



■ BIOMATERIALS

Enhanced release of calcium phosphate additives from bioresorbable orthopaedic devices using irradiation technology is non-beneficial in a rabbit model

AN ANIMAL STUDY

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Objectives

Bioresorbable orthopaedic devices with calcium phosphate (CaP) fillers are commercially available on the assumption that increased calcium (Ca) locally drives new bone formation, but the clinical benefits are unknown. Electron beam (EB) irradiation of polymer devices has been shown to enhance the release of Ca. The aims of this study were to: 1) establish the biological safety of EB surface-modified bioresorbable devices; 2) test the release kinetics of CaP from a polymer device; and 3) establish any subsequent beneficial effects on bone repair *in vivo*.

Methods

ActivaScrew Interference (Bioretec Ltd, Tampere, Finland) and poly(L-lactide-co-glycolide) (PLGA) orthopaedic screws containing 10 wt% β -tricalcium phosphate (β -TCP) underwent EB treatment. *In vitro* degradation over 36 weeks was investigated by recording mass loss, pH change, and Ca release. Implant performance was investigated *in vivo* over 36 weeks using a lapine femoral condyle model. Bone growth and osteoclast activity were assessed by histology and enzyme histochemistry.

Results

Calcium release doubled in the EB-treated group before returning to a level seen in untreated samples at 28 weeks. Extensive bone growth was observed around the perimeter of all implant types, along with limited osteoclastic activity. No statistically significant differences between comparative groups was identified.

Conclusion

The higher than normal dose of EB used for surface modification did not adversely affect tissue response around implants *in vivo*. Surprisingly, incorporation of β -TCP and the subsequent accelerated release of Ca had no significant effect on *in vivo* implant performance, calling into question the clinical evidence base for these commercially available devices.

Cite this article: *Bone Joint Res* 2019;8:266–274.

Keywords: Bioresorbable, Poly(L-lactide-co-glycolide), Electron beam irradiation, Tricalcium phosphate, Orthopaedic screw

Article focus

- To investigate the influence of calcium release from bioresorbable devices in relation to bone healing since the clinical benefits of calcium phosphate (CaP) additives to these devices over normal fracture repair times have not been established.
- We investigated the ability of surface treatment of bioresorbable polymer devices (via electron beam treatment) to

accelerate calcium (Ca) release *in vitro* and the biological response *in vivo* in an orthotopic model.

Key messages

- Ebeam (EB) treatment accelerated *in vitro* release of CaP dissolution products from clinically relevant polymer screws to a timeframe relevant to normal fracture repair.

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doi: 10.1302/2046-3758.86.BJR-2018-0224.R2

Bone Joint Res 2019;8:266–274.

- EB-treated polymers had no detrimental effect on the biological response.
- Enhanced Ca release did not increase bone formation or osteoclast activity *in vivo*.

Strengths and limitations

- Strength: *In vitro* and *in vivo* comparative results.
- Limitation: Animal model limited to two timepoints.

Introduction

The use of bioresorbable fixation devices in orthopaedic surgery began in the mid-1980s to treat displaced fractures of the ankle.¹ They are seen as advantageous over their metallic counterparts because devices degrade gradually during healing, limiting the stress shielding associated with rigid metallic devices that has been shown to result in osteoporosis.² Also, the potential need for future device removal is negated.³ Recently, there has been much interest in the incorporation of therapeutic additives, such as an antibiotic-releasing bioresorbable fixation device,⁴ and there are a number of calcium phosphate (CaP)-containing devices.⁵

Despite this promise, issues surrounding the degradation of implantable bioresorbable polymer devices have limited their success. Poly(α -hydroxy acids) are the most widely investigated bioresorbable polymers due to their tailorable degradation properties and excellent biocompatibility; they include poly(lactic acid), poly(glycolic acid) and a range of poly(L-lactic-co-glycolide) copolymers.⁶ Degradation of these polymers proceeds via hydrolysis of ester bonds,⁷ which results in the release of monomer and oligomer degradation products. If this hydrolytic degradation is slow compared with diffusion, the complete cross-section of the polymer matrix is affected as there is ample water available for hydrolysis, a process known as bulk degradation.⁸ If acidic degradation products build up in the polymer matrix and accelerate hydrolysis, in a process known as autocatalysis,⁹ they can become trapped within the device, and erosion of the polymer surface can then result in sudden release of these products.⁸ It was a degradation-related failure that led to the 2007 recall of the Calaxo interference screw (Smith & Nephew plc, London, United Kingdom).¹⁰

