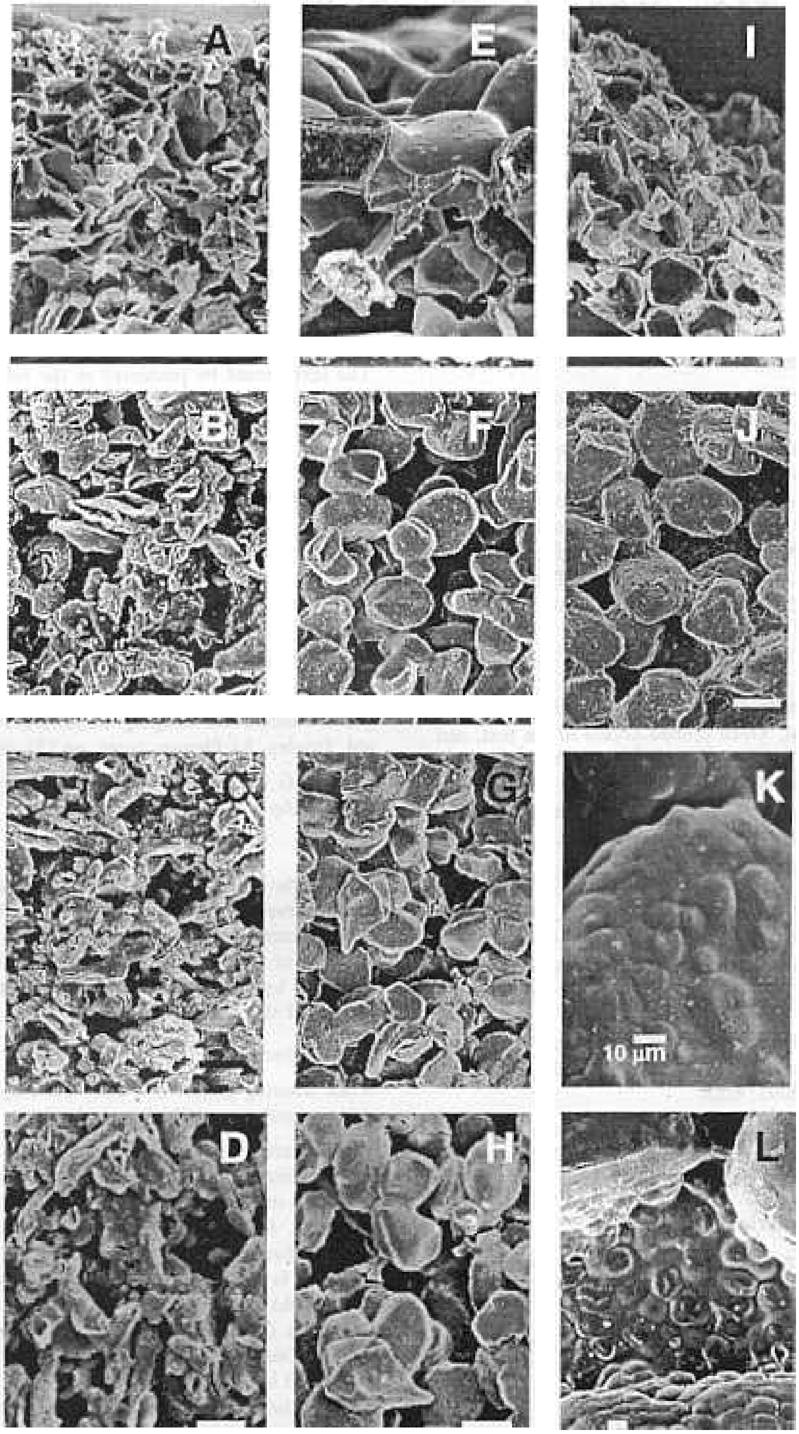
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