STUDY OF THE *IN VITRO* DEGRADATION OF POLY(LACTIDE-CO-GLYCOLIDE) FOAMS PROCESSED WITH SUPERCRITICAL FLUIDS

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ABSTRACT

In the present study we have evaluated the degradation behaviour of poly(lactide-co-glycolide) in a simulated body fluid, before and after foaming with supercritical CO_2. Initial polymer samples, shaped as tablets, and foamed samples were immersed in Sörensen buffer solution and maintained for 10 weeks at a constant temperature of 37°C under mild stirring. Every week samples of degradation medium were collected from each test vial and various tests were performed. The pH of each liquid sample was measured, while precipitation with excess ethanol was used to identify the dissolved polymer. The mass loss of samples at various stages of the study was also determined. A comparison was performed between the *in vitro* behaviours of PLGA samples before and after supercritical fluid processing. It was observed that PLGA foams degrade slightly slower than the tablets. These data are important for evaluating the suitability of various processing methods when designing biodegradable medical devices or implants with well-defined requirements regarding their stability and mechanical properties during specific applications.

INTRODUCTION

A wide variety of natural and synthetic biodegradable polymers have been investigated for drug targeting and release, or for various bioresorbable implants and tissue engineering scaffolds. The biodegradable polymers have advantages over metals or non-degradable polymers and ceramics in that after performing their intended function (releasing the drug or supporting the healing of the tissue) they are eliminated from the organism without the need of a subsequent removal operation. Poly(lactide-co-glycolide) (PLGA) was widely studied and used as biodegradable material for various applications such as drug delivery systems, orthopaedic fixation devices and tissue engineering scaffolds [1, 2]. Recently supercritical fluids have been extensively applied for PLGA processing, especially for the formation of microparticles and of porous devices with various potential applications in medicine and pharmacy [3]. The main advantage of supercritical technologies over the classical processing methods for biomedical devices is found in the lack or limited use of organic solvents and the low temperatures involved. These methods thus overcome the limitations of the classical techniques regarding the incorporation during polymer processing of thermolabile compounds such as drugs, proteins or other bioactive substances.

In the field of foams, supercritical fluids found application as blowing agents and were used to replace traditional compounds, hazardous to health and the environment, such as chlorofluorocarbons or volatile organic solvents. To obtain polymer or composite foams the substrate is saturated with supercritical fluid, followed by rapid depressurization at constant temperature (pressure quench). This method takes advantage of the large depression of the

glass transition temperature observed for many polymers in the presence of dense gases, such as supercritical CO_2. This method allows foam formation with lower energy consumption compared to traditional technologies such as thermally induced phase separation or by using chemical blowing agents. Moreover, the gas is easily removed from the foam at atmospheric conditions, eliminating the need of drying or cleaning steps associated with the use of organic solvents.

The foams obtained from biodegradable polymers, such as PLGA, may be used as tissue engineering scaffolds or grafts, supporting the healing of a damaged tissue. Overtime, the polymeric graft is gradually replaced by the healing tissue, finally being completely resorbed. The most important requirement for such an application is a controlled degradation rate of the polymeric device: the polymer should be resorbed fast enough to allow the growth of the healing tissue but slow enough to provide the necessary mechanical support. Therefore the behaviour of such devices in the presence of biofluids has to be thoroughly assessed before the biomaterial comes in contact with the living tissue. In the present study we have assessed the behaviour in simulated body fluid of PLGA foams obtained by gas foaming with supercritical CO_2. To determine the influence of processing method on degradation, we have compared the results with data acquired for PLGA samples obtained by simple melting and solidification of the initial polymer granules.

MATERIALS AND METHODS

PLGA 50/50 (MW 220 000) was provided by Purac Biomaterials (The Netherlands). CO_2 was obtained from Messer, Slovenia. All materials were used as received, without further purification. The degradation medium (Sörensen buffer solution, pH = 7.4) was prepared according to the standards ISO 13781 and ISO 15814, by dissolving phosphate buffer solution (PBS) 0.13M, NaCl 0.9% and NaN_3 0.02% into distilled water.