Furthermore, this pattern of degradation has a significant impact on the release profile of any products incorporated within the polymer matrix, as release rate is closely related to degradation.¹¹ There are a number of clinically available bioresorbable polymer and ceramic composite interference screws including Bilok (Biocomposites Ltd., Keele, United Kingdom), Biosteon (Stryker Corp., Kalamazoo, Michigan), Biosure HA and Regenesorb (Smith & Nephew, Mansfield, Massachusetts), BioComposite (Arthrex GmbH, Munich, Germany), Genesys Matryx (ConMed Corp., Utica, New York), CompositTCP (Zimmer Biomet Inc., Warsaw, Indiana),

and Milagro (DePuy Synthes, Raynham, Massachusetts). These devices are widely used and display appropriate degradation times,¹²⁻¹⁶ however, the evidence base supporting claims of enhanced clinical performance is weak, and questions remain around the clinical significance of adding osteoconductive materials to bioresorbable screws.¹⁷

In order to improve long-term mechanical properties and additive release profiles, surface degradation should be initiated prior to bulk degradation, permitting bulk strength retention while allowing the release of additives from the surface.¹⁸ This can be achieved through the use of low-energy electron beam (EB) radiation. This emerging technology has seen applications in semiconductor manufacture and food security (food-safe packaging and microbial inactivation) but has never been utilized by the medical device industry for use in clinical products. Potentially, bioresorbable medical devices, such as interference screws, suture anchors, fracture fixation plates, and bone fixation screws, could be treated with EB to ensure surface degradation occurs ahead of bulk degradation, thereby ensuring the release of surface-encapsulated therapeutics in a timely manner. Such therapeutics may include antimicrobials (e.g. antibiotics) and minerals that are essential to bone healing such as calcium (Ca), phosphorous, zinc, and silicon. EB irradiation would typically be applied at higher doses than those used for medical sterilization, thus causing near-surface polymer chain scission, and decreasing molecular weight and accelerating the surface degradation process. *In vitro* studies have shown it to be suitable for medical-grade polymer surface modification and controlled additive release due to its low and tailorable penetration capability,¹⁹⁻²³ but the safety of neither the higher dose, nor the increased surface degradation, has yet been confirmed *in vivo*.

The aims of this study were to investigate EB treatment as a method of enhancing the release of CaP dissolution products from clinically relevant orthopaedic screws and to assess the effect on *in vivo* performance. A rabbit femoral condyle defect model was chosen as the bone is large enough to accommodate implantation of commercially available screws in a clinically relevant anatomical location; many of these polymer screws are designed for ligament repair around the knee joint.

Materials and Methods

Commercial interference screws formulated from a proprietary blend of poly(L-lactide-co-glycolide) (PLGA) (ActivaScrew Interference; Bioretec Ltd, Tampere, Finland) and prototype screws of the same formulation with additional 10 wt% β -tricalcium phosphate (β -TCP; PLGA-TCP) were provided by Bioretec Ltd. A total of 31 PLGA and 31 PLGA-TCP screws were EB-treated using an EBLab electron beam laboratory unit (Comet AG, Flamatt, Switzerland), and equal numbers remained untreated. EB

treatment entailed a bilateral surface dosage of 500 kGy and an accelerating voltage of 115 keV, giving an all-round penetration depth of approximately 50 μm (as simulated by Comet's EBLab software). All screws were then sterilized by γ -irradiation (25 kGy) in a single batch.

In vitro analysis. Dissolution was assessed *in vitro* by monitoring mass and pH changes, and Ca release from the PLGA-TCP screws. Screws were weighed to the nearest 0.1 mg, placed individually in 30 ml phosphate-buffered saline (PBS) solution in sealed containers, and stored at 37°C with no agitation. Timepoints were 4, 12, and 36 weeks, with four treatment groups (PLGA, PLGA+EB, PLGA-TCP, PLGA-TCP+EB), and $n = 5$ for each outcome measure. The mean starting masses of the screws were: PLGA = 3.250 g (SD 0.046); PLGA+EB = 3.270 g (SD 0.040); PLGA-TCP = 3.260 g (SD 0.019); and PLGA-TCP+EB = 3.267 g (SD 0.023), giving an approximate mass:volume ratio of 1:10.

At each timepoint, screws were dried under vacuum at ambient temperature to a constant mass, which was then recorded. Dissolution media pH was measured using an HI-2210 Basic pH Benchtop Meter with an HI-1131B General Purpose Laboratory pH Electrode (both Hanna Instruments Ltd, Leighton Buzzard, United Kingdom). The pH was measured weekly for the first four weeks, and every two weeks thereafter. Calcium release from the PLGA-TCP screws was quantified by inductively coupled plasma mass spectroscopy (ICP-MS) (ELAN DRC-e; Perkin-Elmer Ltd, Seer Green, United Kingdom) with a detecting limit of 0.04 mg/l. Calcium was quantified in a 2 ml sample removed from the test container at two weeks, four weeks, and every four weeks thereafter up to 36 weeks, and cumulative Ca release was calculated.

