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New magnetic-resonance-imaging-visible poly(ε-caprolactone)-based polyester for biomedical applications

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ABSTRACT

A great deal of effort has been made since the 1990s to enlarge the field of magnetic resonance imaging. Better tissue contrast, more biocompatible contrast agents and the absence of any radiation for the patient are some of the many advantages of using magnetic resonance imaging (MRI) rather than X-ray technology. But implantable medical devices cannot be visualized by conventional MRI and a tool therefore needs to be developed to rectify this. The synthesis of a new MRI-visible degradable polymer is described by grafting an MR contrast agent (DTPA-Gd) to a non-water-soluble, biocompatible and degradable poly(ε-caprolactone) (PCL). The substitution degree, calculated by ^{1}H nuclear magnetic resonance and inductively coupled plasma-mass spectrometry, is close to 0.5% and proves to be sufficient to provide a strong and clear T1 contrast enhancement. This new MRI-visible polymer was coated onto a commercial mesh for tissue reinforcement using an airbrush system and enabled in vitro MR visualization of the mesh for at least 1 year. A stability study of the DTPA-Gd-PCL chelate in phosphate-buffered saline showed that a very low amount of gadolinium was released into the medium over 52 weeks, guaranteeing the safety of the device. This study shows that this new MRI-visible polymer has great potential for the MR visualization of implantable medical devices and therefore the post-operative management of patients.

1. Introduction

Polymeric biomaterials are being increasingly used for biomedical applications as implantable devices or drug delivery systems [1–3]. As these biomaterials offer a great variety of structures, and therefore possess a broad range of properties, they can be used in a host of applications. One of the most important applications in the biomedical field is the use of these polymers for medical devices, especially prostheses. Such materials can be used for permanent or temporary applications, depending on their structure [4–6]. Unfortunately, all these polymeric materials are transparent to X-rays and are invisible using magnetic resonance imaging (MRI). This inability to visualize implanted material restricts any evaluation of tissue integration and post-operative fixation, and any determination of fate in the body [7].

A radio-opaque poly(ε-caprolactone) (PCL)-based aliphatic polyester has been developed in our laboratory in which a covalent link has been formed between a radio-contrast dye (typically iodine) and the polymeric backbone [8].

MRI is a non-ionizing technique that is widely used in clinical practice, mainly because of its non-invasive nature, its capability to produce high-definition images and its ability to depict pathological tissues [9–12]. Image contrast is often enhanced by using contrast agents [13,14] and this approach may be used to visualize polymeric material. In a recent report, Kramer et al. have reported the synthesis of surgical textile implant loaded with iron oxide nanoparticles during the polyvinylidene fluoride threads extrusion [15]. The resulting material was visualized by MRI using the positive-contrast inversion recovery with on-resonant water suppression sequence [16]. Positive contrast may also be gained using conventional MRI sequences if paramagnetic agents, such as gadolinium salts Gd(III), are used [17]. Unfortunately, gadolinium salts are toxic and must be chelated [18]. Of all gadolinium chelates, diethylenetriaminepentaacetic acid (DTPA) is the most frequently used in medical imaging [19].

Several methods have been reported in the literature to design macromolecular DTPA-Gd complexes. Applications of these new contrast agents in blood pool imaging are focused on the

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modification of the pharmacokinetics [20], through chelating structures grafted onto water-soluble macromolecules [18,21–26]. However, these water-soluble macromolecular complexes are rapidly excreted and cannot be used for the MRI visualization of non-water-soluble implanted devices.

Very few studies have been conducted on the conjugation of Gd(III) chelates to hydrophobic polymers. In some cases the contrast agent is dispersed within a polymeric matrix [27]. It can diffuse out of the device and therefore enables only transitory visualization, as for example with PLAGA microspheres [28]. In order to overcome this drawback, a chelate of the contrast agent should be covalently linked on a polymer chain, but this approach requires the functionalization of the polymeric backbone prior to the addition of the chelate. Jiang et al. [29] mentioned a surface modification of a polymeric plate by plasma treatment with hydrazine followed by reaction of DTPA on the amine group. However, the resulting hydrolysable amide bond may not be suitable for the long-term visualization requirement. In addition, this method is known to induce possible reactive pendant function rearrangements on the surface, which can decrease the material's reactivity [30,31]. Another study referred to a hydrophobic poly(styrene-maleic acid) (SMA) copolymer grafted onto a gadolinium (HEDTA (hydroxylethylenediaminetetraacetic acid)) chelating agent [32]. In this case a potentially hydrolysable ester bond is created between the chelating agent and the polymer and this again may not be suitable for long-term visualization.

The purpose of the work described herein was to synthesize a new biodegradable MRI-visible polymer in order to provide a tool for surgeons to locate implanted polymeric prostheses. This new polymer needed to ensure that medical devices can be visualized long term by MRI: (i) the polymer must be non-water-soluble in order to remain immobilized on the surface, (ii) it should degrade slowly to provide at least 1-year post-operative MRI visualization, without permanently modifying the characteristics of the medical device, (iii) visualization must be possible until the polymer has completely degraded. Thus, the contrast agent should be linked to the monomeric units in a non-cleavable manner to be finally released concomitantly with the degradation products. To meet these specific requirements, a chelate of a contrast agent (DTPA) was grafted onto a non-water-soluble, degradable and biocompatible polymer by a covalent and non-cleavable bond. Poly(ε-caprolactone) (PCL) was selected for its biocompatibility and FDA approval, and because this polymer is known to be slowly degraded in the human body [4–6]. This polymer is not functionalized, but we have previously described a rapid, easy and versatile method to chemically activate PCL chains in a polycarbanionic form [33]. Briefly, this activation is achieved by using lithium diisopropylamide (LDA) as a non-nucleophilic strong organic base to remove one of the hydrogen atoms at the α-position on the carbonyl groups. In a second step, electrophilic reactants are then bound to the carbanionic sites.