PLGA foams were obtained using a batch pressure quench method and supercritical CO_2 as foaming agent. The foaming was performed as described in a previous study [4], applying pressure 25 MPa, temperature 323 K and depressurization rate 8 MPa/min. The foams were then analysed by scanning electron microscopy, to determine their internal structure and pore size. To obtain PLGA tablets, initial polymer granules were melted, moulded into the desired shape and allowed to solidify. The tablets and foamed samples, having similar mass, were immersed in degradation medium and maintained for 10 weeks at a constant temperature of 37°C under mild stirring. Every week samples of degradation medium were collected from each test vial and various tests were performed. The pH of each liquid sample was measured, while precipitation with excess ethanol was used to identify the dissolved polymer. Every 2 weeks some polymer samples were removed from the degradation medium, dried and then weighed to determine their mass change. Each test was performed in duplicate, and the results offered in this paper represent the average value of the measurements.

RESULTS

PLGA foams were obtained using supercritical CO_2 as blowing agent in a batch pressure quench method and afterwards were analysed by scanning electron microscopy. As it can be observed in Figure 1, the employed foaming conditions (pressure 25 MPa, temperature 323 K and depressurization rate 8 MPa/min) allow the formation of interconnected pores with mean

diameter of about 100 μm. These results suggest a proper structure of the foam for tissue engineering and bone graft applications. The size of the pores is appropriate for cell adhesion, proliferation and differentiation, while the interconnectivity of the pores would allow vascularization and nutrient diffusion.

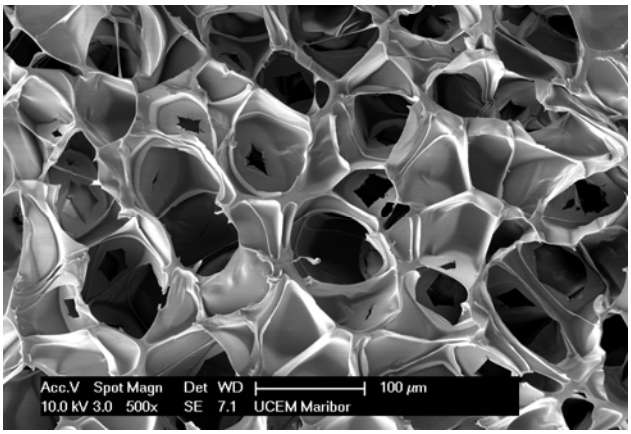

Figure 1. Scanning electron microscopy analysis of poly(lactide-co-glycolide) foams obtained by pressure quench method using supercritical CO_2 as foaming agent. Foaming conditions: pressure 25 MPa, temperature 323 K and depressurization rate 8 MPa/min. Magnification 500x.

Next, the degradation of the obtained foams in a simulated body fluid was monitored for a period of 10 weeks. To study the influence of the processing method on the degradation behaviour of the polymer, simultaneous tests were performed for PLGA tablets moulded from the initial polymer granules. The changes in structure and appearance of the samples during the degradation studies are presented in Figure 2.

