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Irradiation of Bioresorbable Biomaterials for Controlled Surface Degradation

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Highlights

- Electron beam irradiation of bioresorbable polymer.
- Controlled surface degradation.
- Controlled release of incorporated agent.

Abstract

Bioresorbable polymers increasingly are the materials of choice for implantable orthopaedic fixation devices. Controlled degradation of these polymers is vital for preservation of mechanical properties during tissue repair and controlled release of incorporated agents such as osteoconductive or anti-microbial additives. The work outlined in this paper investigates the use of low energy electron beam irradiation to surface modify polyhydroxyacid samples incorporating beta tricalcium phosphate (β-TCP). This work uniquely demonstrates that surface modification of bioresorbable polymers through electron beam irradiation allows for the early release of incorporated agents such as bioactive additives. Samples were e-beam irradiated at an energy of 125keV and doses of either 150kGy or 500kGy. Irradiated and non-irradiated samples were degraded in phosphate buffered saline (PBS), to simulate bioresorption, followed by characterisation. The results show that low energy e-beam irradiation enhances surface hydrolytic degradation in comparison to bulk and furthermore allows for earlier release of incorporated calcium via dissolution into the surrounding medium.
1. Introduction

Bioresorbable polymers have gained increasing interest over recent decades with regards to biomedical applications. Early applications of these polymers were mostly for sutures. However, this has expanded to include implants for trauma surgery; drug carriers; delivery devices in gene therapy and 3D porous scaffolds [Gogolewski, 2000]. These polymers degrade in-vivo to non-harmful by-products which in turn can be metabolised and enter the general metabolic pathways [Venkatraman et al, 2008]. Problems associated with non-degradable implants can be avoided with the use of bioresorbable implants. For example, in comparison to metals, the use of bioresorbable polymers overcomes problems such as corrosion, debris formation and implant removal. The main type of bioresorbable polymers used for implants are polyhydroxyacids, such as poly(lactic acid), poly(glycolic acid) and poly(lactic-co-glycolic acid) [Gogolewski, 2000; Schwach and Vert, 1998].

Polyhydroxyacids mainly degrade via bulk hydrolytic degradation [Grizzi et al, 1995; Hakkarainen et al, 1995; Vert et al, 1990]. Bulk (or homogeneous) degradation occurs when the rate of hydrolytic degradation is slow compared to the diffusion of the medium into the polymeric sample. This causes the entire cross section of the sample to experience degradation followed by erosion [Gopferich, 1997]. Bulk degradation can lead to a heterogeneous phenomenon whereby degradation of the sample’s inner core occurs ahead of the rest of the sample. This has been attributed to acidic degradation products accumulating in the centre of the sample and catalysing the degradation reactions [Cameron et al, 2004; Gopferich, 1996; Gopferich, 1997; Schwach et al; 1999].

Bulk degradation is detrimental to the long term preservation of mechanical properties and to tissue in-growth as the implant retains mass but no useful strength. Furthermore, this can lead to a late acid burst caused by a build up of degradation by-products within the sample [Cairns et al, 2011a; Leonard et al, 2006]. Bulk degradation can also inhibit controlled release of incorporated agents such as osteoconductive or anti-microbial additives as their release rate tends to follow the pattern of degradation [Cameron et al, 2002; Cameron et al 2004; Hurrell et al, 2003]. Therefore, in order to improve the long term mechanical properties and the release profiles of additives, surface degradation should ideally be initiated ahead of bulk degradation. This would allow the implant to retain inner strength, whilst releasing bioactive agents from the surface [Gopferich, 1996].
Literature shows that low energy electron beam (e-beam) irradiation has been successfully utilised to initiate surface degradation ahead of bulk degradation for polyhydroxyacids. This is due to e-beam irradiation causing polymer chain scission, decreasing molecular weight, therefore advancing the surface hydrolytic degradation process [Cairns et al, 2011b]. Low energy e-beam irradiation is suited for surface modification due to its low (and controllable) penetration capability although higher e-beam irradiation doses than used for conventional sterilisation procedures are preferable to cause appropriate levels of chain scission of the irradiated area of the sample per unit volume [Cairns et al, 2011a, 2011b; Leonard et al, 2006; Loo et al, 2005].

