Acta Biomaterialia 20 (2015) 94–103

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Non-invasive deep tissue imaging of iodine modified poly(caprolactone-*co*-1-4-oxepan-1,5-dione) using X-ray

Timothy R. Olsen [a], Lundy L. Davis [c], Samantha E. Nicolau [c], Caroline C. Duncan [c], Daniel C. Whitehead [b,*], Brooke A. Van Horn [c,*], Frank Alexis [a,d,*]

[a] Department of Bioengineering, Clemson University, Clemson, SC 29634-0905, United States
[b] Department of Chemistry, Clemson University, Clemson, SC 29634-0905, United States
[c] Department of Chemistry and Biochemistry, College of Charleston, Charleston, SC 29401, United States
[d] Institute of Biological Interfaces of Engineering, Department of Bioengineering, Clemson University, Clemson, SC 29634-0905, United States

ARTICLE INFO

Article history:
Received 12 November 2014
Received in revised form 12 March 2015
Accepted 19 March 2015
Available online 25 March 2015

Keywords:
Imaging polymers
Polymeric imaging contrast agents
X-ray
Polymeric biomaterials

ABSTRACT

When biodegradable polyester devices, like sutures and screws, are implanted into the body, it is very challenging to image them in deep tissue, monitor their degradation, and detect defects. We report our recent findings on non-invasive deep tissue imaging of polyester degradation, stability and integrity using an iodinated-polycaprolactone (i-P(CLcoOPD)) X-ray imaging contrast agent. The results of experiments performed with i-P(CLcoOPD) demonstrate the feasibility to quantify *in-situ* polyester degradation *in vitro* and *in vivo* using rats. We also demonstrate that X-ray imaging could be used to identify and quantify physical defects, such as cracks, in polymeric implants using rabbit animal models. This approach enables non-invasive monitoring of polyester materials and is expected to become an important technology for improving the imaging of polymers at clinically relevant depths.

1. Introduction

In 2008, it was projected that the biocompatible materials market had an $11.9 billion market, with 88% of the sales projected to be from polymeric biomaterials [1]. Issues with permanent implantable devices include long-term biocompatibility, metal sensitivity and the chance of requiring secondary revision surgeries. For some applications, implantable devices are not needed for the lifetime of the patient, but only for the time period after injury to provide structural support and stability. Remarkably, metallic implant removal occurs 19% to 54% of the time, depending on the fracture type, which makes resorbable implants an attractive alternative [2]. Many of the permanent implantable devices used for temporary therapeutic treatment will be replaced with biodegradable polymeric devices that can help repair and regenerate damaged tissues [1]. Devices composed of degradable polyesters (i.e. polylactic acid, polycaprolactone, polyglycolic acid, poly(lactic-co-glycolic acid) have been approved by the FDA [3]. Examples of commercially available degradable polyester implants include fracture fixation devices (ComposiTCP Interference Screw by BIOMET), spinal fixation devices (Inion S-2 Biodegradable Anterior Thoraco-Lumbar Fusion System), and abdominal wall repair devices (Bio-A Hernia Plug by W.L. Gore and Associates). Nonetheless, *in vivo* performance of degradable polyester implants cannot always be predicted by mathematical modeling or common *in vitro* studies due to the complex biological environment associated with tissues and patient health.

While degradable polyester products are available for use as implantable devices, they lack the imaging contrast properties to locate the devices, monitor changes in morphology, detect cracks and defects, and quantitatively determine the degradation kinetics *in situ* using non-invasive imaging. Further, there are no suitable polyester imaging agents commercially available. Edeleman et al. developed a non-invasive imaging technique that tracks material erosion *in vivo* through a fluorescent tag covalently attached to PEG amine and dextran aldehyde, which were model biodegradable materials [4]. While novel fluorescent biodegradable polymers have addressed some of these challenges, they are not yet relevant

* Corresponding authors at: Department of Chemistry, Clemson University, 467 Hunter Laboratories, Clemson, SC 29634, United States. Tel.: +1 864 656 5765; fax: +1 864 656 6613 (D.C. Whitehead). Department of Chemistry and Biochemistry, College of Charleston School of Science and Math Building, 202 Calhoun Street, Charleston, SC 29401, United States. Tel.: +1 843 953 3690; fax: +1 843 953 1404 (B.A. Van Horn). Department of Bioengineering, Clemson University, 301 Rhodes Research Center, Clemson, SC 29634, United States. Tel.: +1 864 656 5003; fax: +1 864 656 4466 (F. Alexis).