In vivo analysis. *In vivo* screw performance was assessed using a New Zealand White rabbit model. All rabbits (Thrush Hall Supplies, Co. Antrim, United Kingdom) were female, 28 to 30 weeks old, skeletally mature with a mean weight of 3.7 kg (SD 0.32). All procedures had ethical approval, were performed under a licence issued by the Department of Health, Social Services and Public Safety of Northern Ireland, and were carried out in accordance with the regulations as laid down in the UK Animals (Scientific Procedures) Act 1986. A total of 32 rabbits underwent surgery and received implants bilaterally in the distal femoral condyles, giving 64 experimental samples in total, and eight samples per experimental condition per timepoint. Implantation sites were randomly assigned an implant type (PLGA, PLGA+EB, PLGA-TCP or PLGA-TCP+EB). Rabbits were sacrificed at 12 weeks and 36 weeks post-implantation. Animals assigned to the later timepoint were operated on first but otherwise implant order was randomized. A power calculation showed that eight animals per group would allow us to detect an effect size of an increase in percentage bone area of 10% with 80% power.

The surgical procedure was similar to that used by Palmer et al.²⁴ Rabbits were anaesthetized using Hypnorm (VetaPharma Ltd, Leeds, United Kingdom), intramuscularly, at 0.25 ml/kg, and midazolam (Hypnovel; Roche Products Ltd, Welwyn Garden City, United Kingdom), intravenously, at 0.5 mg/kg to 1.5 mg/kg^{25,26}. An incision (approximately 2 cm) was made medially to the patella, allowing lateral dislocation of the patella. The femoral condyle was visualized and a defect slightly smaller than the size of a screw was created by reaming with a custom-made handheld steel drill. The slow speeds involved meant that there was minimal heat generation, and, prior to implantation, the defect was lavaged with a saline solution to remove any debris from the cavity. The implant was then screwed into place using the supplied driver so that the base of the screw was flush with the cortical bone before the patella was reduced. All implants were fitted correctly. The incision was closed in two layers using bioresorbable sutures (Coated Vicryl; Ethicon Inc., Livingston, United Kingdom). Post-surgery, rabbits were given a broad-spectrum antibiotic (Baytril; Bayer HealthCare AG, Leverkusen, Germany) at 2 ml/kg subcutaneously and an analgesic (Temgesic; Reckitt & Colman PLC, Hull, United Kingdom) subcutaneously at 0.5 ml/kg mixed 1:9 with saline solution. Animals were weight bearing within approximately two to three hours and had access to food and water *ad libitum*. Animals were sacrificed with a lethal intravenous injection of sodium pentobarbital (Euthatal; Merial Animal Health Ltd, Woking, United Kingdom).

At sacrifice, femora were cleaned of soft tissue, fixed in 4 wt% paraformaldehyde (Sigma-Aldrich Co., Gillingham, United Kingdom) in PBS for two days at 4°C, then dehydrated through graded alcohols, cleared in xylene (Sigma-Aldrich), and embedded in a poly(methyl methacrylate) (PMMA)-based resin (Technovit 9100 Neu; Heraeus Kulzer GmbH, Wehrheim, Germany) according to the manufacturer's instructions. Embedded specimens were sectioned using an Accutom-50 precision cut-off machine (Struers Ltd, Rotherham, United Kingdom). Longitudinal sections of 300 μm thickness were mounted on fully frosted microscope slides (Fisherfinest; Fisher Scientific UK, Loughborough, United Kingdom). Sections were then polished with an Alpha 2 Speed Grinder/Polisher fitted with a Vector Power Head (both Buehler, Coventry, United Kingdom) prior to staining.

A section from the centre of each implant showing a complete cross-section was stained with a 0.25% w/v Toluidine Blue (TolBlue) solution (Sigma-Aldrich), then rinsed with water and allowed to air dry before viewing. A second section from each implant was used for enzyme histochemistry to stain for tartrate-resistant acid phosphatase (TRAP), a commonly used technique for the detection of osteoclasts. Prior to staining, resin was removed from the sections by soaking in acetone

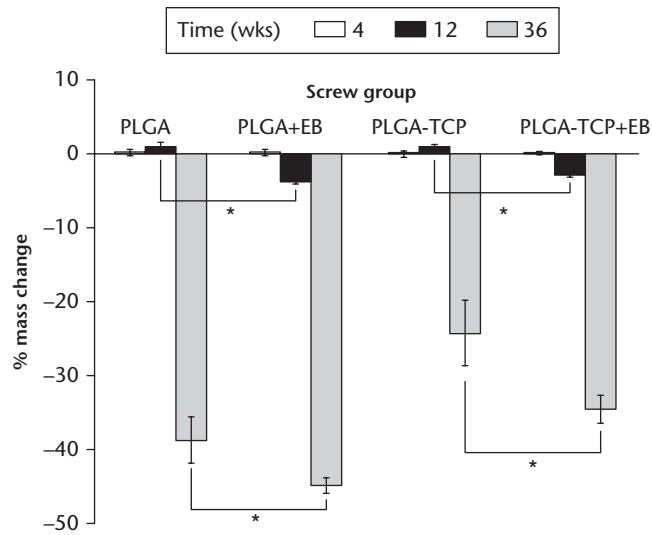


Fig. 1

Percentage mass change over dissolution time for the four screw groups ($n = 5$; mean (SD); * $p < 0.05$). PLGA, poly(L-lactide-co-glycolide); EB, electron beam; TCP, tricalcium phosphate.

