J Mater Sci: Mater Med (2008) 19:53–58
DOI 10.1007/s10856-007-3134-2

In vivo degradation of poly(DTE carbonate) membranes. Analysis of the tissue reactions and mechanical properties

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Received: 2 May 2005 / Accepted: 24 July 2006 / Published online: 19 June 2007

Abstract Different bioabsorbable polymers and their co-polymers have been used to construct an optimal material for guided bone regeneration applications. Our aim was to evaluate a novel bioabsorbable material in a soft tissue environment. In this study, a poly(DTE carbonate) membrane (0.2–0.3 mm) was implanted into 20 NZW rabbits' subcutaneous pouches for 6, 12, 24 and 52 weeks. The material was evaluated by means of histological reactions to the material and mechanical properties of the membrane. Based on this study, it can be concluded that poly(DTE carbonate) elicited a very modest foreign body reaction in the soft tissues. This reaction was uniform throughout the study. Varying amounts of calcification was seen in the fibrous capsule surrounding the implant. The number of calcified bodies did not correlate to healing time.

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Introduction

Most of the early studies about bioabsorbable implant materials have been on the use of these materials in fracture fixation and surgical sutures [1–4]. Applications for thin plates, foils and membranes were also introduced in the early 1970s [5]. Clinical studies have concentrated mainly on applications of bioabsorbable membranes in dentistry or cranio-maxillofacial surgery. Thin implants have been especially required in places where there is limited space for the implant, which is often the case in the dento-facial area. Therefore, the material should not expand or create an unnecessary space-consuming connective tissue capsule. So far, thin plates have been successfully used to reconstruct orbital floor fractures, to support bone augmentation in periodontal surgery and to cover defects of the calvarium [6–8].

Different bioabsorbable membranes have been tested for guided bone regeneration purposes. These include polylactides, polyglycolides, polydioxanone, e-caprolactone, trimethylene carbonate, collagen and their co-polymers [9–12].

Bioabsorbable materials are developed for different purposes. However, the main goal is to avoid the problems encountered with non-absorbable materials, and the second goal is to avoid material removal operations. By avoiding the removal of the implant, risk of infection, cost and time of the treatment, and patient morbidity have been reduced [13].

Tyrosine-based pseudo-peptide polymers were first introduced by Kohn and Langer in 1987 [14]. Ertel and Kohn have described polymeric amino acid systems that contain non-amide linkages pseudo-peptides. Various tyrosine polycarbonates derived from diverse alkyl esters of desaminotyrosyl-tyrosine, can be prepared by condensation

polymerization. All polymers manufactured in this fashion are amorphous and no evidence of crystallinity is seen by X-ray diffraction [15]. Studies of various tyrosine polycarbonates, derived from diverse alkyl esters of desaminotyrosyl-tyrosine have indicated that poly(DTE carbonate) is the most suitable polymer to construct scaffolds for guided bone regeneration [16]. In the literature, the use of poly(-DTE carbonate) in ACL(anterior crucial ligament) reconstruction [17] and cancellous bone fracture fixation [18] has been previously reported in rabbits.

Tyrosine-derived polycarbonates incorporate two in - vivo hydrolytically labile bonds in each repeat unit, a carbonate bond that connects the monomer units and an ester bond connecting a pendent chain. Degradation rate and products of the polymer are determined by the relative hydrolysis rate of these two labile bonds. Of these two bonds, the carbonate bond is hydrolyzed at a faster rate than the pendent chain ester bond [19]. No enzyme or cellular activity has been shown to participate to the degradation process [15, 20]. Shape and porosity of the implant has not been shown to affect degradation significantly either. Because the hydrolytic cleavage of the carbonate backbone is the rate-determinating step in degradation, the surface to volume ratio has only a minimal effect [21]. At neutral or slightly acidic pH, the hydrolysis rate of the carbonate bond is faster than the rate of ester bond cleavage. As the degradation continues, the amount of acidification of the interior of the polymer matrix increases and the further degradation of the polymer can be expected to slow down. Tangpasuthadol et al. describe this as the ''lag period'' of degradation. However, as more pendent chains are eventually hydrolyzed, the reaction rate increases after the pH within the polymer matrix sinks below three. Only under pH 3, the acid catalyzed hydrolysis of the ester bond becomes a dominant factor and pendent chain ester hydrolysis outpaces the rate of hydrolysis of the backbone carbonate bonds [19]. This leads eventually to disintegration and complete degradation of the implant. However, degradation process can take up to 4 years before resorption is complete [21].

