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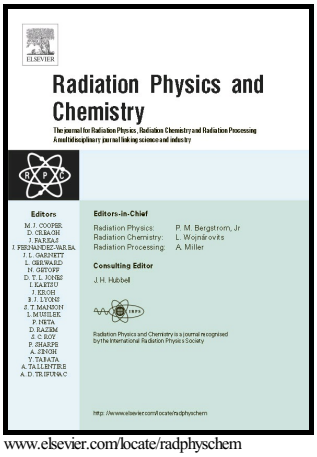

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Low Temperature Gamma sterilization of a Bioresorbable Polymer, PLGA

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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 40kGy 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.

Keywords: Poly(lactide co-glycolide) (PLGA), Gamma Irradiation, Bioresorbable, Low Temperature, Inert 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 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

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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. (2005b,a, 2004) 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 x 100 mm x 1 mm, with a polytetrafluoroethylene (PTFE) base sheet, of 0.23 mm thickness , supported by a 120 mm x 150 mm x 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 hours 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 minutes 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 ($\triangle$H) was measured between 95 and 165°C for each sample.

2.4. Tensile Testing

Tensile testing was carried out according to ISO 527-1 to investigate changes in the mechanical properties of all samples. Tensile bar samples were tested at a strain rate of 10 mm/min on an Instron 5564 with a 2 kN load cell and mechanical wedge grips at room temperature, n = 5. Tensile strength, percentage strain to failure and Young's modulus were calculated from the load-extension data.

2.5. Gel Permeation Chromatography (GPC)

GPC measurements were carried out in chloroform on a Malvern Viscotek GPC Max using a Malvern triple detector array 305 detector. Approximately 20 mg of PLGA were dissolved in 10 ml of chloroform overnight, then filtered through 0.45 μm PTFE syringe filters (Phenex 15 mm) and used for analysis. The system was calibrated with poly(methyl methacrylate) (PMMA) standards (Easivial, batch no. VPM-007, Agilent Technologies). GPC was carried out on all sample treatments. Two PLgel mixed bed columns with dimensions 300 x 7.5 mm, a flow rate of 1 ml/min and a temperature of 30°C were used for measurements. Weight average molecular weight (M_w), number average molecular weight (M_n) and polydispersity index (PDI) values of the samples were determined.

2.6. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR measurements were carried out using a Perkin Elmer Spectrum 100 spectrometer. The spectrometer had a diamond topped ZnSe crystal and the scanning technique used was attenuated total reflectance. A razor blade was used to create shavings (from fractured tensile bars) which were used as samples; 32 scans were completed per sample at room temperature. The acquisition range used was 4000-650 cm^{-1}.

2.7. Proton Nuclear Magnetic Resonance (^1H NMR) Spectroscopy

All ^{1}H NMR spectroscopy measurements were performed on a Bruker Ultrashield Plus 400 Hz spectrometer. For the analysis, a concentration of 3 mg/ml of PLGA (both irradiated and unirradiated samples) was dissolved in deuterated chloroform.

2.8. Statistical Analysis

One way ANOVA was performed on SPSS Statistics 20 to define if differences were significant between the irradiated samples, controls and raw material (significant if $p \leq 0.05$). Tukey post hoc tests were used.

Design of experiments was used to detect interaction or trends between the variables; irradiation dose and irradiation temperature. A full factorial design was used in DX8 software, Stat-Ease Inc., USA. The dependent variables that were used were glass transition temperature, melting temperature, change in enthalpy, percentage strain ($\%\varepsilon$) and M_n.

3. Results

3.1. DSC

The T_g, T_m and $\triangle$H were measured for all samples. The T_g decreased by 3-4°C from 59 to 55 or 56°C (low temperature and room temperature, respectively) when the samples were irradiated at 40 kGy, as seen in Figure 1. The difference in T_g between unirradiated and irradiated samples was significant, $p < 0.001$.

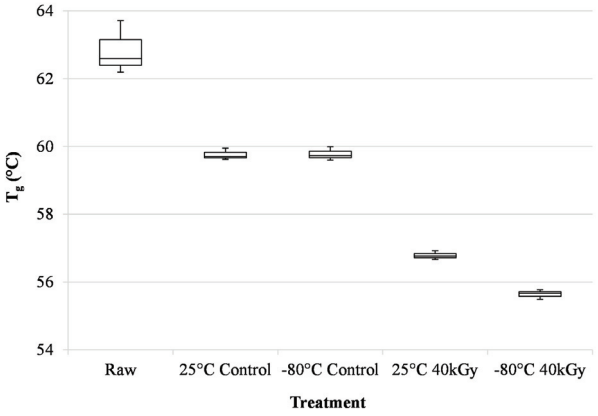

Figure 1: Glass transition temperature of raw, control and irradiated samples

After irradiation, a second peak appeared on the DSC graphs (Figure 2 and 3) in the crystal melting area at a higher temperature of 156°C, in comparison to a melting temperature of 144°C prior to irradiation. This was a difference of 11-12°C due to the irradiation process (Figure 2). There was no difference between the samples irradiated at 25°C and -80°C which can be seen in Figure 2. For the T_m the difference between the unirradiated and irradiated samples was significantly different, $p < 0.001$.