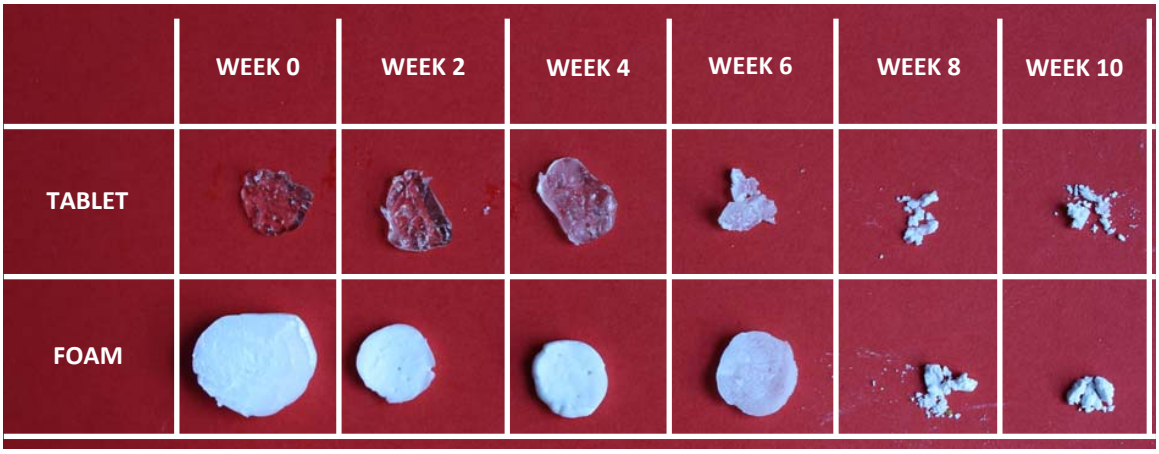

Figure 2. Changes in structure and appearance for poly(lactide-co-glycolide) samples (tablets and foams) during degradation studies in Sörensen buffer solution (phosphate buffer solution 0.13M, NaCl 0.9%, NaN_3 0.02%, pH = 7.4). Incubation conditions: 37°C, mild agitation.

It can be observed that both tablets and foamed samples were strongly degraded after 10 weeks of incubation. However, their behaviour during the incubation period slightly differs. In the case of tablets, the PLGA samples maintained their size and appearance for the first 2 weeks. After the 4^{th} week the first signs of degradation appear under the form of a slight change in the colour. Afterwards the degradation progresses fast, the samples losing their

shape and being fragmented into smaller and smaller parts. On the other hand, the foams suffer a first transformation in the first week, when they undergo shrinking. Afterwards they maintain their shape and appearance longer than the tablets, the first signs of degradation occurring around the 6th week, when the tablets were already fragmented. Although they also suffer fragmentation after the 6th week, at the end of the study the solid residues following the degradation of the foams were still larger than the ones remaining after tablet decomposition.

These results were also confirmed by additional tests such as pH measurements and weight loss determination. The results of these tests are presented in Figures 3 and 4.

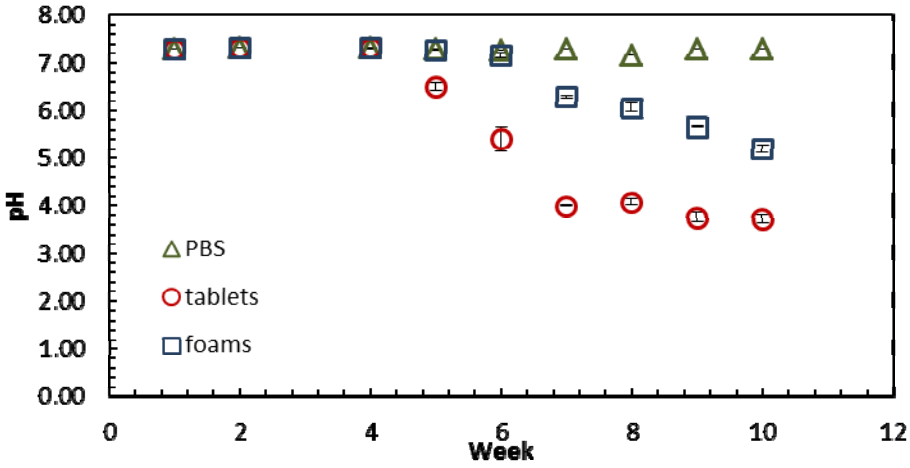

Figure 3. pH variation during the degradation of for poly(lactide-co-glycolide) samples (tablets and foams) in Sörensen buffer solution (phosphate buffer solution-PBS-0.13M, NaCl 0.9%, NaN3 0.02%, pH = 7.4). Incubation conditions: 37°C, mild agitation.