While degrading in vivo, poly(DTE carbonate) rods have been shown to maintain their shape at least until 1,280 d and changes in appearance (turning opaque) has been seen only inside the material. James et al. have suggested that the opaque appearance of the implant is related to the small amount of water uptake into the polymer matrix [16]. With tyrosine-derived polycarbonates, no noticeable swelling has been evident; they do not take up more than 5% of water during any stage of degradation [16, 20].

Besides the water uptake, another difference between tyrosine-derived polymers and poly(alpha hydroxyacid) is related to the beginning of mass loss. Tyrosine-derived monomers are not readily water-soluble and the mass loss occurs very slowly at the end of the degradation process [16]. Tangpasuthadol et al. have suggested that the only degradation products formed will be pendent alkyl alcohol and desaminotyrosyl-tyrosine [19]. Even though the material looses molecular weight significantly during degradation, the implants' mass loss has not been evident before 1,090 days of implantation. Further, before 180 days of degradation, no significant amounts of degradation products have been detected [16]. For example, poly lactic acid starts to loose mass when molecular weight decreases to about 20,000 D [20].

Based on the properties presented above and the results published by Ertel and Kohn [15], we decided to investigate the behavior of poly(DTE carbonate) membrane in a rabbit subcutaneous model. In this study, biaxially oriented membranes 0.2–0.3 mm thick were fabricated for use in guided bone regeneration applications. These membranes were implanted in rabbit soft tissue to evaluate the soft tissue reactions to thin implants. The material was also studied for mechanical properties and shape maintenance when no bone tissue fixation was available.

Materials and methods

Batches of poly(PDTE) carbonates were supplied by Integra LifeSciences Corporation (New Jersey, USA). These polycarbonates having molecular weights from 2,00,000 to 2,20,000 D (weight averages) were prepared according to previously published procedures [22, 23].

Materials were stored in the form of powder at –18 °C temperature prior to processing in airtight containers. Three days before processing, this powder was ground in liquid nitrogen to eliminate visible clots. The homogenated powder was dried in vacuum at 53 °C for 48 h.

Solid plates (85 × 85 × 3.3 mm) were compression molded from the raw material powder at 165 °C. Molded plates were biaxially oriented in one phase to a size of 150 × 150 mm at 75 °C with a plate-stretching machine Karo IV (Brueckner GmbH, Germany).

Animals

This experimental study was approved by The Research Animal Commission of the University of Helsinki and by The Provincial Administrative Board, according to Finnish law.

About 20 adult female New Zealand White rabbits (HsdPoc strain) weighing 2,500–3,000 g were used as experimental animals. No preoperative fasting was required. The animals were divided in three groups ($n = 6$) according to the intended healing time (6, 12 and

24 weeks) and two animals for 52 weeks. National guidelines for care of laboratory animals were followed.

Surgical procedure

Preoperatively, the animals received trimethoprim-sulfadiazine (Duoprim vet®, Schering-Plough, Brussels, Belgium) 0.3 mg/kg subcutaneously (s.c.) for infection prophylaxis. Anesthesia was induced with medetomidine (Domitor®, Orion Pharma, Turku, Finland) 300 μg/kg and ketamine (Ketaminol vet®, Intervet International, Boxmer, The Netherlands) 25 mg/kg (s.c.).

