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Low temperature gamma sterilization of a bioresorbable polymer, PLGA

Lisa Davison[a,*], Efrosyni Themistou[b], Fraser Buchanan[a], Eoin Cunningham[a]

[a] School of Mechanical and Aerospace Engineering, Queen's University Belfast, Belfast BT9 5AH, UK
[b] School of Chemistry and Chemical Engineering,Queen's University Belfast, Belfast BT9 5AG, UK

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ABSTRACT

Medical devices destined for insertion into the body must be sterilised before implantation to prevent infection or other complications. Emerging biomaterials, for example bioresorbable polymers, can experience changes in their properties due to standard industrial sterilization processes. Gamma irradiation is one of the most reliable, large scale sterilization methods, however it can induce chain scission, cross-linking or oxidation reactions in polymers. sterilization at low temperature or in an inert atmosphere has been reported to reduce the negative effects of gamma irradiation. The aim of this study was to investigate the impact of low temperature sterilization (at −80 °C) when compared to sterilization at ambient temperature (25 °C) both in inert atmospheric conditions of nitrogen gas, on poly(lactide co-glycolide) (PLGA).

PLGA was irradiated at −80 and 25 °C at 40 kGy in a nitrogen atmosphere. Samples were characterised using differential scanning calorimetry (DSC), tensile test, Fourier transform infrared (FTIR) spectroscopy, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy and gel permeation chromatography (GPC).

The results showed that the molecular weight was significantly reduced as was the glass transition temperature, an indication of chain scission. FTIR showed small changes in chemical structure in the methyl and carbonyl groups after irradiation. Glass transition temperature was significantly different between irradiation at −80 °C and irradiation at 25 °C, however this was a difference of only 1 °C. Ultimately, the results indicate that the sterilization temperature used does not affect PLGA when carried out in a nitrogen atmosphere.

1. Introduction

In medicine, solutions for patient care are continuously being improved. This can take the form of new products, new designs or new materials. Materials which have caught the attention of medical device manufacturers are bioresorbable polymers such as poly(lactide co-glycolide) (PLGA), polycaprolactone (PCL) and polyhydroxyalkanoate (PHA) Ulery et al. (2011). These are materials that break down in a specific time frame, which can be adapted for their purpose and are compatible with the body Makadia and Siegel (2011). They have the added advantage that, after implantation, they do not need a second, invasive removal surgery and will not affect medical imaging or future surgeries once they have broken down. These polymers have potential applications as tissue engineering scaffolds and drug delivery vehicles and are currently used for bone screws (S & N and DePuy), arterial stents (Abbott) and bone fixation plates (DePuy Synthesis). The alternatives to using bioresorbable polymers for these applications are metals such as titanium or stainless steel, which do not resorb and can cause bone resorption due to the disparity in the moduli of bone and metal Huiskes et al. (1992), Sivakumar et al. (1993).

The development of these new applications for bioresorbable polymers has led to issues with the current sterilization methods available. The favoured sterilization techniques used for industrial scale sterilization are ethylene oxide (EtO), gamma irradiation and electron beam irradiation MDDI (2004). EtO requires a typical relative humidity of 35–80% Ellab (2015) and has an optimum sterilization temperature of 55 °C Sandle (2013b). The temperature is similar to that of the glass transition temperatures of poly(lactic) acid and poly(glycolic) acid, Agarwal et al. (1997), Nakafuku and Takehisa (2004), Garlotta (2001) constituents of PLGA, and therefore it can cause changes in the polymer structure. Moreover, the humidity could initiate hydrolytic degradation. Electron beam is effective for thin, low density items Sandle (2013a), however, does not have the penetration power that gamma irradiation has. A 10 MeV beam can pass through 3–5 cm of average density material Sandle (2013a), however 10.9 cm of aluminium are required to reduce gamma radiation dose to half from a source with a dose rate of 10 kGy/h Allen et al. (1995).

Gamma irradiation is an important sterilization method and has proven very effective for single use devices, such as syringes and gloves, however, it has been found to change the properties of PLGA and other

* Corresponding author.
E-mail addresses: ldavison10@qub.ac.uk (L. Davison), e.themistou@qub.ac.uk (E. Themistou), f.buchanan@qub.ac.uk (F. Buchanan), e.cunningham@qub.ac.uk (E. Cunningham).

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lactide based polymers Jo et al. (2012). This can be a change in the Young's modulus, strain or glass transition temperature, resulting in a change in product performance. In many polymers changes in these properties can be linked to changes in the chemical structure as a result of chain scission or cross-linking during irradiation Jo et al. (2012). These changes can affect the degradation time. It was found by Konan and Haddad that when Smith and Nephew's Calaxo bone screw was used (PDLGA and calcium carbonate) its degradation profile was not as predictable in humans as it had been in the animal model, leading to a high complication rate. They observed that a lump formed under the skin of 29% of the 59 patients in their study; this ultimately caused the product to be removed from circulation Konan and Haddad (2009). In addition to this, irradiation has been found to advance the degradation, which leads to a more rapid loss in strength. Implants, therefore may not maintain sufficient mechanical strength in the body in a weight bearing application to allow the body to heal before it is broken down Jo et al. (2012), Yixiang et al. (2008).