E-mail addresses: dwhiteh@clemson.edu (D.C. Whitehead), vanhornba@cofc.edu (B.A. Van Horn), falexis@clemson.edu (F. Alexis).

http://dx.doi.org/10.1016/j.actbio.2015.03.021
1742-7061/

to deep tissue imaging, which is required for human scale use, and do not allow for monitoring of implant defects. To address the issue of deep tissue imaging of polymeric materials, radio-opaque contrast agents have been developed. The conventional approach to provide polymeric implants with X-ray contrast properties is by addition of radio-opaque fillers, like barium salts or zirconium dioxide, into the matrix of the polymer [5,6]. An attractive alternative is to attach iodine atoms on the polymer which leads to a filler-free radio-opaque polymer. Examples of this technology are the ReZolve and ReZolve2, which are coronary drug eluting stents manufactured by REVA Medical [7]. These stents are composed of a resorbable tyrosine-derived polycarbonate polymer that is radiopaque after the chemical modification of tyrosine to incorporate iodine molecules, which enables visualization with X-ray and fluoroscopy [8]. Iodine compounds have been approved by the FDA for use as a contrast agent and is commonly used in computed tomography and X-ray imaging applications [6,9,10]. An iodinized PCL was described by Nottelet et al. using a one pot reaction [11]. Here, mechanical properties, thermal properties, crystallinity, *in vitro* degradation and opactiy to x-rays were reported [11] but, three challenges still remain for their possible biomedical applications: (1) identifying and quantifying cracks, defects and changes in morphology of the polyester and (2) measuring the degradation of the polyester and (3) imaging through deep tissue. Given the common use of polymers for biomedical applications, there is a need to develop novel biodegradable polyester imaging contrast agents for non-invasive deep tissue imaging and quantification of degradation and morphology.

Here, we report a biodegradable polyester, iodine poly(caprolactone-*co*-1-4-oxepan-1,5-dione) i-P(CLcoOPD) imaging contrast agent via a grafting strategy that allows the non-invasive imaging and measuring of material characteristics through deep tissue. We hypothesized that a chemically-modified poly(caprolactone-*co*-1-4-oxepan-1,5-dione) P(CLcoOPD) could provide X-ray contrast in deep tissue and that the contrast would decrease during degradation because of the gradual decrease of iodine content in the implant [12]. The rationale of iodine functionalized P(CLcoOPD) as radiographic contrast agent is based on the fact that polycaprolactone is approved by the FDA for clinical applications and iodine is commonly used in the clinic as a radiographic contrast agent to distinguish water from soft tissues. Iodine compounds have been FDA approved for use as contrast agents, iodine is not toxic at high concentrations (600 mg/kg), and it is cleared rapidly through urine [6,9,10,13]. Most commercial organic iodine sources are aryl iodides and they have been approved by the FDA. Devices composed of PCL that have been FDA approved include Monocryl sutures (Ethicon) and Capronor contraceptive (Research Triangle Institute) [14,15]. Recently, a 510 k premarket submission has been made in which PCL is used as a bone filler for craniofacial applications [15]. In this application, iodinated benzylhydroxylamines are grafted to P(CLcoOPD) to make i-P(CLcoOPD). This approach goes beyond current polymeric imaging contrast agents by allowing for non-invasive deep tissue imaging and measurement of polymer defects and degradation profile. This contribution is significant because there are currently no methods to non-invasively measure the stability and integrity of polyester implants in deep tissue.