Increasingly additives such as calcium fillers and anti-microbials are being incorporated into polyhydroxyacid implants for use in orthopaedics [Ashammakhi et al, 2006; Daculsi et al, 2011]. Integrating bioactive ceramics into biodegradable polymers is advantageous for the biocompatibility and osteointegration of the implant. One common example is beta-tricalcium phosphate (β-TCP) filler particles within polyhydroxyacid polymers. These particles exhibit osteoconductive properties and allow for the formation of a bone bonding interface [Deculsi et al, 2011]. However, they are trapped within the polymer matrix until such time as sufficient degradation has occurred to expose them to the in-vivo environment. It is hypothesised that the application of e-beam irradiation to biodegradable polymers, in particular polyhydroxyacids, has the potential to improve control of the release of such additives.

The aim of this study was to investigate the use of e-beam irradiation for surface modification of polyhydroxyacid samples incorporating β-TCP in order to enhance the early release of the calcium phosphate.
2. Materials and methods

2.1 Materials

The PLGA, incorporating β-tricalcium phosphate (PLGA β-TCP), used in this study was processed by Smith and Nephew (Mansfield, MA, USA) by melt compounding and was supplied in pellet form (Smith and Nephew proprietary formulation). The composition of the product used was, by weight, 65% Poly-L-lactide-co-glycolide (85:15) and 35% calcium sulphate and β-tricalcium phosphate (20:15). The grain size of the product was 4 microns.

2.2 Sample Preparation

Using a Collins P200 P computer controlled Platen Press the pellets of PLGA β-TCP were compression moulded into sheets of dimensions 100x100x1mm. A moulding temperature of 200°C and a pressure of 10MPa were used, followed by crash cooling. Samples were compression moulded at 200°C for 5 minutes, 30 seconds. Gel Permeation Chromatography (GPC) was carried out in order to ascertain whether the PLGA experienced any significant degradation during the compression moulding. Table 1 shows the molecular weight results for samples of the PLGA β-TCP pre, and post compression moulding.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Molecular Weight Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Compression Moulded</td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>266,614</td>
</tr>
<tr>
<td>A2</td>
<td>268,996</td>
</tr>
<tr>
<td>A3</td>
<td>261,811</td>
</tr>
<tr>
<td>A4</td>
<td>269,358</td>
</tr>
<tr>
<td>A5</td>
<td>271,718</td>
</tr>
<tr>
<td>Compression Moulded</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>261,806</td>
</tr>
<tr>
<td>B2</td>
<td>258,114</td>
</tr>
<tr>
<td>B3</td>
<td>254,891</td>
</tr>
<tr>
<td>B4</td>
<td>240,389</td>
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The GPC results show that there has been a minor decrease in molecular weight after compression moulding, suggesting that a minor degree of polymer degradation has taken place. However, the decrease is small and is therefore likely not have had a significant effect on the polymer properties.
Flexural bar samples of dimensions 80 x10x1mm (conforming to ISO6602) were then cut using the Ray Ran RR/HCP hand press. The flexural bar samples were annealed at 100°C for 4 hours.

2.3 Surface Irradiation

Flexural bar samples were irradiated using a DC-beam electron accelerator (80-125keV) at Risø High Dose Reference Laboratory, Technical University of Denmark. Samples were irradiated with 125keV electrons at surface-doses [Helt-Hansen et al. 2010] of either 150kGy or 500kGy under ambient pressure and temperature in air. Samples irradiated at 500kGy were irradiated in four passes to ensure that the surface temperature of the test pieces was kept well below 100°C. Only one 80x10mm² surface was irradiated for each sample.

