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Full Length Original Research Paper

Degradation and i*n Vitro* Biocompatibility studies of Citric acid based polyesters

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Abstract:

The suitability of a polymer for a particular application is dependent on its physical and chemical properties. The monomers used play a significant role in the synthesis of polymers for biomedical applications. In the present work two copolyesters, poly (1,12-dodecanediol citrate-co-1,12-dodecanediol sebacate) (PP1); poly (1,12-dodecanediol citrate-co-1,12-dodecanediol itaconate) (PP2) were synthesized by catalyst-free melt polyesterification using monomers - citric acid, sebacic acid, itaconic acid and 1,12-dodecanediol. The polymers exhibited mechanical and thermal properties that made them suitable candidates for degradation and biocompatibility studies. In the present work the degradation and *in vitro* biocompatibility studies were carried out. The degradation and biocompatibility data support the potential use of the elastomers in tissue engineering applications as well as other clinical procedures that may require a biodegradable elastomeric implant.

Keywords: itaconic acid; citric acid; bioelastomer; sebacic acid, polyester

Introduction

Monomers play a pivotal role in synthetic biopolymer syntheses because they are essential for determining and controlling the degradability and biocompatibility of the polymers. Synthetic polyester elastomers based on molecules that are endogenous to the human metabolism have been designed (1). In earlier studies, several investigators have reported elastic polyesters based on citric acid, in particular polyoctanediol citrate (POC) (2), poly(alkenylene maleate citrate) (3), poly(xylitol-co-citrate) (4) and poly(mannitol citric dicarboxylate) (5). Although a number of biodegradable elastomers have been developed, most of them require complex and expensive synthetic procedures, which translate into higher manufacturing costs and hinder the commercial and clinical implementation of their use in tissue engineering (6). Also, as more stringent material requirement in tissue engineering is made, there is a continuous need for newer materials' design and synthesis (7). In an earlier work published elsewhere this group has synthesized and studied polyesters based on polyfunctional monomer-citric acid and other diacids such as sebacic acid and itaconic acid; diols such as 1,12-dodecanediol and cyclohexanedimethanol, that have better mechanical and thermal properties under mild synthesis conditions. These polymers had Young's modulus closer to that of human human thoracic

aorta (0.60 MPa), elastin(1.1 MP) and myocardium of human (0.02-0.5 MPa)(8-10). In the present paper two copolyesters poly (1,12-dodecanediol citrate-co-1,12-dodecanediol sebacate) (PP1); poly (1,12-dodecanediol citrate-co-1,12-dodecanediol itaconate) (PP2) with good mechanical and thermal properties have been investigated for their degradation and in vitro biocompatibility.

Materials and Methods

Water uptake experiment

Disc samples (10 mm in diameter, approximately 1mm thickness) were placed in a 100 ml beaker containing deionized water (20 ml) and were kept for 24 h. The samples were removed after 24 h, wiped gently with filter paper to remove excess liquid on the surface, and immediately weighed using an analytical balance (W_2). Then, the samples were washed with deionized water and dried to a constant weight (W_1) in vacuum oven. The water uptake (expressed as a percentage) was calculated according to the formula:

Water uptake = $W_2 - W_1/W_1$ (g/g)

In vitro Degradation

Degradation studies were conducted in NaOH solution (0.05 M). NaOH degradation was used to screen the polymer degradation in a relatively short period of time. Disk specimen (10 mm in diameter, approximately 1-1.5 mm thickness) was weighed and placed in a small bottle containing 15 ml of NaOH. The bottle was incubated at 37 °C for certain periods of time. After incubation the film was washed with water and dried to a constant weight at 40 °C in vacuum. Weight loss was calculated by comparing the initial mass (W_o) with the mass measured at a given time point (W_t), as shown in equation below (11).

Weight loss (%) = $((W_0 - W_t)/W_0) \times 100$

In vitro Cell Compatibility

Qualitative *in vitro* cytotoxicity studies were performed on polymer discs. Vero cells were used as model cells for cytotoxicity evaluation. The cells were cultured in 50 mL tissue culture flasks with Dulbecco's modified eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS). The culture flasks were kept in an incubator maintained at 37 °C, 5% CO_2 and 95% humidity. The polyester films were cut into discs (5 mm) and sterilized by treatment with 70% ethanol for 30 min, followed by another 30 min of UV light exposure. VERO cells were added to the top of the polyester films in tissue culture flasks. Approximately 30 min after cell seeding, 10 mL of culture medium were added to the culture flasks. The morphology of attached cells was observed and recorded at 24 h and 72 h after cell seeding (12).The positive control had the VERO cells grow freely on the medium.

Results and Discussion

Water uptake Experiments

The water uptake experiment of the bioelastomers demonstrated that the water swelling values were low. Between the two polymers, PP2 showed 19% swelling whereas the polymer PP1 swelled to only 3.5 %. This trend suggested that the swelling in water was directly related to the number of hydrophobic aliphatic chains. PP1 showed lower hydration due to the presence of two longer hydrophobic moieties namely DD and SA whereas PP2 had one longer hydrophobic moiety only. The

samples retained their physical integrity during swelling in water.

