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In vitro and in vivo degradation profile of aliphatic polyesters subjected to electron beam sterilization

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ABSTRACT

Degradation characteristics in response to electron beam sterilization of designed and biodegradable aliphatic polyester scaffolds are relevant for clinically successful synthetic graft tissue regeneration. Scaffold degradation in vitro and in vivo were documented and correlated to the macroscopic structure and chemical design of the original polymer. The materials tested were of inherently diverse hydrophobicity and crystallinity: poly(L-lactide) (poly(LLA)) and random copolymers from L-lactide and ε-caprolactone or 1,5-dioxepan-2-one, fabricated into porous and non-porous scaffolds. After sterilization, the samples underwent hydrolysis in vitro for up to a year. In vivo, scaffolds were surgically implanted into rat calvarial defects and retrieved for analysis after 28 and 91 days. In vitro, poly(L-lactide-co-1, 5-dioxepan-2-one) (poly(LLA-co-DXO)) samples degraded most rapidly during hydrolysis, due to the pronounced chain-shortening reaction caused by the sterilization. This was indicated by the rapid decrease in both mass and molecular weight of poly(LLA-co-DXO). Poly(L-lactide-co-ε-caprolactone) (poly(LLA-co-CL)) samples were also strongly affected by sterilization, but mass loss was more gradual; molecular weight decreased rapidly during hydrolysis. Least affected by sterilization were the poly(LLA) samples, which subsequently showed low mass loss rate and molecular weight decrease during hydrolysis. Mechanical stability varied greatly: poly(LLA-co-CL) withstood mechanical testing for up to 182 days, while poly(LLA) and poly(LLA-co-DXO) samples quickly became too brittle. Poly(LLA-co-DXO) samples unexpectedly degraded more rapidly in vitro than in vivo. After sterilization by electron beam irradiation, the three biodegradable polymers present widely diverse degradation profiles, both in vitro and in vivo. Each exhibits the potential to be tailored to meet diverse clinical tissue engineering requirements.

1. Introduction

A key element in the field of tissue engineering is the development of temporary, three-dimensional, porous scaffolds, which function not only as cell carriers, but also as acellular matrices, providing mechanical support for the newly formed and developing tissues. All scaffolds developed for tissue engineering purposes must meet stringent requirements with respect to biocompatibility, porosity, pore size, surface properties, tissue specific differentiation, mechanical suitability and biodegradation [1,2].

Once implanted into the body, the scaffold should provide the required mechanical support and structural integrity during the formation of new tissue, i.e. the scaffold should adequately withstand initial stress and strain in vivo. As the newly formed tissue matures and gains in strength, requiring less support, the scaffold should gradually degrade and be completely absorbed by the body [1]. Ideally, the rate of degradation and associated loss of mechanical properties should be synchronized with the formation and maturation of the new tissues. Thus the degradation characteristics of the scaffolding material are vital to the success of a temporary tissue engineering scaffold. One group of materials with great potential in the biomedical engineering field are the synthetic biodegradable aliphatic polyesters, with their great versatility, and ease of both customization and fabrication of three-dimensional scaffolds [3,4].

We have developed porous three-dimensional scaffolds for bone tissue engineering, based on random aliphatic copolyesters from L-lactide (LLA) and ε-caprolactone (CL) or 1,5-dioxepan-2-one (DXO) [5]. These copolymers were selected because both their mechanical properties and surface chemistry can readily be modified to meet the requirements of specific tissue engineering applications.

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Our preliminary studies confirmed that these scaffolds have properties desirable for bone tissue engineering. Biocompatibility was confirmed according to ISO/EN 109935 guidelines [6] and a series of studies has demonstrated that cell lines with osteoprogenitor potential, such as rat- and human-derived bone marrow stromal cells (MSC), periodontal ligament cells and human osteoblast-like cells, attach, spread and proliferate well on these constructs [7–10]. Moreover, the scaffolds stimulate osteogenic differentiation of rat and human bone marrow stromal cells and human osteoblast-like cells [7,8,10]. A recent animal study, using a rat calvarial defect model, showed that implantation of scaffolds seeded with endothelial cells and MSC promoted rapid bone formation [11].

An unresolved question in relation to scaffolding material is the influence of clinical relevant sterilization on degradation rates. Standard sterilization by heat and moisture causes deformation of degradable polyesters [12]. Chemical sterilization avoids increased temperatures but has the disadvantages of lengthy degassing times, potentially toxic residues and geometric changes in the materials [12,13]. An efficient, appealing alternative is radiation sterilization.

In earlier studies we undertook comprehensive investigations of chemical changes to various polyesters following electron beam (EB) and gamma radiation [14,15]. Radiation-induced chemical alterations, crosslinking and/or chain shortening are dependent on the dose and compositional ratios of the incorporated comonomers. Because the polymer chain length is strongly affected by irradiation, the scaffold property most significantly altered by irradiation is the degradation rate, which in turn governs mechanical stability [16–18]. Both properties are critically important to clinical success, because ideally they should be synchronized with tissue regeneration and this may vary considerably, depending on the specific tissue engineering application. Thus, an understanding of the changes induced by irradiation sterilization on degradation rates, both in vitro and in vivo, is important in the design of scaffolds for different tissue engineering applications, which require materials with tailored degradation profiles.