2.4 Mass Loss Study

Irradiated and non-irradiated samples were statically degraded for 28 days to determine the degree of mass loss from the respective samples. Individual samples were pre-weighed and then immersed in 120ml of phosphate buffered saline solution (PBS) at pH 7.4. The study was conducted at 47°C, within an air-circulating oven, to accelerate degradation. Both pH and temperature were monitored throughout the study with samples being removed from the PBS at time points of 3, 7, 17 and 28 days in order to record their wet and dry masses (with the residual PBS being retained for analysis). Prior to dry mass measurement, samples were placed on Grade 1 filter paper and rinsed with distilled water. The samples were then placed in the SL Shel Lab vacuum oven and dried at a temperature of 37°C and a pressure of 70mm/Hg for 24 hours.

2.5 Inductively Coupled Plasma Mass Spectrometry (ICP)

Using the Perkin Elmer Optima 4300DV ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer), ICP was carried out on the residual PBS solution used during the mass loss study once each sample had been removed. This was to ascertain the extent of calcium release from the samples that occurred at each time point. PBS solution tested was diluted by an appropriate factor using deionised water to achieve a concentration range within the detection limits of the instrument.
2.6 Four Point Bend Analysis

Using the Lloyds EZL600R testing machine, four point bend testing was conducted on vacuum dried samples to ascertain the effect that irradiation and degradation testing had on the mechanical properties of the samples. A test rig was used with an upper span of 10mm and a lower span of 20mm. A crosshead speed of 5mm/min was used and testing was stopped upon samples failure or at a maximum deflection of 3mm if complete failure had not occurred. Irradiated samples were tested with their irradiated side in contact with the lower span (i.e. in tension).

2.7 Scanning Electron Microscopy (SEM)

Microscopy (JEOL 6500 FEG SEM) was carried out to qualitatively observe the extent of surface degradation that occurred on the non-irradiated and irradiated samples during the mass loss study. Samples were prepared for SEM by sputter gold coating following vacuum drying. Secondary electron images (SEI) were collected at an accelerating voltage of 3keV.

2.8 Sessile Drop Testing – Contact Angle Measurement

Sessile drop testing was carried out in order to determine the contact angle and hence the wettability of irradiated and non-irradiated samples’ surfaces. Using the FTÅ 200 apparatus, one droplet of deionised water was applied to the sample surface. Using an APPR B/W camera an image was taken of the droplet immediately after contacting the surface. The contact angle was calculated using FTÅ 32 software.

2.9 Statistical Analysis

Tests for statistical significance were performed using GraphPad InStat, One-Way ANOVA at thresholds of $P>0.05$, $P<0.05$, $P<0.01$ and $P<0.001$. $P>0.05$ shows that the results have no significant difference, $P<0.05$ shows that there is reasonable significant difference, $P<0.01$ shows that there is a good degree of significant difference and finally $P<0.001$ shows there is very high significant difference.
3. Results

3.1 Mass Loss Study

Non-irradiated (NI) samples lost no significant mass during the study (Fig. 1) (P>0.05). Samples irradiated at 150kGy showed an increasing trend in mass loss, but this was not found to be statistically significant when compared to the non-irradiated samples (P>0.05). Samples irradiated at 500kGy showed significant mass loss at 28 days when compared to the non-irradiated samples at all time points (P<0.001).

![Graph showing mass change over time for different treatments](image)

**Fig. 1a** % Mass Change for Vacuum Dried Samples, Non-Irradiated (NI) or Irradiated at 150 or 500 kGy, n=5, Average ± Standard Deviation (SD)
The pH of the PBS solutions containing the test specimens decreased over time (Fig. 1b). However, this was more pronounced for the irradiated samples, especially the samples irradiated at a dose of 500kGy. When compared to the non-irradiated samples there was a significant decrease at 12, 17 and 28 days (P<0.001) for both irradiation doses.