The back of the rabbit was shaved and the skin was rinsed and scrubbed with chlorohexidine digluconate (Klorhexol® 5 mg/mL, Leiras, Turku, Finland). A skin incision was made at midline on the back and subcutaneous pouch was created for poly(DTE carbonate) membrane (10 × 20mm). The membrane was inserted in the pouch but was not fixed by any other means. The incision was closed in layers with absorbable sutures (Vicryl® 3-0, Ethicon, Somerville New Jersey, USA).

For postoperative pain control, the animals received 0.02–0.05 mg/kg (s.c.) buprenorphinum (Temgesic®, Schering-Plough, Brussels, Belgium) immediately after the operation and every 12 h for the next 2 days. For euthanasia, pentobarbital (Mebunat®, Orion Pharma, Turku, Finland) 30 mg/kg was used intravenously (i.v.).

Samples were carefully dissected, fixed in 70% ethanol and embedded in plastic.

Histological analysis

Five μm thick sections were prepared and stained with hematoxylin and eosin. Masson-Goldner staining was carried out as well.

Mechanical testing and molecular weight determination

Mechanical testing and molecular weight determination was done at the Institute of Biomaterials, Tampere University of Technology, Tampere, Finland. Weight average molecular weight (M_w), number average molecular weight (M_n) and polydispersity (PD) of the poly(DTEcarbonate) membranes were studied using conventional gel permeation chromatography (GPC) with narrow polystyrene standards using chloroform as solvent and eluent. The equipment consisted of differential refractometer detector (Waters 410 RI) and HPLC-pump (Waters 515). The injection volume of the samples was 150 μL, and the flow rate in the columns was 1 mL min^{-1}. Each data point presents the mean of four measurements taken from two samples at the same time point.

The biaxially oriented membranes were die cut to achieve samples for tensile testing (Fig. 1). Tensile strengths of the membranes were measured following the standard ISO 527. The samples were in form of standard tensile specimen except the size 5 × 50 mm. The membranes were tensile tested using an Instron 4411 (Instron Ltd, High Wycombe, England) materials testing instrument using a crosshead speed of 10 mm/min.

Results

Tissue reactions

The implanted membranes elicited a fairly uniform tissue reaction throughout the study. The membrane can be seen as an opaque structure in all histological sections. After the first 6 weeks of implantation, the membranes became surrounded by a 200–300 μm thick fibrous capsule containing mature fibroblasts. The fibrous capsule was thicker at the edges of the membrane. Presence of a few macrophages was confirmed in only one 24 week sample.

After 6 weeks of implantation, varying amounts of calcified deposits (Fig. 2) were seen uniformly distributed at the membrane site in the surrounding fibrous capsule. The number of calcified bodies was not found to correlate with implantation time. There was no concomitant bone formation or inflammation in the surrounding tissue.

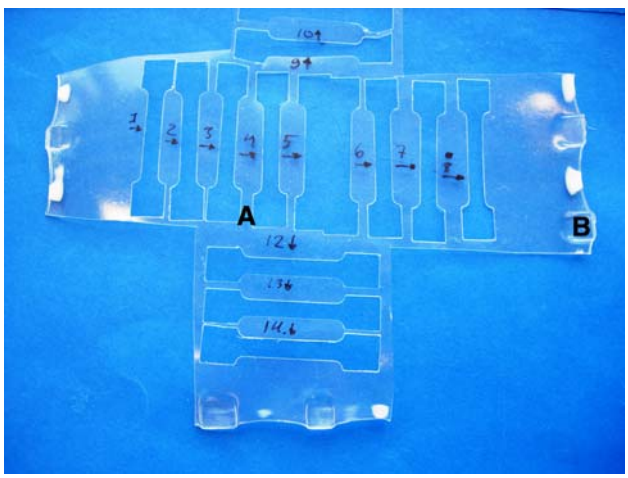

Fig. 1 Bi-axially oriented plate, samples for mechanical testing removed. Decreasing the implants thickness results in an increased orientation inside the implant's structure (A). Grip positions of the biaxial stretching machine are shown (B)