(Sigma-Aldrich). Sections were adhered to fresh slides and rehydrated through graded alcohols to distilled water. The staining solution was prepared from a kit (Acid Phosphatase, Leukocyte; Sigma-Aldrich) according to the manufacturer's instructions, then covered in an aqueous-based permanent mounting medium (CC/Mount; Sigma-Aldrich) and dried at 70°C .

Both TolBlue- and TRAP-stained sections were viewed under a Nikon SMZ800 stereomicroscope (Nikon UK Ltd, Kingston Upon Thames, United Kingdom). Using Photoshop CC (Adobe Systems Europe Ltd, Maidenhead, United Kingdom) and blind to the experimental group, a Region of Interest (RoI) was drawn 0.8 mm around the perimeter of the implant, and the percentage of bone in this area was measured. To quantify TRAP activity, the sections were divided into two areas: within $500\mu\text{m}$ of the implant and more than $500\mu\text{m}$ from the edge of the implant. The number of TRAP-stained pixels in each area of the sections was labelled as 0 (no TRAP activity), 1 (low TRAP activity), or 2 (moderate TRAP activity).

Statistical analysis. One-way analysis of variance (ANOVA) was used to identify significant differences between groups with pairwise comparisons using Tukey's honestly significant difference tests to assess intergroup differences, provided the sample sizes were the same. If assumptions of ANOVA were not met, Kruskal–Wallis multiple comparisons stepwise stepdown analysis was performed. All statistical analyses were performed using PASW Statistics 18.0 (IBM Corp., Armonk, New York). A p -value of ≤ 0.05 was considered significant.

Results

As expected from bioresorbable polymers, there was a trend towards decreasing mass over *in vitro* dissolution

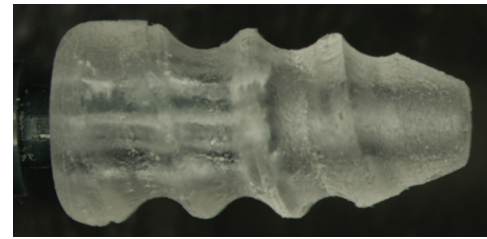


Fig. 2a

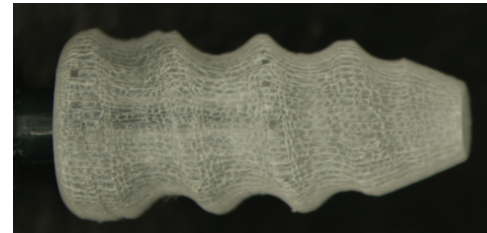


Fig. 2b

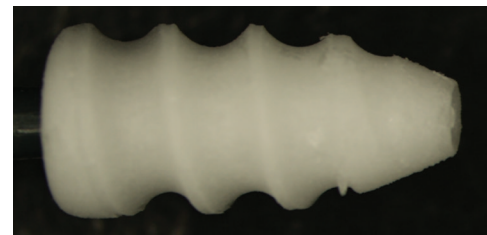


Fig. 2c

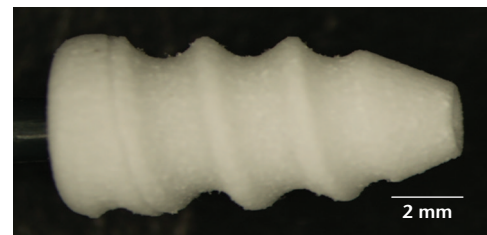


Fig. 2d

Optical microscopy images of screws following 12 weeks of *in vitro* degradation. a) PLGA; b) PLGA+EB; c) PLGA-TCP; d) PLGA-TCP+EB. PLGA, poly(L-lactide-co-glycolide); EB, electron beam; TCP, tricalcium phosphate.

time (Fig. 1). After four weeks, no significant mass changes were observed in any group or between groups ($p = 0.654$). After 12 weeks, both EB-treated groups showed a statistically significant reduction in mass when compared with the initial screw mass and the screw mass after four weeks of dissolution. This was not the case in the untreated groups, which showed no significant mass change. The largest mass changes were observed after 36 weeks. Between groups, the EB treatment resulted in a significantly greater mass loss in both the PLGA and the PLGA-TCP screws. Screws at 12 weeks were visually compared after drying (Fig. 2). Shrinkage cracks occurred during the drying process for the PLGA+EB group screws, but surface cracking was not apparent for the non-treated PLGA screws. Surface cracking was not obvious for the PLGA-TCP+EB group, however, they appeared distinctly rougher than the non-treated PLGA-TCP group at 12 weeks.