Here, in this present study, we describe the synthesis of a new MRI-visible polymer based on the grafting of a DTPA derivative onto activated PCL, followed by the complexation of Gd^{3+} onto the grafted DTPA. The characterization of all compounds, by-products and final polymer are reported herein. In addition, the potential of this MRI-visible polymer for biomedical applications was investigated by (i) assessing the stability of the Gd^{3+} polymeric complex and the polymer coating on a polypropylene mesh, (ii) experimentally determining the device's MRI visibility and its cytocompatibility.

2. Materials and methods

PCL (Mn = 42,500 g mol^{-1}; Mw 65,000 g mol^{-1}) was obtained from Aldrich (St Quentin Fallavier, France), benzyl alcohol (purity = 99%) and palladium on activated carbon (10% Pd) were purchased from ACROS (Gell, Belgium). $MgSO_4$ and diethylenetriaminepentaacetic acid dianhydride (DTPA-diA) were supplied by Carlo Erba (Milan, Italy) and were used as received.

Lithium di-isopropylamide (LDA) (2 M in tetrahydrofuran/n-heptane); gadolinium(III) chloride hexahydrate, (99%), thionyl chloride ($SOCl_2$), dried tetrahydrofuran (THF); and dichloromethane, diethyl ether, methanol, dry dimethylsulfoxide were purchased from Sigma-Aldrich (St Quentin Fallavier, France). All chemicals and solvents were used without further purification, except THF, which was treated over benzophenone sodium until a deep blue color was obtained, and then distilled.

Polymer molar masses were determined by size exclusion chromatography (SEC) on a Waters Inc. system fitted with a PLgel 5 μm mixed-C (60 cm) (Polymer Laboratories, Les Ulis, France) column as stationary phase and a Waters 410 refractometric detector, eluted with THF at 1 ml min^{-1}. Typically, samples were dissolved in THF at 10 mg ml^{-1} and filtered through a Millex®-FH PTFE filter, pore size 0.45 μm (Millipore Corporation, Billerica, MA, USA) and 20 μl of the polymer solution were injected. The SEC was calibrated with poly(styrene) standards. A Waters PDA 2996 photodiode-array detector was added on-line for the specific detection of aromatic groups.

ATR-FTIR spectra were obtained on polymeric films cast on NaCl and recorded on a Perkin–Elmer Spectrum 100 FT-IR spectrometer using attenuated total reflectance (ATR).

^{1}H NMR spectra were recorded in d_6-DMSO on an AMX300 Bruker spectrometer operating at 300 MHz. Chemical shifts were expressed in ppm with respect to tetramethylsilane (TMS).

Relaxation times for polymers dissolved in d_6-DMSO were measured on an AMX400 Bruker spectrometer operating at 400 MHz. T1 measurements were obtained using T1 inversion/recovery sequence. This analysis was carried out at 80 °C to facilitate the molecular mobility of the polymer.

Gd^{3+} was quantified using an Element XR sector field ICP-MS (inductively coupled plasma-mass spectrometry) at Géosciences in Montpellier (Montpellier II University). Internal standardization used an ultra-pure solution enriched with indium.

2.1. Synthesis and characterization of the MRI-visible polymer (DTPA-Gd-PCL)

2.1.1. Synthesis of benzylated DTPA (Bn_2-DTPA)

Commercial DTPA dianhydride (14 mmol, 5 g) was dispersed in DMSO (20 ml) and benzyl alcohol (35 mmol, 3.6 ml) was added dropwise for complete dissolution of the mixture within a few hours. The reaction was monitored by infra-red analysis until complete disappearance of the anhydride band at 1820 cm^{-1}. After 5 h the DMSO was evaporated off and the residue precipitated in diethyl ether before being washed several times with diethyl ether.

2.1.2. Chlorination of Bn_2-DTPA (Bn_2-DTPA-Cl)

Bn_2-DTPA (5.3 mmol, 3 g) was dissolved in thionyl chloride (5 ml) and the reaction conducted at room temperature for 2 h under a flow of argon. Thionyl chloride was then evaporated under vacuum. Bn_2-DTPA-Cl was used immediately for the next reaction without any further purification.

2.1.3. Reaction of Bn_2-DTPA-Cl with PCL (Bn_2-DTPA-PCL)

Typically, PCL (26.3 mmol, 3 g) was dissolved in anhydrous THF (200 ml) by stirring in a reactor that had previously been dried overnight. The solution was carefully kept under a flow of dry argon at −40 °C. A 2 M LDA solution (35 mmol, 17.5 ml) was poured through a septum, while stirring, for 30 min. A solution of Bn_2-DTPA-Cl (5 mmol, 3 g) in dry THF was then poured into the mixture. After 30 min of reaction, the mixture was neutralized with