At the minute, industrial scale gamma irradiation is carried out in air at ambient temperature, unless a device has been vacuum packed, for example. The temperature in an irradiation chamber is not controlled and when the source rack is in the active position the chamber will heat up due to the radiation. The temperature can increase to 40 °C or more depending on the outside temperature. The only way this temperature has been reduced in a standard irradiator is by putting ice or dry ice around the device and insulating it Craven et al. (2012). There are therefore limited temperature options and temperature can affect the sterilization efficacy Kennedy et al. (2005).

As a means to prevent the negative effects of gamma irradiation on polymers, it can be carried out under different conditions, such as in an oxygen free atmosphere (e.g. nitrogen or vacuum) or in low temperature environments Brown and O'Donnell (1979), Kennedy et al. (2005). The literature has indicated that sterilization under these conditions can reduce the negative effects of the irradiation on other polymers such as ultra high molecular weight polyethylene (UHMWPE) Premnath et al. (1996). There are very few studies that look at the combined effects of both low temperature and nitrogen atmosphere sterilization on polymers, in particular bioresorbable polymers.

Loo et al. (2005a, 2005b, 2014) have reported on the effects of electron beam (e-beam) irradiation on PLGA and Poly(l,lactic acid) (PLLA). The purpose of their work was to understand the degradation effects of the e-beam irradiation on these polylactide based polymers. They have found that crystallinity affects degradation due to the cage effect and that the mechanism of degradation changes from chain scission to hydrogen abstraction at higher doses (>200 kGy). However, this was the case for electron beam, not gamma radiation and they did not investigate temperature effects.

Montanari et al., (2001, 1998) have identified the radicals which are produced during gamma irradiation of PLGA using electronic paramagnetic resonance (EPR) spectroscopy. They compared irradiation at room temperature in air to −196 °C in a vacuum in one study and included room temperature in a vacuum in a second study. They identified that the radicals changed depending on the temperature and whether or not the polymer was in a vacuum or oxygen.

Bittner et al. (1999) studied tetracycline-HCl-loaded and placebo poly(DL-lactide-*co*-glycolide) microspheres at −80 °C in nitrogen, but did not carry out a “standard” process at room temperature for comparison.

The aim of this study was to quantify the effects of gamma irradiation on PLGA when irradiated at room temperature and at low temperature (−80 °C) in a nitrogen atmosphere.

2. Materials and methods

2.1. Sample preparation

PLGA 85:15, batch number 0912000786, supplied by Corbion Purac (Netherlands), was compression moulded in a Collins P200P platen press; the regime is shown in Table 1 as developed by Simpson et al. (2014).

Table 1
Platen Press Regime for PLGA.

Stage	1	2	3
Pressure (MPa)	0	10	crash
Temperature (°C)	200	200	cool
Time (s)	180	240	

A mass of 17 g of PLGA was placed in a square mould, dimensions 100 mm × 100 mm × 1 mm, with a polytetrafluoroethylene (PTFE) base sheet, of 0.23 mm thickness, supported by a 120 mm × 150 mm × 2 mm steel plate. A second PTFE sheet and steel plate were placed on top of the mould, then this was put into the platen press. Samples were stored in a desiccator after preparation to prevent prolonged exposure to moisture. Twenty samples were cut from the sheets into tensile bars using a cutter and a Ray-Ran Hand Operated Test Samples Cutting Press. Offcuts were kept for testing with various other characterisation methods. The cutter had the following gauge dimensions: length = 20 mm, width = 5 mm and depth = 1 mm.

The samples, along with some offcuts, were placed on a steel plate. A second flat steel plate (0.33 kg) was also placed on top of the samples to ensure they did not deform during annealing. They were then annealed in an oven for 4 h at 100 °C.

2.2. Irradiation treatment

Irradiation was carried out on tensile dumb-bell samples with additional 250 mg offcuts for GPC, NMR and DSC analysis. The samples were irradiated in Nordion Inc.'s modified Gamma Cell 220 (Ottawa, Canada) at 40 kGy and at temperatures of −80 or 25 °C in a nitrogen atmosphere at a dose rate of 16.4 kGy/h. The gamma cell irradiation chamber was calibrated and is traceable to a national standard laboratory.

The samples were placed in a holder, designed for the Gamma Cell 220, which was then placed in a custom-made chamber and inserted in the gamma cell. The exposure time was then set according to the activity of Co-60 and the temperature allowed to adjust to the target temperature, −80 or 25 °C. The samples were lowered into the irradiation chamber and the timer started. Samples were automatically ejected from the irradiation chamber when they had been in for a sufficient time to reach the specified dose.

Four types of samples were measured; controls at room temperature (25 °C) and low temperature (−80 °C) and irradiated specimens at the same two temperatures.

2.3. Differential scanning calorimetry (DSC)

DSC analysis was performed on a Perkin Elmer DSC 6. A heating rate of 10 °C per minute from 30 °C up to 180 °C was applied in a nitrogen purge gas. The samples were then held at 180 °C for 3 min before being cooled to the initial temperature, at the same rate. They were then reheated once at the same heating rate. DSC was performed on control, irradiated samples and on the raw material for comparison, n = 3. Samples weighing approximately 15 mg were sealed in aluminium pans before being inserted into the DSC. Glass transition temperature (T_g) was calculated using the half width of the T_g peak using the Perkin Elmer software - Pyris 6. Melting temperature (T_m) was recorded from the highest point of the crystallisation peak and change in enthalpy (ΔH) was measured between 95 and 165 °C for each sample.