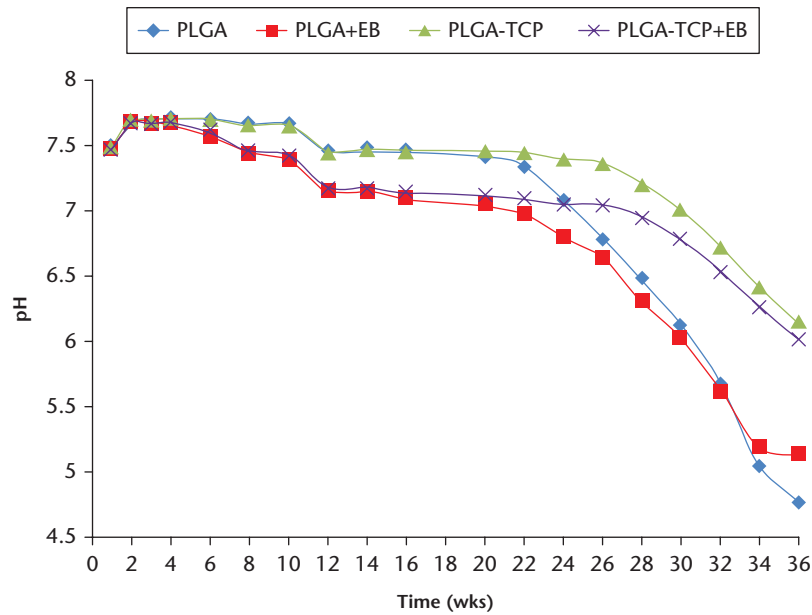


Fig. 3

pH change over dissolution time for the four treatment groups (n = 5; mean (sd)). PLGA, poly(L-lactide-co-glycolide); EB, electron beam; TCP, tricalcium phosphate.

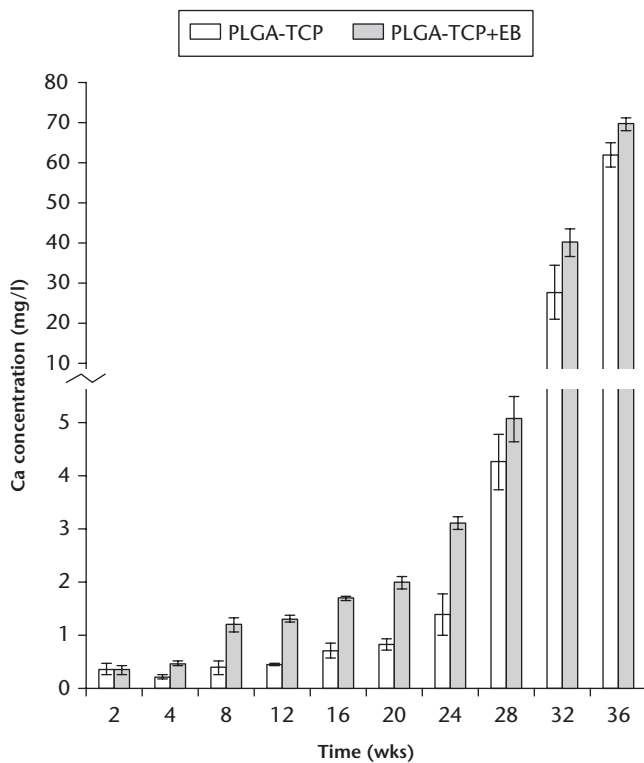


Fig. 4

Calcium (Ca) release over dissolution time for the two tricalcium phosphate (TCP)-containing screw groups (n = 4; mean (sd)). PLGA, poly(L-lactide-co-glycolide); EB, electron beam.

Differences in pH between treated and untreated samples were apparent from around four weeks (Fig. 3). EB treatment accelerated the drop in pH of PLGA+EB samples to 7.4 at ten weeks, while the pH of PLGA samples

remained relatively constant. The difference in pH between treated and untreated PLGA samples continued to increase, reaching 7.3 and 7, for PLGA and PLGA+EB, respectively, by 22 weeks. A similar trend is seen in the TCP-containing samples, although slightly delayed compared with the PLGA only.

Marked differences in Ca release were observed between PLGA-TCP and PLGA-TCP+EB (Fig. 4). After two weeks, the amount of Ca released is similar for untreated and treated samples, however between weeks 4 and 24, significantly more Ca was released from the EB-treated samples (p < 0.001). At each timepoint between four and 24 weeks, Ca release increased by over 100% following EB treatment. From 28 weeks onwards, the effect of EB treatment on Ca release was less marked.

Before surgery, all rabbits were in good health and were given at least seven days of acclimatization. All animals tolerated the procedure well with no adverse events and no gross pathology upon implant retrieval. During processing for histological analysis, three implants were lost, one from the 36-week PLGA+EB group and two from the 36-week PLGA group. Due to the technical challenges of removing the resin while retaining section integrity before TRAP staining, the numbers per group were reduced for this outcome measure. The exact number per group is provided in the relevant figure legend.