Fig. 1b Change in pH of PBS Solution containing Non-Irradiated (NI) and Irradiated (150 and 500 kGy) Specimens, n=5, Average ± SD
3.2 Mechanical Analysis

Maximum bending strength (Fig. 2) decreased significantly in over time for all samples (P<0.05). The greatest decreases occurred between 17 and 28 days. Maximum bending strength also decreased significantly for irradiated samples compared to non-irradiated samples at the same time points, including the initial strength at day 0 (P<0.001). However, there was no significant difference between the two sets of irradiated samples (P>0.05).

Fig. 2  Average Maximum Bending Strength for Non-Irradiated (NI) and Irradiated Samples, n=6, Average ± SD
3.3 Inductively Coupled Plasma Mass Spectrometry Analysis (ICP)

Calcium release displayed an increasing trend over time for all samples, however, this was shown not to be statistically significant for the non-irradiated and irradiated (150kGy) samples (P>0.05). Samples irradiated at 500kGy did generally show a significant increase in calcium release over time (P<0.001). The samples irradiated at 500kGy showed significantly greater calcium release at 28 days compared to non-irradiated and irradiated (150kGy) samples (P<0.001).

Fig. 3  ICP Monitoring of Calcium Release into PBS Solution during Degradation Studies, n=3, Average ± SD
3.4 Scanning Electron Microscopy (SEM)

Fig 4a shows that the non-irradiated side of the sample underwent no visible degradation. Fig 4b shows that the irradiated side of the samples underwent considerable surface degradation. This is shown by the cracking on the surface of the irradiated side which was very evident.
3.5 Wettability Analysis

Contact angle (Fig. 5) decreased to a greater extent over time for irradiated samples compared to non-irradiated samples (P<0.001). This was the case for all time points. However, there was no significant difference between the contact angles for the irradiated samples at 3 and 7 days (P>0.05). Furthermore, there was little difference between the contact angles for the two sets of irradiated samples for these times (P<0.05).

Fig. 5  Contact Angles for Non-Irradiated and Irradiate Samples, n=3, Average ± SD
4. Discussion

The mass loss study was carried out at an elevated temperature of 47°C. The rationale behind this was that it would lead to an acceleration in the hydrolytic degradation of the sample, and thereby the release of the incorporated β-TCP. Weir et al. [Weir et al, 2004] conducted hydrolytic degradation experiments on PLLA at an elevated temperature of 50°C and 70°C. These studies showed that by elevating the temperature the rate of hydrolytic degradation increased exponentially, with no change in mechanism above the polymer’s glass transition temperature. Samples that were hydrolytically degraded at 70°C took only 23 days to degrade, whereas samples degraded at 50°C took over 115 day to degrade to the same extent. Samples were also degraded at 37°C, taking nearly 1 year to degrade to the same extent. Agrawal et al. [Agrawal et al, 1996] conducted hydrolytic degradation experiments on 50:50 PLGA at a number of different temperatures, these being 25°C, 37°C, 44°C, 54°C, 65°C and 80°C. This study showed that rate of decrease in molecular weight correlated with the elevation in temperature. Samples degraded at 37°C took 42 days to degrade to the same extent as samples degraded at 44°C, which took only 16 days.