Degradation studies

Accelerated degradation studies were performed to ascertain if the material's hydrolytically liable bonds were accessible and that the degradation products were completely soluble. Complete degradation of the polyesters was confirmed by degradation in the presence of 0.05 M NaOH. The polyester PP1 displayed a slower degradation rate than PP2. The existence of longer hydrophobic chains and higher crosslink density hindered the penetration of the solvent into the structure thus decreased the cleavage of ester bonds. The diffusion of solvent into the bulk is also governed by the T_g of the polymer. Lower the T_g, the greater the diffusion rate of solvent into the bulk (13). Thus PP1 with higher T_g value degraded only upto 50% in 48 h whereas PP2 degraded up to 99% under similar conditions. Concurrently, T_g was also affected by crosslink density. Thus, increase in crosslink density of the elastomer also resulted in an increase the degradation time (8-10). The degree of crosslinking also influenced the nature of the hydrolytic degradation. The highly cross-linked elastomer, PP1 was found to degrade in a manner consistent with a surface erosion mechanism that is an almost constant mass loss with time, whereas less cross-linked elastomer, PP2 degraded in a bulk degradation fashion (14). This difference in degradation mechanism was attributed to the slower rate of solvent penetration into the bulk of the highly cross-linked elastomer, PP1 compared to the rate of hydrolysis of the ester bonds.

In vitro Cell Compatibility

Experimental trials were first performed on polyester films seeded with VERO cells. The films were washed prior to cell culture in order to remove any soluble diol/citrate/diacid oligomer that has been detected in the swelling-in-water experiments. These soluble materials could alter the pH of the culture medium and could be toxic to the cells. Further optimization experiments were conducted and it was concluded that the polyester films required washing for at least four days prior to cell seeding, two days in phosphate-buffered saline and two days in serum-free cell growth medium. After this washing step, the cells attached and grew on the films for longer periods of time.

Initial cell adhesion of the polyesters was observed 24 h after VERO cell seeding. The cultured VERO cells showed no negative response to any of the polyesters after the first day. After 3 days of cell culture, the relative cellular vitality was found to be greater on PP2 whereas PP1 displayed moderate toxicity (Figures 1a-c).

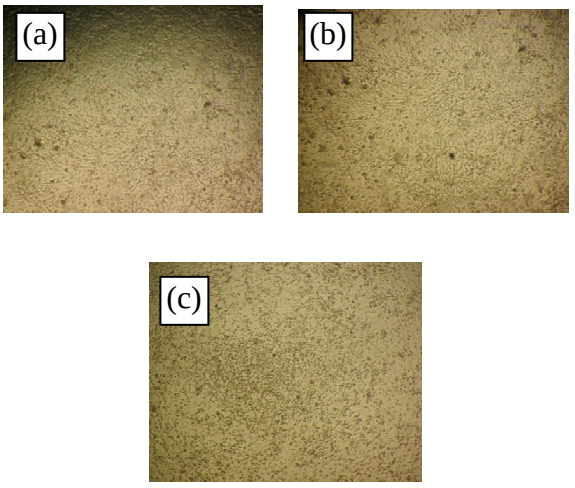

Fig. 1 Phase contrast micrographs of VERO cells on (a) control after 72 h (b) PP2 film (c) PP1 film. Magnification 10X

Better adhesion, spreading and growth of cells was observed on polymer film surface with moderate hydrophilicity, i.e., on PP2. It could be noticed that a relationship exists between hydrophilicity and cell adhesion. The more hydrophilic substrates tend to provide a better interaction with cells. The experimental results showed that the adhesion of cells was found to increase with increasing polarity of the substrate(15). Thus, it could be observed that PP1 which had non-polar hydrophobic alkyl groups (1,12-dodecanediol and sebacic acid) in its monomers experienced moderate toxicity when compared to PP2 which had less non-polar moieties.

It has been noted that the polymer films did not require pre-coating or pre-treatment with adhesion proteins such as fibronectin or laminin. Such pre-treatment steps have been often considered essential to obtain cell adhesion onto biomaterials. The fact that the unmodified elastomers can support cell adhesion and confluence without additional treatments is therefore a significant advantage. Thus this biological data indicates that the test elastomers could be explored further as novel biomaterials.

Conclusions

The two copolyesters poly (1,12-dodecanediol citrate-co-1,12-dodecanediol sebacate) (PP1); poly (1,12-dodecanediol citrate-co-1,12-dodecanediol itaconate) (PP2) showed promising degradation profile. The in vitro biocompatablity studies show that these polymers could be favorably fabricated into biomaterials. This creates the possibility of newer materials that could be used as scaffolds for tissue engineering applications and drug delivery vehicle.

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