Like the melting temperature, the $\triangle$H was also found to increase after irradiation, by approximately 5 J/kg, this is shown in Figure 4.

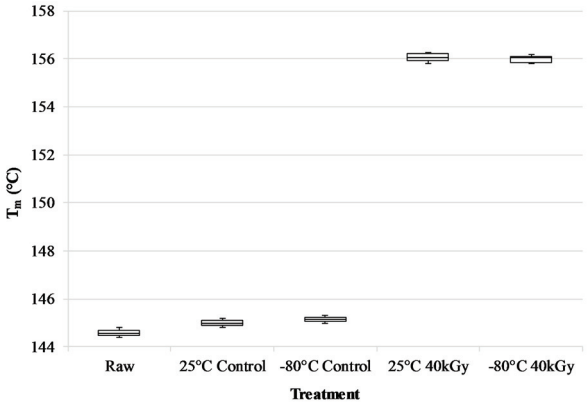

Figure 2: Melting temperature of raw, control and irradiated samples

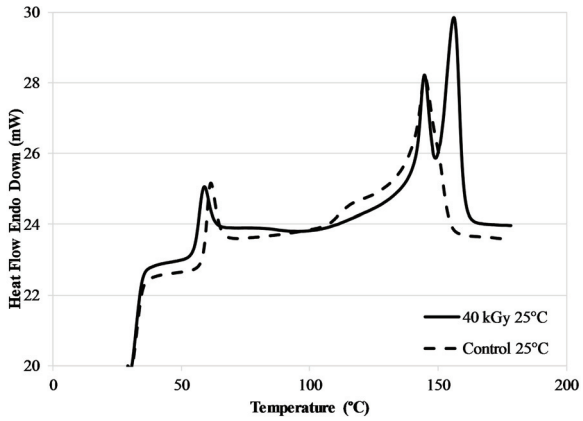

Figure 3: DSC plot of room temperature control and irradiated sample

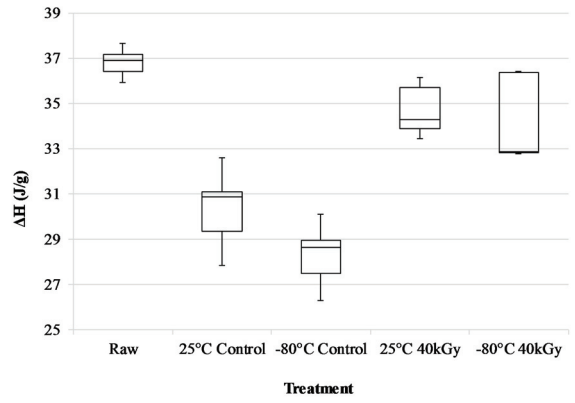

Figure 4: Change in enthalpy of raw, control and irradiated samples

3.2. Tensile Testing

The percentage strain to failure and modulus results are shown in Figure 5, one way ANOVA showed no significant difference. The -80°C irradiated samples percentage strain results did not fit to the binomial distribution condition, therefore this sample was not included in the ANOVA calculations, all other samples were found to be significantly similar ($p > 0.999$).

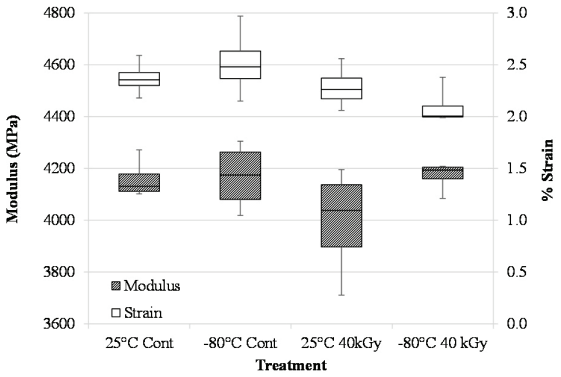

Figure 5: Modulus and percentage strain before and after irradiation

3.3. GPC

The M_w and M_n decreased to approximately a third of the initial value after irradiation as can be seen in Table 2 and Figure 6. The M_n dropped from approximately 162 800 g/mol at room temperature and 187 200 g/mol low temperature to 48 300 at room temperature and 49 700 g/mol low temperature. There was no significant difference in molecular weight as a result of irradiation temperature.