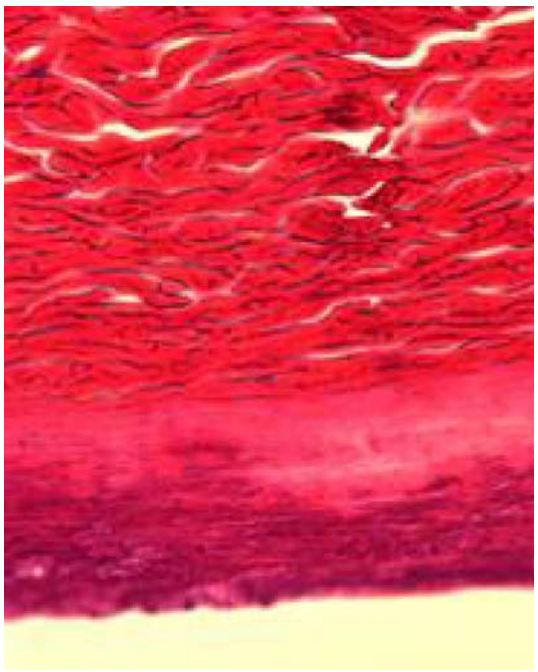

Fig. 2 The opaque poly(DTE carbonate) membrane is shown at the bottom of the picture. Large amounts of calcified tissue (dark staining) is evident next to the membrane (original magnification × 200)

Mechanical testing

No changes in appearance or shape of the membranes were recorded. All samples remained transparent and were not bent or otherwise distorted.

The tensile strength of the membranes varied from 84 to 142 MPa (average 114, standard deviation 26). The strength values varied due to the fact that the original plates had been compression molded to different thicknesses. The subsequent orientation step resulted in membranes with the same size but varying degree of orientation and thickness (Fig. 3). Consequently the thinnest membranes exhibited the highest mechanical properties. Determined strength levels were in the same range as high performance engineering polymers but had no significant contribution in the animal subcutaneous model used.

Retrieved membranes from weeks 12, 24 and 52 were tested to evaluate molecular weight reduction. After

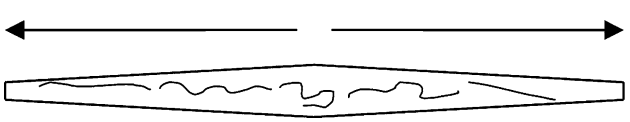

Fig. 3 Schematic drawing of the subsequent orientation step that resulted in same sized membranes of varying degree of orientation and thickness. At the edges of the drawing the molecules are more oriented than in the middle

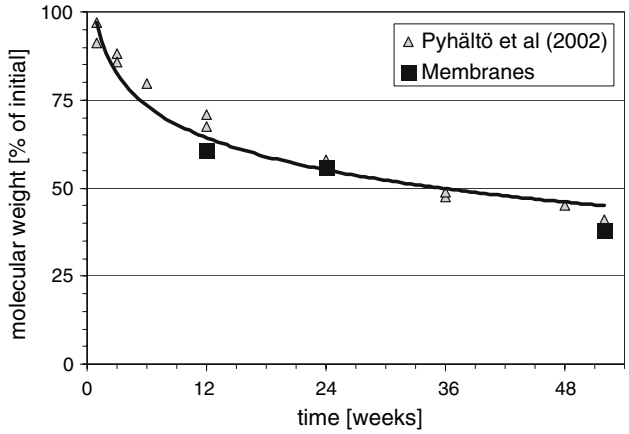

Fig. 4 The molecular weight retention of membranes retrieved in vivo in comparison to the rods used by Pyhältö et al. [25]. The trend line is fitted to data using logarithm equation as suggested by Hooper et al. [20]

52 weeks, the viscosity average molecular weight (M_w) was reduced to 38% of the initial value (Fig. 4 and Table 1).

