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# Effect of annealing on the mechanical properties and the degradation of electrospun polydioxanone filaments

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ABSTRACT

Annealing, or heat treatment, has traditionally been used as a treatment to improve the strength and stiffness of electrospun materials. Understanding the extent to which annealing can improve the mechanical properties and alter the degradation rate of electrospun polydioxanone filaments could influence the range of its potential clinical applications.

In this study, we investigated the effect of annealing electrospun polydioxanone filaments at varying times and temperatures and subsequently subjecting them to *in vitro* degradation in phosphate buffer saline for up to 6 weeks. Fibre alignment, tensile strength and thermal properties were assessed.

It was determined that annealing at 65 °C for 3 h only marginally improved the tensile strength (9 ± 2%) but had a significant effect on reducing strain and rate of degradation, as well as maintaining fibre alignment within the filament. The filament retained significantly more of its force at failure after 4 weeks (82 ± 15%, compared to 61 ± 20% for non annealed filaments) and after 6 weeks of degradation (81 ± 9%, compared to 55 ± 13% for non annealed filaments). Conversely, annealing filaments at 75°C improved the initial tensile strength of the filament (17 ± 6%), but over 6 weeks, both samples annealed at 75 °C and 85 °C otherwise performed similarly or mechanically worse than those not annealed.

These findings suggest that annealing at low temperatures is more useful as a method to tailor degradation rate than to improve mechanical properties. The ability to modulate the degradation profile with annealing may become useful to tailor the properties of electrospun materials without altering the chemistry of the polymer used. This might better match the degradation of the implant and gradual loss of mechanical properties with the new matrix deposition within the structure, enabling multiple regenerative strategies within a single biomaterial system.

## 1. Introduction

There is a need to improve the outcome of soft tissue repairs and it is indicated that the use of biomaterials will better support and guide tissue regrowth. Biomaterials made by electrospinning, a process by which microscale fibres are drawn out from a polymer solution using electrical charges, has received recent attention in the field of tissue engineering (Reneker and Yarin, 2008). These materials have been shown to have potential in mimicking the major structural components of native extra-cellular matrix, in particular collagen and elastin (Boland et al., 2005). While electrospinning allows for the manipulation of key biophysical parameters (such as fibre diameter and alignment), these materials often lack the necessary mechanical properties for eventual biomedical applications, such as scaffolds, sutures and drug delivery devices (Venugopal and Ramakrishna, 2005). Post-spinning treatments are often needed to enhance the strength and stiffness of the fibres (Tan and Lim, 2006a, 2006b; Srithep et al., 2013; Weir et al., 2004a, 2004b). Annealing, or heat treatment, is one post-spinning treatment known to change the mechanical properties of electrospun materials (Cho et al., 2011; Tan and Lim, 2006a, 2006b; Barber et al., 2013). Its effects on crystal structure, fibre alignment and on mechanical properties are documented in the literature for some polymers (Baji et al., 2010; Bonnet et al., 1999; Takayama et al., 2011; Yeh et al., 1976). Annealing some biodegradable polymers such as poly-L-lactide (PLLA) (Weir et al., 2004a, 2004b; Srithep et al., 2013; Ali et al., 1993; Tan and Lim,

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2006a, 2006b) and polycaprolactone (PCL) (Ali et al., 1993) has led to improvements in their mechanical properties. Recently, polydioxanone (PDO) is emerging as an attractive biodegradable polymer for use as a biomaterial, as it has a good safety profile with mild foreign body reaction and complete degradation between 5 months to one year, depending on manufacturing and processing conditions (Goonoo et al., 2015; Mouthuy et al., 2015; Sabino et al., 2000). It is known that heat treatment changes the microstructural properties and degree of crystallinity of aliphatic polyester polymers (like PDO), which affects the absorption rate of the polymer (Cho et al., 2011). The extent to which annealing can improve the mechanical properties and alter the degradation rate has not been explored for PDO electrospun materials but could influence the range of its potential clinical applications. Hence, the objective of this study was to quantify the effect of annealing on the mechanical and degradation properties of PDO filaments manufactured according to a method recently described (Mouthuy et al., 2015). We hypothesized that annealing will improve the mechanical properties and alter the degradation rate of PDO electrospun materials.

## 2. Materials and methods

### 2.1. Electrospun filaments

Polymer solutions were made by dissolving polydioxanone (PDO, Sigma-Aldrich; viscosity 1.5–2.2 dL/g; $T_g$ −10 to −5 °C; $T_m$ 110–115 °C) in HFIP solvent (Apollo Scientific; 1,1,1,3,3,3, -hexafluora-2-propanol) at a 9% weight to volume ratio and stirred for 24 h before electrospinning. HFIP was chosen for its easy dissolution in the polymer and its fast evaporation in electrospinning conditions (Xie et al., 2008).