The PDI values of the polymer samples are also shown in Table 2. It can be seen that the irradiated samples also had slightly higher PDI values, however, the results were not significant, $p = 0.090$.

Table 2: Molecular weight and polydispersity index values of PLGA before and after irradiation determined by GPC ($CHCl_3$)

	25°C Control	-80°C Control	25°C 40 kGy	-80°C 40 kGy
M_n	162 800	187 200	48 300	49 700
PDI	2.4	2.1	2.8	2.5

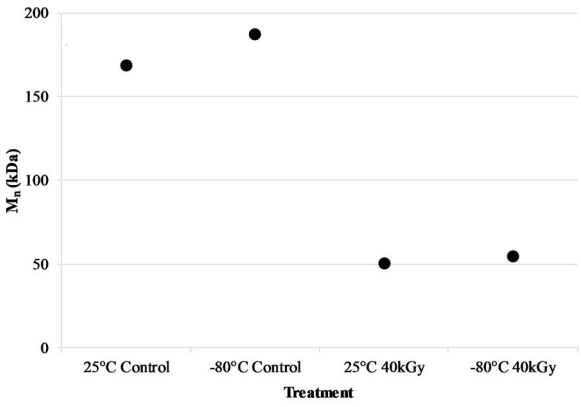

Figure 6: Number average molecular weight of control and irradiated samples by GPC ($CHCl_3$)

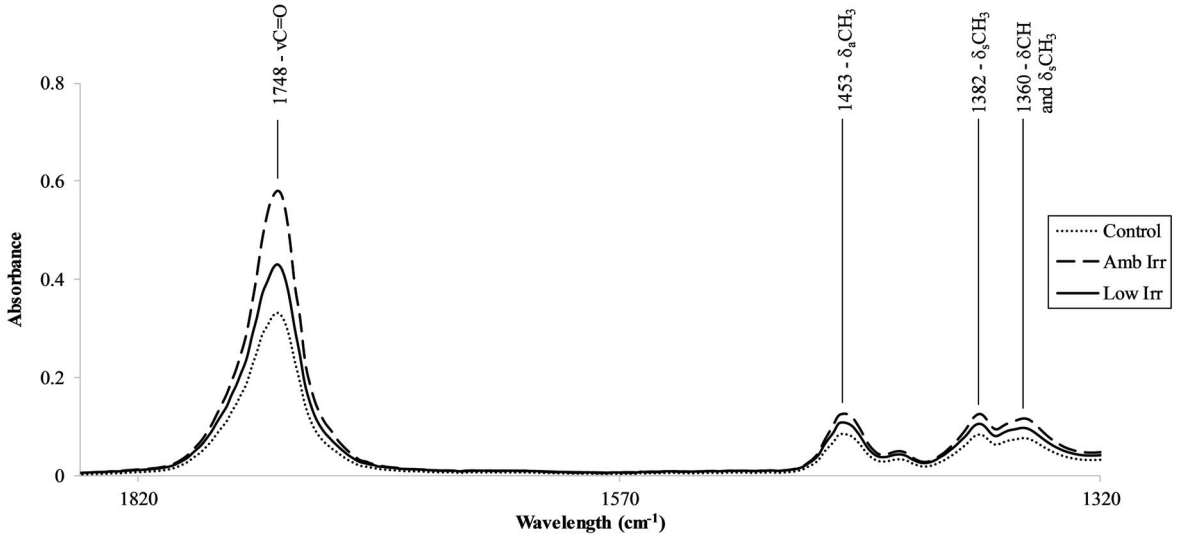

Figure 7: FTIR spectrum of unirradiated PLGA

3.4. FTIR

The key bonds identified from the FTIR spectra are shown in Figure 7. A number of changes were observed in the spectra after irradiation, especially for methyl groups, CH_3 and CH, and carbonyl bonds, C=O. Changes in the peak heights were observed as well as changes in the peak shapes.

Figure 7 shows broadening and splitting of the carbonyl peak at 1748 cm^{-1} for an ambient irradiated sample and the CH/CH_3 at 1360 cm^{-1}. There was a noticeable change in peak height and peak shape of the low temperature irradiated sample. The low temperature irradiated sample had a more domed shape than the other samples and was smaller in height relative to the 1382 cm^{-1} peak. These trends however were not consistent as the control sample had slight splitting of the peak in Figure 7.