In general, the implants were well tolerated *in vivo*, with evidence of new bone growth and little indication of inflammatory response. By 36 weeks, in approximately one-third of cases in every treatment group, cracking of the screws was evident with bone growth into the cracks (Fig. 5). There was no significant difference in the amount of bone surrounding the screws between treatments at

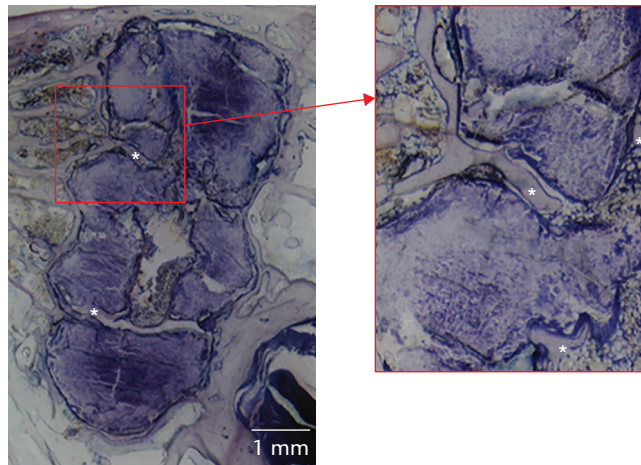


Fig. 5

Toluidine Blue (TolBlue)-stained section showing typical host response to an implanted screw (PLGA-TCP after 36 weeks in this instance), with bone ingrowth into cracks indicated with * and highlighted in the magnified area of interest. PLGA, poly(L-lactide-co-glycolide); TCP, tricalcium phosphate.

either timepoint (Fig. 6). Within the treatment groups, significant decreases in percentage of bone in the RoI were observed between 12 and 36 weeks in both the PLGA and the PLGA-TCP+EB groups ($p = 0.004$ and $p = 0.038$, respectively). No significant change over time was observed in the PLGA+EB or the PLGA-TCP groups ($p > 0.999$ and $p = 0.798$, respectively).

More TRAP activity was observed in the 12 weeks group compared with the 36 weeks group (Fig. 7). More TRAP activity was also observed within 500 μ m of the implants than in regions further away from the implants. No differences in TRAP were apparent between treated and untreated samples or between TCP-containing samples and PLGA only samples.

Discussion

EB irradiation has been used in other industries and has potential to be applied to the medical device industry as it has been shown to enhance the release of additives to bioresorbable polymers. The aims of this study were to establish the safety of EB treatment on clinically relevant orthopaedic devices in an orthotopic model and to identify any clinical benefit in the form of enhanced bone growth associated with the use of CaP as a bioactive.

The *in vitro* mass loss study showed that screws from all groups largely retained their original mass after four weeks of dissolution, and untreated screws retained their mass after 12 weeks. This is in line with reported times for typical onset of PLGA degradation ranging from 14 weeks to 22 weeks, depending on the L-lactide residual monomer content.²⁷ The EB treatment had the anticipated effect of accelerating degradation as evidenced by the significant mass loss associated with both PLGA+EB and PLGA-TCP+EB in comparison with the untreated samples. This was supported by the observed shrinkage

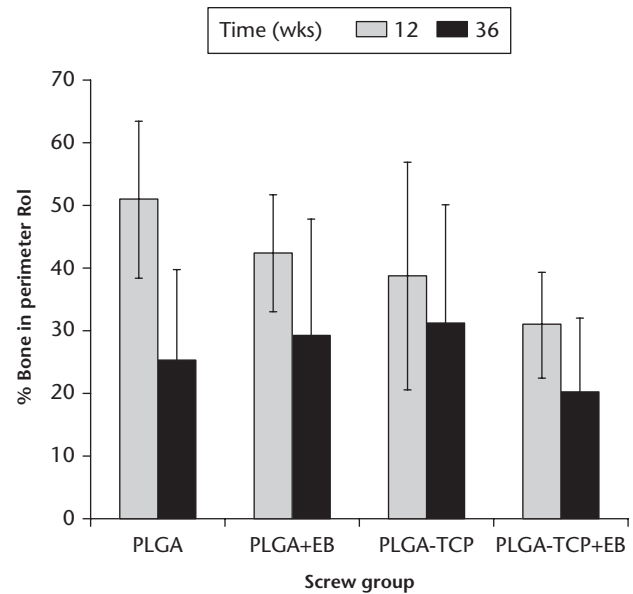


Fig. 6

Percentage of bone in the perimeter region of interest (RoI) surrounding the implants from each group after 12 and 36 weeks' implantation ($n = 8$, apart from PLGA and PLGA+EB at nine months where $n = 6$ and $n = 7$, respectively; mean (SD)). PLGA, poly(L-lactide-co-glycolide); EB, electron beam; TCP, tricalcium phosphate.

cracks for the vacuum-dried PLGA+EB at 12 weeks, indicating a decrease in surface volume. Changes in pH of this system can be attributed to either polymer breakdown, releasing oligomers of acidic nature, or dissolution of TCP, of basic nature (i.e. tends to cause a pH increase). The divergence in pH values between the EB- and non-EB-treated screw types at four to 26 weeks is associated with more rapid surface polymer breakdown for the EB groups whereas, beyond 26 weeks, differences are likely to be a result of a pH-buffering effect associated with the release of TCP from the polymer.²⁸