A custom electrospinning apparatus with a single nozzle and a stainless steel wire collector was used to fabricate continuous electrospun filaments (made up of submicron fibres). The solution feed rate was 1 mL/h, wire feed rate was 0.5mm/s and the filaments spun under an electric field of 6.9 kV. These filaments were then manually stretched until resistance was felt (around 3.5 times their length), to increase the length and align the submicron fibres in the direction of the thread (Mouthuy et al., 2015). These drawn filaments were kept at room temperature of 25 °C in a dessicator until use.

### 2.2. Annealing

Electrospun filaments were thermally annealed at 65 °C, 75 °C, and 85 °C. It has been proposed that most polymer annealing changes occur in a range below melting temperature (Tm), generally between Tm-60 and Tm (Yeh et al., 1976). While PDO has a melting temperature of about 110 °C (Goonoo et al., 2015), the 65–85 °C temperature range was chosen for this experiment to avoid melting of the polymer chains. The filaments were heat treated for 3, 6, 9, 12 and 24 h. This annealing time range was chosen following optimization work done at 65 °C.

### 2.3. In vitro degradation

Prior to *in vitro* degradation, filaments were cut and sterilized for 2 h in 70% ethanol. Degradation of the annealed filaments was carried out in phosphate-buffered saline solution (PBS) under pH 7.4 at 37 °C in an incubator. Each set of annealed samples was removed for mechanical testing, scanning electron microscopy (SEM) and differential scanning calorimetry (DSC) characterization at time points of 3 weeks, 4 weeks and 6 weeks. Every week, the samples were washed and replaced with fresh PBS and the pH was monitored with a pH meter. Prior to analysing the degraded filaments, they were washed twice with deionized $H_2O$ and placed in a vacuum for 3 h to dry the filaments.

### 2.4. Mechanical characterization

Annealed filaments were dried and cut to 2 cm sections. Samples were tested until failure with a 20N load cell using a uniaxial tensile testing machine (Deben Stage Tensile Compression Stage, UK). Both undegraded and degraded filaments were tested and maximum force at failure (N) and clamp-to-clamp breaking strain (%) were recorded. Work done until failure (Nmm) was calculated based on the force-displacement data. Ten specimens were tested for each condition and for each experimental repeat.

### 2.5. Scanning electron microscopy (SEM)

Scanning electron microscopy (Carl Zeiss Evo LS15 VP-Scanning Electron Microscope) images from each annealing and degradation time point were taken. The samples were washed with phosphate buffered saline (PBS, Sigma-Aldrich, St. Louis, MO, USA), cut and coated with gold using a SC7620 Mini Sputter Coater System (Quorum Technologies Ltd, Laughton, UK), prior to mounting on the SEM machine. Samples were analysed in a high vacuum mode to examine changes in morphology due to degradation, such as fibre fusing or breaking. Fibre diameter was determined using ImageJ software (National Institute of Health, Bethesda, MD, USA).

### 2.6. Differential scanning calorimetry (DSC)

Polydioxanone is a semi-crystalline polymer with the glass transition temperature of about −10 °C and melting temperature of around 110 °C (Goonoo et al., 2015). A differential scanning calorimeter (TA Q2000-1275 Differential Scanning Calorimeter) was used to examine the thermal properties of PDO samples annealed at different times and temperatures. From each annealing and degradation condition, 3 to 4 mg of sample was cut and mounted in aluminium (TZero Aluminium) pans, along with an empty tin as the reference standard. The pans were heated from 25 °C to 140 °C at a heating rate of 10 °C/min in a nitrogen atmosphere. From DSC thermograms, the melting temperature (Tm), measured heat of fusion (ΔHf) and measured area of the annealing peak (derived from ΔHc), the endothermic peak found about 10–20 °C below the melting temperature (Bonnet et al., 1999), were determined.

### 2.7. Statistical analysis

Data are expressed as means with standard deviations. The statistical significance was determined by the analysis of variance (two-way ANOVA) and Tukey post-hoc test at the significance level of less than 0.05 ($p < 0.05$) using GraphPad Prism version 7 software (GraphPad Software Inc., La Jolla, CA, USA).

## 3. Results

### 3.1. Visual & SEM analysis

The morphology of electrospun PDO filaments and changes following degradation are shown in Fig. 1, for samples annealed for 3 h. Filaments in each set were of similar diameter (1.3 ± 0.5 μm). No differences in fibre arrangement could be seen due to the annealing temperature (Fig. 1a-c). Samples that were annealed at 65 °C maintained a high degree of fibre alignment and there were few visible breaks in the fibres up to 6 weeks (Fig. 1g). Samples annealed at 75 °C maintained a high level of fibre alignment after 4 weeks of degradation (Fig. 1e), but lost much of the linear organization by 6 weeks. These samples took on a more wavy appearance with visible breaks along the length of the filament (Fig. 1h). Samples annealed at 85 °C exhibited these signs of degradation by 4 weeks (Fig. 1f). By 6 weeks, the fibres had fused together, making the samples brittle and difficult to handle