3.5. ^{1}H NMR

The ^{1}H NMR spectra showed the characteristic peaks of PLGA which were seen in the literature Zhou et al. (2003). The structure of PLGA is shown in Figure 8. The peaks observed were from the protons in the polymer structure and the solvent peak at 7.26 ppm (in Figure 8): CH at 5.12 ppm, the CH_2 at 4.77 ppm and CH_3 at 1.51 ppm. No changes were observed in peak height, area or shift after irradiation at either temperature

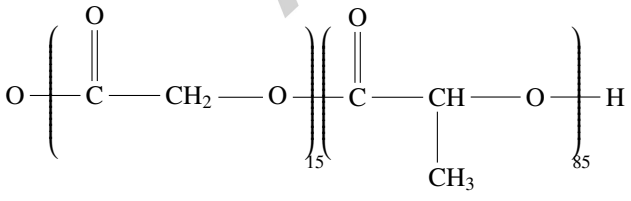

Figure 8: Chemical Structure of PLGA

4. Discussion

The effect of gamma radiation on different materials varies. In this study, PLGA 85:15 was investigated at different irradiation temperatures in a nitrogen atmosphere and the results showed that the polymer was affected by the irradiation. The 3-4°C reduction in T_g seen in Figure 1 was due to chain scission. Chain scission causes an increase in the free volume, this is created at chain ends. This results in more free space within the structure, allowing more chain movement. Due to this increase in chain movement the temperature must be lower than previously to stop the movement and form a glassy structure Uskokovic and Ignjatovic (2007). The same pattern was observed irrespective of irradiation temperature. The decrease in molecular weight after irradiation (observed by GPC analysis), seen in Figure 6, also confirms this. The difference between the raw and unirradiated control samples was considered to be the result of the heat introduced during sample preparation. A reduction in T_g due to the irradiation could cause issues when implanting this polymer into the body as it brings the T_g closer to body temperature. This could cause the polymer to lose its structural stability and therefore deform under load instead of supporting the tissue.

A significant difference of 1°C was observed in the T_g between the samples irradiated at low temperature and room temperature. Whilst this difference was statistically significant, a 1°C reduction change in T_g will not have an effect on the properties or how the polymer behaves in the body. The irradiation dose used in this study - 40 kGy - assures sterility in the majority of cases, therefore lower doses should have less of an effect.

An additional peak was observed in the DSC thermographs after irradiation for all samples, an example of which is shown in Figure 3. This peak was in the melting temperature range and was at a higher temperature of 156°C compared to 144°C for unirradiated samples. This is very similar to the results of Wang and Mano (2009) who found that annealing time and temperature can affect height, number and melting temperature of peaks. It was significantly higher by 11-12°C and had a larger enthalpy, as shown in Figure 4. The new peak indicates that there was an increase in either the crystal size, the perfection

of the crystals (i.e. ordered in the lowest energy structure) or a combination of both in some areas of the polymer Hohne (2002). The control and irradiated samples were exposed to the same conditions before and after irradiation, for the same length of time, therefore this increase in melting temperature has been attributed to the irradiation process. A possible reason for this change is that in both the ambient and low temperature treatments, the temperature at which the irradiation treatment was carried out was below the glass transition temperature of the polymer, so the polymer chains were equally rigid in both situations. When samples were irradiated, the energy provided by the radiation then gave the chains energy to realign and form larger/more ordered crystals, irrespective of the actual temperature.

The area under the melting peaks (enthalpy) was also found to increase after irradiation as mentioned previously. It can be seen in Figure 4 that before irradiation, the enthalpy was much less than the raw material, but the irradiation process increased it. The area under the melting peak is an indication of the degree of crystallinity (DoC), therefore this was increased by irradiation. In the work by Wang and Mano (2009) the melting temperature of PLGA was found to be affected by the annealing time and temperature. In this study therefore it seems like the irradiation process is having an effect similar to the annealing process. An increase in the degree of crystallinity could cause a change in the degradation profile *in vivo* as crystalline regions take longer to break down than amorphous regions Masatsugu and Katsuyuki (1997).

As mentioned, the control samples have a lower DoC than the raw material, therefore, it can be concluded that the annealing process did not provide fully crystalline samples. In order to achieve the initial DoC, it might be necessary to change the annealing temperature or time.