The idea that differences in mass loss and pH between TCP-containing screws and PLGA-only screws were a result of a buffering effect associated with dissolution of TCP was confirmed by measuring the Ca released from the screws. It has been shown that the incorporation of Ca compounds delays PLGA degradation with the basicity of Ca compounds proportional to the delay.²⁹ Calcium release from EB-treated samples was at least double that observed in untreated samples between four and 24 weeks, and almost 200% greater at eight and 12 weeks. This increased Ca release coincides with typical healing times of common fractures where bioresorbable screws may be clinically deployed.³⁰ However, PLGA-TCP composites have been shown to degrade more rapidly under dynamic conditions.³¹

In vivo results demonstrate that there was no detrimental effect of EB treatment on the biological response to the screws. There was no significant difference in bone formation nor any inflammatory response found in the EB groups compared with the experimental controls.

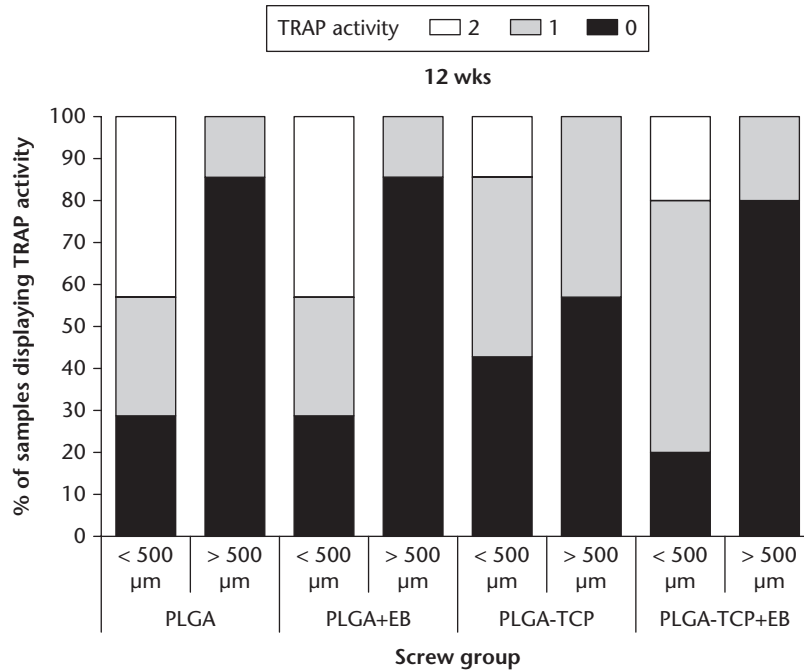


Fig. 7a

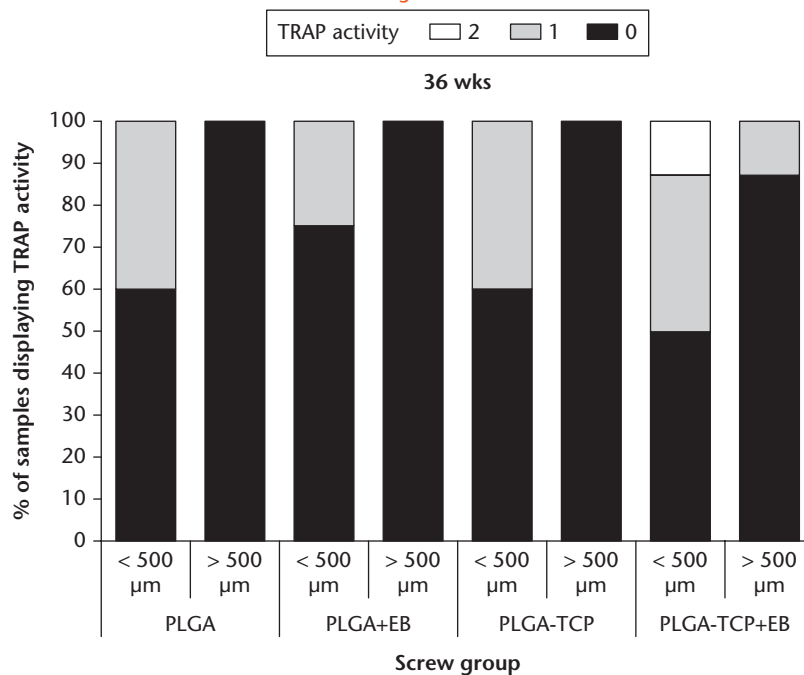


Fig. 7b

a) and b) Percentage of samples displaying tartrate-resistant acid phosphatase (TRAP) activity both within, and further than 500 μm from the implants after 12 and 36 weeks. The TRAP activity was quantified on an arbitrary scale of 0 (no TRAP activity), 1 (low TRAP activity), or 2 (moderate TRAP activity). n = 7 (PLGA 12 weeks, PLGA+EB 12 weeks, PLGA-TCP 12 weeks); n = 5 (PLGA-TCP+EB 12 weeks, PLGA 36 weeks, PLGA-TCP 36 weeks); n = 4 (PLGA+EB 36 weeks); n = 8 (PLGA-TCP+EB 36 weeks). PLGA, poly(L-lactide-co-glycolide); EB, electron beam; TCP, tricalcium phosphate.