From the results (Fig. 1a) it is evident that increased e-beam irradiation dosage increases the rate of surface hydrolytic degradation, hence the rate of mass loss [Witschi and Doelker, 1998]. This corresponds to results by Loo et al. and Cairns et al. [Cairns et al, 2011a, 2011b, Loo et al, 2005]. In the study carried out by Cairns et al. a number of different bioresorbable polymeric samples were exposed to e-beam irradiation of energy 0.5MeV. Two different doses were used, these being 150kGy and 500kGy. In concurrence with this study, the samples exposed to the higher dose experienced greater mass loss [Cairns et al, 2011a]. In all of the previous studies it was found that that e-beam irradiation decreased the average molecular weight. As a result of this a faster rate of water absorption into the polymer occurred, which in turn led to a faster rate of degradation [Cairns et al, 2011a, 2011b; Loo et al, 2005; Witschi and Doelker, 1998]. The larger pH decreases can also be associated with greater mass loss due to carboxylic acid monomers and oligomers are released into the solution which in turn lowers the pH [Cairns et al, 2011a, 2011b; Leonard et al, 2006; Li, 1999]. However, unlike the studies carried out by Cairns et al. this study used low e-beam irradiation energy to allow for modification of surface properties only. The samples irradiated in this study experienced a maximum penetration of approximately 100µm (10% sample thickness). It should be noted that with the study carried out by Loo et al., low energy e-beam irradiation (175keV) was used. However, bioresorbable films were irradiated in this study were of approximate thickness 55 to 65µm which therefore allowed for full e-beam penetration of the sample at the low e-beam energy [Loo et al, 2005].
The results (Fig. 2) showed that e-beam irradiation led to a greater decrease in mechanical properties over all time points. This is again due to a decrease in molecular weight which reduced the mechanical properties of the sample. This corresponds to studies carried out by Leonard et al. [Leonard et al, 2006] and Cairns et al. [Cairns et al, 2011a, 2011b]. Low energy e-beam irradiation has a low penetration capability. As a result only surface modification takes place, in this case polymer degradation caused by chain scission [Cairns et al, 2011a, 2011b; Leonard et al, 2006; Loo et al, 2005]. Irradiated samples experienced a decrease in mechanical properties due to degradation of the irradiated surface layer, causing weakening via a brittle surface layer. The decrease in mechanical properties was exacerbated by static degradation. There is little difference between the results for the two sets of irradiated samples for the first four time points. However, at 28 days there is noticeable difference due to embrittlement of the entire sample profile brought about by static degradation.

The degree of calcium release can also be attributed to the change in molecular weight caused by the e-beam irradiation, followed by hydrolytic degradation during the mass loss study. PLGA hydrolytically degrades via reaction-erosion fronts and degrades over four stages, these being diffusion of water into the sample; hydrolysis and insertion secondary crystallisation; diffusion of oligomers from the surface of the sample and finally; reaction-erosion fronts meet at the centre of the sample and degradation becomes more homogeneous. The majority of incorporated agent release occurs during the third stage of degradation [Hurrell et al, 2003]. With PGA and PLGA reaction-erosion fronts do not form until a crucial molecular weight is reached [Cameron et al, 2004]. As e-beam irradiation causes a decrease in molecular weight at the exposed surface, this advances the degradation process in the affected area and causes the third stage of the degradation process to occur sooner. As a result the incorporated agent, in this case calcium, is released sooner. Cameron et al. [Cameron et al, 2004] conducted a study into the release profiles of drugs from four types of PGA samples, each with a different average molecular weight. This study found that PGA samples with lower molecular weights released the incorporated drug much sooner than PGA samples with higher molecular weights. The greater degree of calcium release from the samples that were irradiated has occurred from the areas that were affected by the electron beam irradiation, in this case the modified surface. This has enhanced the early release of the β-TCP from the samples’ irradiated surface followed by release of the β-TCP from the bulk of the sample. This is important for potential in-vivo applications, such as bone repair where non-pre degraded PLGA can take up to 6 months to degrade [Millar et al, 1977], as β-TCP is a bioresorbable ceramic material that gradually dissolves in physiological conditions, releasing calcium and acting as an
An earlier commencement of release of the β-TCP would allow for earlier osteosynthesis, during the crucial phase on bone healing (the first 8 weeks in vivo) [Kalfas, 2001] as there is greater control over the timing of the release of the β-TCP [Daculsi et al., 2003; Daculsi et al. 2011]. The accelerated study was conducted for 28 days, and it is possible to speculate that, once the modified surface has degraded, degradation of the remainder of the sample will proceed as normal. Once the third stage in the degradation process has been reached for the remainder of sample, a sudden burst of the β-TCP release is likely be occur [Hurrell et al, 2003]. The results suggest that this has already commenced for the samples irradiated at 500kGy for the final, 28 day time point.