Discussion

Tyrosine derived polycarbonates are a relatively new category of bioabsorbable polymers. Therefore, there are only a few reports about tissue reactions in different applications. We have been testing the material in guided bone regeneration application in rabbit mandibles [24]. These membranes enhanced the bone regeneration in the experimentally created defects, and it seemed that the bone was growing along the membrane. Small radiologically detectable calcifications were also seen at the outside of the membrane. Therefore, we decided to evaluate poly(DTE carbonates) reactions in a soft tissue model to find out whether the material has osteoinductive properties in vivo. The lack of water uptake of the tyrosine-derived implants explain the fact that they maintain their profile for longer periods than derivatives of lactic or glycolic acid, and thus may be used in applications where swelling could result in failure of implantation or cause esthetic problems [16, 20]. As a result, poly(DTE carbonate) was considered a suitable material for membrane application.

Table 1 Measured reduction in molecular weight in percentages

Weeks	MW (%)	Standard deviation × 3
12	60.60	1.35
24	56.10	1.35
52	37.90	0.64

For better accuracy, the standard deviation is multiplied by three

The mechanical data observed is consistent with the previous publications, in which identical polymers have been used [25]. The trend line in Fig. 4 was fitted to the data using logarithm equation, suggested by Hooper et al. [20]. The molecular weight levels that have been reached in previous studies and in this study did not provide information about any mechanisms responsible for final absorption. However, the rod-formed implants used by Pyhältö et al. [25] did have comparable degradation rates with the membranes in this study. Therefore, it can be concluded that the surface area of the implant appears to have negligible effect on the degradation rate. Our results are consistent with earlier publications [21].

Previous results from studies concerning soft tissues indicate that this material elicits a very modest foreign body reaction and becomes surrounded with a thin (29–42 μm) fibrous capsule [20], and by 48 weeks of implantation in vivo, no indication of cell mediated immunology has been evident [26]. We found that this material caused a very similar reaction throughout the follow-up period. This supported the earlier findings, except the thickness of the fibrous capsule was notably wider in this study. This might be explained by the shape of the implant. Hooper et al. used a small diameter rod, but in this study, we used a membrane with rather sharp edges. So, the thicker fibrous capsule noticed adjacent to the edges of the implants could be the result of the tissues' reaction to the mechanical stress caused by these edges.

However, we found that poly(DTE carbonate) membranes elicited a formation of small calcified bodies in the connective tissue capsule around the implant material. This phenomenon was seen as early as after 6 weeks of implantation. The amount of calcified bodies was not found to correlate to healing time. To our knowledge, no such reaction has been described with any in vivo implanted bioabsorbable polymer so far. The origin of the calcified bodies remains unsolved in this study. This might be due to the relatively short follow-up time.

In in vitro studies, it has been shown that the deposition of the calcium salts can result from either osteoblast derived ossification, dystrophic or non-metabolic calcification. The presence of osteoblasts and the formation of type I collagen containing extracellular matrix marker has been considered to be the marker of osteoinductive capacity of the material [27]. In this study, we failed to demonstrate the presence of osteoblasts in the fibrous capsule and, hence, the effect of calcification in relation to possible bone formation remains unsolved. Further studies are needed to confirm the nature of calcification.

Conclusions

Poly(DTE carbonate) membranes elicit a very modest foreign body reaction. After 6 weeks of implantation, small calcifications were seen in the membrane surrounding fibrous capsule. The implantation time did not correlate with the amount of calcified bodies. Based on the results retrieved from mechanical testing and earlier publications, it can be concluded that the surface area of the implant appears to have negligible effect on degradation rate.

Acknowledgments This study was supported by the State Subsidiary Grant (TYH1331 and TYH4307), Finnish Dental Society Apollonia and National Technology Agency of Finland. Authors wish to thank Histola Ltd, Tampere, Finland for preparing the samples. Finally, we would like to thank Antti Kourula, MD, for English proof reading of this article.

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