2. Materials and methods

2.1. Materials

D,L-lactide ($C_6H_8O_4$, PURASORB DL) was supplied by Purac Biomaterials. ε-Caprolactone (CL, 97% Sigma Aldrich) and benzyl alcohol (BnOH, >99.0% Sigma Aldrich) were distilled from calcium hydride (CaH_2) and stored under nitrogen. Tin(II) 2-ethylhexanoate ($[CH_3(CH_2)_3CH(C_2H_5)CO_2]_2Sn$, ~95%), *Meta*-chloroperoxybenzoic acid (*m*-CPBA, ⩽77%), 1,4-cyclohexandione monoethylene acetal (97%), 2-iodobenzyl bromide (97%), *N*-hydroxyphthalimide (⩾97%), triethylamine (⩾99%), and hydrazine monohydrate (64–65%) sodium sulfate (Na_2SO_4, >99%), anhydrous magnesium sulfate ($MgSO_4$, >99.5%), anhydrous toluene ($C_6H_5CH_3$, 99.8%), methanol (CH_3OH, >99.9%), and chloroform ($CHCl_3$, >99.8%) were supplied by Sigma–Aldrich. *Para*-toluenesulfonic acid monohydrate (TsOH, Sigma Aldrich) was dissolved in tetrahydrofuran (THF) to afford a 0.01 M solution. PrestoBlue Cell Viability Reagent was supplied by Life Technologies.

2.2. Polymer synthesis

Poly(D,L)-lactic acid (PDLLA) was synthesized using ring opening polymerization using lactic acid as the initiator and tin (II) 2-ethylhexanoate as the catalyst. Lactic acid, lactide monomer, and Na_2SO_4 were vacuum-dried overnight in the reaction vessel before use. Reagents were dissolved by stirring in 120 °C anhydrous toluene under N_2 gas and reflux. Tin(II) 2-ethylhexanoate was added and the reaction vessel was stirred at 120 °C for 24 h under N_2 and reflux. The next day, the polymer product was mixed three times in a chloroform and water solution, with the chloroform phase collected after each wash. Following, the polymer was dried over $MgSO_4$ and precipitated in cold methanol.

Traditional thermal ring-opening polymerization with tin (II) 2-ethylhexanoate was employed to create polycaprolactone copolymers with a predictable incorporation of co-monomer. The copolymerization of ε-CL and 1,4,8-trioxaspiro-[4,6]-9-undecanone (TOSUO) was achieved using tin (II) 2-ethylhexanoate as catalyst and residual water as the initiator at 20 wt.% monomer in dry toluene at 110 °C for 18 h. The final products were isolated from precipitation in methanol non-solvent and dried under vacuum. Subsequent removal of the ketal units was affected using trityltetrafluoroborate in dichloromethane, followed by precipitation in methanol, and isolation and drying of the solid product to yield poly(caprolactone-*co*-1,4-oxepan-1,5-dione), abbreviated P(CLcoOPD). The copolymer was characterized using ^{1}H NMR spectroscopy to determine the monomer conversion and the composition of the copolymer, P(CLcoTOSUO). Attachment of synthesized *O*-(2-iodobenzyl) hydroxylamine to the P(CLcoOPD) polymer backbones was achieved through *p*-toluene sulfonic acid-catalyzed oxime formation for 24 h in THF solution, followed by precipitation into cold methanol, isolation by filtration and drying under vacuum to yield a white solid graft copolymer, abbreviated as i-P(CLcoOPD) (Fig. 1a).

2.3. Cell culture

Primary rat aortic smooth muscle cells were cultured using Dulbeco's Modified Eagle Medium:F-12 (ATCC) supplemented with 10% fetal bovine serum (Atlanta Biologics) and 1% penicillin–streptomycin-amphotericin (MediaTech, Inc.) at 37 °C and 5% of CO_2.

2.4. Cell viability testing

Polymers were dissolved in acetonitrile (50 mg mL^{-1}) and dispensed into a 96-well plate (125 μL, 6.25 mg). Chemical compatibility was checked to ensure acetonitrile would not degrade the tissue culture plastic. Plates were left overnight under a chemical hood to evaporate the acetonitrile. Cells were seeded (50,000 cells per well) into well plates with no polymer films, PDLLA films and i-P(CLcoOPD) films and incubated for 24, 48 and 72 h. At each time point, a PrestoBlue cell viability assay was performed to quantify cell viability. The control for this study was smooth muscle cells