The effects of irradiation on the mechanical properties of the PLGA were found to be insignificant. As seen in Figure 5, all of the strain values were between 2 and 3%. The moduli also showed no significant differences, with the majority of the results between 4 and 4.2 GPa. The moduli and strain were expected to decrease, in accordance with the literature Ghosh et al. (2015), however this was not the case. It is possible that there was an increase in strength due to the crystallinity balanced by a similar decrease in strength and strain, caused by the chain scission. However, this lack of change means that the mechanical strength of the polymer has not decreased, so it will still be suitable for a structural role in the body. The drop in molecular weight after sterilization could, however, cause the degradation rate to increase, so the device would not retain its strength as long as an unirradiated device. The effect of the irradiation treatment (low temperature, nitrogen atmosphere, etc.) on the properties during degradation is therefore an area which warrants further investigation.

The increase in PDI value observed in the GPC data would be expected when random chain scission occurs Hsu et al. (2012). The small change was not significant but could be an indication of random scission, as opposed to the scission of chain ends which occurs in hydrolytic degradation. This is in agreement with the ^{1}H NMR results which did not show indications of monomer presence.

The differences in the FTIR were subtle and the main changes occurred in the carbonyl and methyl groups as shown in Figure 7. FTIR did not allow identication of which bonds were broken down during the irradiation process, this could be due to the chain scission process being random and therefore there was no pattern in in where the chain scission occurred Oliveira et al. (2012). The spectra did indicate that the double oxygen bonds and the hydrogen bonds were most affected, which could be due to recombinations of radicals as a result of the cage effect - where radicals are trapped in the structure by the crystalline portions and recombine with the polymer Loo et al. (2005a). Loo et al. (2005a) proposed that at higher electron beam radiation doses (> 20 MRad), hydrogen abstraction occurs due to alkyl free radicals reacting with oxygen trapped in the structure . According to Kurtz (2015) oxygen-oxygen bonds are most susceptible to breaking in air at 70-80°C (Bolland's cycle), therefore the radicals could react with the double oxygen bonds in the polymer or hydrogen abstraction may have occurred. The FTIR results also showed that the oxygen bonds in the spectra were less consistent and therefore may have been more affected by the irradiation process, however, there were no trends with irradiation temperature identified.

The γ and δ CH and CH_3 methyl groups are the most affected by irradiation; these are the peaks at 1382 and 1360 cm^{-1} in Figure 7. The change in hydrogen bonds could be a result of hydrogen extraction by radicals to form ROOH, peroxy radicals Kurtz (2015). When a hydrogen was extracted from a CH_3, the chemical structure changed subtly, therefore the peaks did not change position but may have experienced more slight changes, such as the development of shoulders or splitting of peaks. Again there were no trends found as a result of sterilization temperature.

The ^{1}H NMR ($CDCl_3$) spectra appeared to be very similar before and after irradiation. After irradiation, no apparent changes in the area, position or shape of the peaks corresponding to the three characteristic protons of the polymer structure were observed in the spectra of the two different repeating monomer units of PLGA (Figure 8).

In the study by Montanari et al. (2001) on a similar polymer, they proposed from their EPR results that in the vacuum, hydrogen abstraction occurs. Upon the admission of air, and therefore oxygen, peroxy radicals are created which degrade the polymer via chain scission.

Design of experiments software was used to analyse the interaction between the irradiation dose (0 or 40 kGy) and the irradiation temperature (-80 and 25°C). The software indicated that there was no combined effect of the dose and temperature on the polymer for any of the output variables - glass transition temperature, melting temperature, change in enthalpy, molecular weight and percentage strain. There was no benefit of irradiating the polymer at a temperature other than room temperature.

5. Conclusions

In summary, the results showed that gamma irradiation caused property changes commonly associated with chain scission in PLGA when irradiated in nitrogen at 40 kGy compared

to unirradiated samples. The extent of the chain scission was not affected by the irradiation temperature according to the characterisation methods carried out in this study.

FTIR spectra showed that the carbonyl and methyl groups tended to be affected by irradiation, as changes in the spectra were observed for their characteristic peaks. The ^{1}H NMR and mechanical test data did not show any significant differences before and after irradiation or as a result of sterilization temperature.

Design of experiments (DX8) did not show an interaction between the irradiation dose and irradiation temperature.

Whilst low temperature irradiation has been found to reduce the impact on other polymers or bone, it had minimal effect for this PLGA. A comparison of room temperature irradiation in air and in nitrogen will be carried out to see if the nitrogen is an improvement on the standard process. However, it is worth noting that the mechanical strength was not affected, so for PLGA devices which have a structural role, gamma radiation sterilization is suitable. If it is not possible to change the process, then the final option would be to modify the polymer properties as lactide based polymers have been found to be highly customisable.

6. Acknowledgements

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7. References

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