SEM showed that e-beam irradiation had a clearly observable effect on the surface of the samples that underwent degradation. As shown in Fig. 4, after being subjected to 28 days degradation, sample surfaces that had been irradiated with 500kGy exhibited considerable erosion compared to non-irradiated surfaces. The cracked surface is attributed to surface mass loss and water absorption during degradation. As the water is removed during drying, surface tensile stress develops, resulting in shrinkage cracks. The wettability results (Fig 5) for the irradiated samples at 3 and 7 days can be attributed to an increase in hydrophilicity brought about by exposure to the e-beam irradiation [Cairns et al, 2012]. With electron beam irradiation under atmospheric conditions, the air becomes ionised, thereby producing a plasma. As result of this was that the polymer was subjected to radicals and ions in the gaseous plasma discharge. When the PLGA polymer comes into contact with this plasma, this can potentially increase hydrophilic groups on the affected surfaces. Cairns et al. [Cairns et al. 2012] has shown that electron beam irradiation of a bioreabsorbable polymer, in this case PLLA, led to an increase in surface oxygen content, and a decrease in surface carbon content suggesting polymer chain scission due to a decrease in C-C /C-H bonds. As a result of polymer chain scission on the surface of the samples an increase in hydrophilicity is likely to have occurred, therefore leading to an increase in wettability. However the contribution of chemical surface modification is considered to be relatively small compared to the gross physical changes to the surface (shrinkage cracks) that were observed by SEM at the later time points. For example the maximum reduction in contact angle reported from Cairns et al. [Cairns et al. 2012] was approximately 25° (post-irradiation), whereas the current study reports much higher reductions (of up to 70°) for time points of 17 and 28 days. It is concluded that chemical modification may play a role in early time points (3 and 7 days) where reductions of up 25° were observed. At later time points, when surface cracking occurs, the gross physical changes dominate. These wettability results show that surface e-beam irradiation, followed by static degradation increases the wettability of the surface. This
is due to e-beam irradiation causing a decrease in average molecular weight by causing polymer chain scission. This causes surface degradation, and therefore leads to faster degradation at the surface of the samples during the static degradation study, which in turn leads to greater erosion [Cairns et al, 2011a; Hurrell et al, 2002; Leonard at al, 2006].

It should be noted that despite the advantages of low energy electron beam irradiation of biodegradable polymer samples in order to induce controlled release of bioactive additives, there are possibly some potential drawbacks to consider. For example the formation of debris on the surface from the degradation process could cause inflammation around the surrounding tissue if implanted due to phagocytosis [Yang et al, 2008]. SEM showed that the irradiated surfaces exhibited extensive cracking, thereby showing considerable surface erosion. However, in an in-vivo environment the implant would be subjected to a more dynamic setting, rather than a static one. This may aid in the expulsion of the degradation debris from the implants surface, therefore preventing inflammation.

5. Conclusion

Previous studies have shown the potential of using e-beam irradiation to modify biodegradable polymeric samples to a depth of 1mm or greater in order to influence degradation properties. This study has uniquely shown the potential of low energy e-beam irradiation to modify a surface layer of approximately 100µm with regards to influencing the release of incorporated agents. The low energy e-beam irradiated alters the surface properties of polyhydroxyacid samples through polymer chain scission which in turn allows for greater control of the release of an incorporated agent over a period of time, rather than a late burst that has been described in previous studies. However, further study is needed into this field such as evaluation in an in-vivo environment to provide evidence for the influence on bone healing.
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References


Alexis, F., 2005, Factors affecting the degradation and drug-release mechanism of poly(lactic acid) and poly[(lactic acid)-co-(glycolic acid)], Polymer International 54, pp. 36-46.


Hakkarainen, M., 1996, Weight losses and molecular weight changes correlated with the evolution of hydroxyacids in simulated in vivo degradation of homo-and copolymers of PLA and PGA, Polymer Degradation and Stability 52, pp. 283-291.