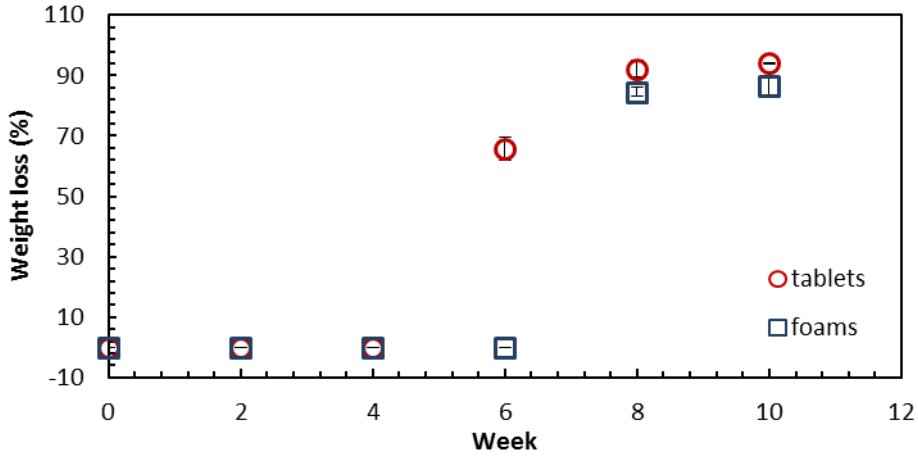

Figure 4. Weight loss for poly(lactide-co-glycolide) samples (tablets and foams) during degradation in Sörensen buffer solution (phosphate buffer solution 0.13M, NaCl 0.9%, NaN3 0.02%, pH = 7.4). Incubation conditions: 37°C, mild agitation.

The degradation of PLGA occurs by hydrolytic cleavage of its backbone ester linkages. It degrades into lactic and glycolic acids, which change the pH of the degradation medium. In the human body, lactic acid is metabolized and excreted, while glycolic acid is either

excreted, or may react to form glycine, which is further metabolized to water and CO_2 [5]. As it can be observed in Figure 3, the first change in the pH value occurs for the liquid medium containing the PLGA tablets. This change takes place after the 4th week of incubation, therefore after the observation of changes in the sample appearance presented in Figure 2. The pH of the degradation medium containing the foam samples starts decreasing after the 6th incubation week. As in the case of the tablets, this change happens after the first modifications in the sample appearance were observed. These results suggest that the sample degradation starts by modifications inside the bulk of the samples, such as chain cleavage into smaller, insoluble fragments, which subsequently undergo transformations into acidic, soluble oligomers or monomers.

These results were further confirmed by measuring the weight loss of the PLGA samples during different stages of the incubation (Figure 4). The biostability of all samples during the first 4 weeks of the study is reflected by the lack of changes in the weight of all samples. The tablets started degrading soon after the 4th week, exhibiting a large and fast weight loss. The foams started losing their mass later; however until the end of the study all samples suffered around 90% weight loss.

A precipitation test was also performed to identify the dissolved polymer. An excess of ethanol was used as antisolvent, followed by filtration of the samples and gravimetrical determination of the amount of precipitate. The results however were inconclusive, most likely because of the loss of solid fraction during the transfer and filtration of the suspensions.

It can be concluded that the processing method influences the *in vitro* and *in vivo* degradation behaviour of the medical device obtained from PLGA. Foams processed by supercritical CO_2 have slightly better biostability than the initial polymer, as reflected by its slower degradation rate. This may be due to the effect of supercritical CO_2 foaming on the conformation of the polymer chains. To obtain polymer foams the substrate is saturated with supercritical CO_2 which has a plasticizing effect on the polymer, increasing chain mobility and significantly decreasing its glass transition temperature [6]. By lowering the pressure, the system is brought to the supersaturated state resulting in the nucleation and growth of pores inside the substrate and volume expansion due to the rearrangement of chain segments. At the same time the amount of gas absorbed by the polymer is decreased. Therefore the glass transition temperature begins to rise reaching the point where it is higher than the foaming temperature. At this point the polymer vitrifies, the chains are “frozen” in the new conformations and the cellular structure can grow no further. The chain conformation resulting from the vitrification of the polymer during supercritical CO_2 foaming may be responsible for the shrinking of the foams during the first week of our study and for the slight delay in foam degradation compared to the tablets.

CONCLUSION

In the present study we have evaluated the degradation behaviour of poly(lactide-co-glycolide) in a simulated body fluid, before and after foaming with supercritical CO_2. The results showed that the samples maintain their shape and appearance up to 4 weeks, followed by degradation. PLGA foams degrade slightly slower than the tablets, suggesting that the processing methods influence the degradation behaviour. Degradation starts by modifications in the bulk of the polymer, followed by decomposition into smaller fragments and subsequent

dissolution in the medium. The decomposition is accompanied by changes in the pH of the liquid medium and after 10 weeks of incubation all samples lost about 90 % of their initial mass. Such studies are necessary to evaluate the suitability of biodegradable polymers for certain applications, such as tissue engineering scaffolds and bone grafts.

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