Our previous screening for biocompatible scaffolding materials has disclosed that certain aliphatic copolyesters have great potential in bone tissue engineering. We have also concluded that during clinical EB sterilization these polyesters undergo fundamental chemical changes in the polymer chains. Thus it is important that the effect of irradiation on the degradation rates and mechanical properties of potential tissue engineering scaffolding materials is factored into the design of scaffolding with tailored degradation profiles.

In the process of developing aliphatic polyester scaffolds for clinical application in bone tissue engineering, the next phase was to determine the long-term influence of radiation-induced chemical changes on the degradation rate and mechanical properties of aliphatic polyester scaffolds with diverse pristine properties. Our hypothesis was that the effects of EB sterilization on the degradation rate and mechanical properties will vary according to the chemical, physical and macroscopic structure of the aliphatic polyesters in question.

In the present study, random copolymers with varying thermal properties, hydrophobicity, mechanical stability, etc. were synthesized from LLA and CCL and DXO. Porous scaffolds and non-porous samples of these materials were then sterilized by 25 kGy EB and subjected to in vitro hydrolysis for up to 1 year: poly(LLA) served as a reference material. For comparison, porous scaffold samples were also subjected to in vivo degradation for 28 and 91 days in a rat calvarial defect model.

The aim of the study was to determine the effect of polymer design and chemical changes induced by radiation sterilization on degradation rates and loss of mechanical properties in aliphatic random copolymers.

2. Materials and methods

2.1. Materials

All monomers were carefully purified, dehydrated and subsequently stored in an inert atmosphere. LLA (Cat. No. 80691934, Boehringer Ingelheim, Germany) was purified by recrystallization from dry toluene and subsequently dried under reduced pressure. CL (Cat. No. 16736-3, Sigma–Aldrich, Germany) was dehydrated over calcium hydride for 24 h and then distilled under reduced pressure. DXO was synthesized through Bayer–Villiger oxidation, as described elsewhere [19]. Stannous 2-ethylhexanoate (SnOct2; Cat. No .S3252, Sigma–Aldrich) was dried over molecular sieves and stored in an inert atmosphere. Ethylene glycol (Cat. No. 33068, Sigma–Aldrich) and sodium chloride (Cat. No.106404, Merck, Germany) were used as received. Dulbecco's phosphate-buffered saline, pH 7.4 (PBS, Cat. No. H15-011) was purchased from PAA.

2.2. Polymerization technique

The copolymers Poly(L-Lactide-co-epsilon-caprolactone) (poly (LLA-co-CL)) and poly(L-Lactide-co-1,5-dioxepan-2-one) (poly (LLA-co-DXO)) were synthesized by a previously described method [7]. Briefly, monomer, initiator and catalysts were weighed in the required amounts and bulk polymerized at 110° C for 72 h in an inert atmosphere. The polymers were precipitated three times in cold hexane and methanol.

2.3. Sample preparation

Non-porous samples were produced through casting a 4% (w/v) polymer solution in chloroform in glass moulds, followed by slow evaporation of chloroform. The three-dimensional porous scaffolds were prepared by the solvent-casting-particulate-leaching method, described previously [7,20]: 4% (w/v) polymer solution was prepared in chloroform and poured into glass moulds containing NaCl at a ratio to the polymer of 10:1 by weight. Prior to casting, the salt crystals were sieved into the size range 90–500 μm. The chloroform was allowed to evaporate slowly, leaving a solid composite from which the scaffolds were punched. Salt particles were leached from the composites by repeated soaking in deionized water. Finally the salt-free scaffolds were vacuum dried. Scaffold porosity, ϕ, was calculated according to Eq. (1), in which ρ_p is the density of the scaffold and ρ_s is the density of non porous sample from the corresponding copolymer.

$$\phi = \left(1 - \frac{\rho_p}{\rho_s}\right) \times 100 \quad (1)$$

The average thickness of the non-porous samples was 0.15 mm, and was 1 mm for the porous samples. Two geometric types of samples were made: discs (diameter = 12 mm) for chemical analysis and strips for mechanical testing. The strips were produced with an EP04/80 × 5 mm punch. Solid non-porous samples and three-dimensional porous scaffolds were produced in both geometric types. Samples were sterilized by exposure to a 25 kGy dose using an electron beam in an inert atmosphere before hydrolysis (Mikrotron, Acceleratorteknik, The Royal Institute of Technology, Stockholm), operating at 6.5 MeV.

2.4. In vitro hydrolysis

The samples were pre-wetted in ethanol for 1 h and then soaked repeatedly in PBS [21].The presoaked samples were placed in vials containing PBS and stored in a thermostatically controlled incubator at a temperature of 37 °C with a rotation rate of 60 rpm.