In vitro, 12 weeks was one of the timepoints that showed the largest effect of EB treatment on Ca release, yet there was no significant difference between implant types *in vivo*. Therefore, we did not detect an effect of Ca release on *in vivo* performance.

In vitro results showed that, by 36 weeks, there was significant mass loss, between approximately 25% and

45%, observed in all groups by this timepoint. There were similar levels of Ca release across all treatment groups and a convergence of pH between the PLGA-TCP and PLGA-TCP+EB groups. Therefore, we did not expect to see, nor did we see, any significant difference in bone growth around the implants between treatment groups. However, a difference between pure

PLGA- and TCP-containing screws was expected, given the osteoconductive nature of β -TCP but this hypothesis was not supported by our results, and all implant types were well tolerated.

Similarly, the presence of Ca resulted in no clear differences in the level of TRAP activity between pure PLGA- and TCP-containing samples, regardless of EB treatment. The TRAP activity is a marker for osteoclast activity, and thus, this finding suggests that the presence of β -TCP did not significantly stimulate osteoclast activity around the implants in this model. The reduction in TRAP activity over time is likely to be due to waning of initial trabecular bone repair.

There are few studies comparing *in vivo* outcomes associated with TCP incorporation into bioresorbable polymers. Incorporation of 60% β -TCP into poly-L-lactic acid (PLLA) has been shown to cause less inflammatory reaction and to promote osteogenesis in a rabbit model when compared with pure PLLA.³² A polycaprolactone (PCL)/PLGA scaffold with incorporated β -TCP resulted in significantly greater bone density compared with a PCL/PLGA scaffold without β -TCP in a rabbit model,³³ and PLGA fibres containing 40% amorphous TCP resulted in a significantly increased fraction of newly formed bone in a non-critically sized rabbit calvarial defect model compared with PLGA alone³⁴ but we were unable to replicate these effects in this model. This is perhaps due to the lower level of TCP addition and lower surface-to-volume ratio in our study. A randomized controlled trial comparing Milagro (30% β -TCP in PLGA) and Calaxo (PLGA + 35% calcium carbonate) screws in anterior cruciate ligament (ACL) reconstruction surgery indicated that inclusion of osteoconductive materials into PLGA screws was not associated with bone formation at the screw site at up to two years.¹⁷ These clinical results agree with our experimental model, and highlight the current lack of relevant evidence of the benefits of TCP incorporation into ACL screws.

The bone response to bioresorbable polymers is complex and can be related to a number of factors such as absorption rate, material, age, sex, and health of the patient and the device's location in the body.^{35,36} Adding osteoconductive materials adds further variables with their varied absorption profiles. Modelling this complexity in the rabbit obviously has limitations and the choice of timepoint can be crucial. We chose 12 and 36 weeks as timepoints that would be able to show enhanced healing around the implant and enhanced long-term resorption of the implant, respectively. We cannot rule out the possibility that an effect of Ca may have been measurable earlier in the healing, however, it could be argued that the clinical significance of this may be limited if the effect was not maintained at 12 weeks.

In conclusion, EB treatment allows early surface degradation of bioresorbable polymer devices. For the first time, we have shown that pretreatment of device surfaces can

significantly increase the early release rate of incorporated therapeutic agents without adversely affecting biological response. Incorporation of β -TCP into the polymer formulation under investigation did not influence *in vivo* performance over the timescales investigated in a rabbit model, even after accelerating Ca release through EB treatment. Although the evidence does not support the clinical use of CaP-containing bioresorbable devices, the ability to control and accelerate the release of therapeutic additives is a valuable tool. Therefore, the technology has potential for use in future orthopaedic device development, where beneficiary additives may include antimicrobials (such as antibiotics) and mineral components.

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Author contribution

- I. Palmer: Designed the research, Acquired, analysed, and interpreted the data, Drafted the manuscript.
- S. A. Clarke: Designed the research, Interpreted the data, Revised the manuscript critically.
- F. J Buchanan: Designed the research, Interpreted the data, Revised the manuscript critically.

Funding statement

- The author or one or more of the authors have received or will receive benefits for personal or professional use from a commercial party related directly or indirectly to the subject of this article.

Conflict of interest statement

- I. Palmer declares money received from the COMET group to attend WBC 2016 to present work associated directly with this paper.

Acknowledgements

- This work was funded by Invest Northern Ireland (grant number POC 410). COMET Group provided technical assistance with the electron beam treatment and Bioretec Ltd donated the orthopaedic screws.

Ethical review statement:

- This research was reviewed by Queen's University Animal Welfare Ethical Review Board and performed under a licence issued by the Department Health, Social Services and Public Safety Northern Ireland.

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