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Development of a methacrylate-terminated PLGA copolymer for potential use in craniomaxillofacial fracture plates

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Short title: MA-terminated PLGA for use in fracture plates.

Key Words: Biodegradable; PLGA; resorbable polymer, fracture fixation.

Abstract

We synthesised methacrylate-terminated PLGA (HT-PLGA, 85:15 LA:GA, 169 kDa), for potential use as an adhesively attached craniomaxillofacial fracture fixation plate. The *in vitro* degradation of molecular weight, pH and flexural modulus were measured over 6 weeks storage in PBS at 37°C, with commercially available high (225 kDa, H-PLGA) and low (116 kDa, L-PLGA) molecular weight 85:15 PLGAs used as comparators. Molecular weights of the materials reduced over 6 weeks, HT-PLGA by 48%, H-PLGA by 23% and L-PLGA by 81%. HT-PLGA and H-PLGA exhibited a near constant pH (7.35) and had average flexural moduli in excess of 6 GPa when produced, similar to that of the mandible. After 1 week storage both exhibited a significant reduction in average modulus, however, from weeks 1-6 no further significant changes were observed, the average modulus never dropped significantly below 5.5 GPa. In contrast, the L-PLGA caused a pH drop to below 7.3 by week 6 and an average modulus drop to 0.6 from an initial 4.6 GPa. Cell culture using rat bone marrow stromal cells, revealed all materials were cytocompatible and exhibited no osteogenic potential. We conclude that our functionalised PLGA retains mechanical properties which are suitable for use in craniofacial fixation plates.

1 Introduction

Fractured bones in the craniomaxillofacial region typically require reduction of the bone segments for healing to proceed, with surgically implanted plates attached to the fractured bone being the most common approach to achieve fixation. The superior mechanical properties of titanium alloys compared to bone, makes them the most commonly used material in current fracture plate systems [1]. The high elastic modulus of titanium alloys is particularly useful in protecting the bone segments during healing. However, once healing is complete, the metallic plates cease to have a useful function and a number of post-operative complications have been reported [2], such as bone resorption due to stress shielding [3] , release of metal ions [4, 5] and paediatric bone development problems [6]. In addition, post-operative infections are frequently attributed to loosening of the screws used to attach the plate [2]. As a consequence of these post-operative complications a significant number of revision operations to remove the plates and screws are required each year, with each operation associated with a risk in patient morbidity and cost to health-care providers.

To reduce the potential problems associated with titanium fracture plates alternative materials have been investigated, in particular poly(α-hydroxy acids) such as poly(lactic acid (PLA)), poly(glycolic acid (PGA)) and copolymers of PLA:PGA [7]. These materials are soluble in aqueous environments. PGA is more crystalline than PLA, which has methyl groups within the polymer backbone, making PLA more hydrophobic and consequently slower to degrade than PGA. Therefore, by altering the relative concentrations of PLA and PGA it is possible to make polymers with controllable degradation times. Fracture plates made from these materials degrade progressively post-operatively leading to a gradual transferal of stress to the previously fracture bone, reducing the potential for stress-shielding of the bone once healing has ended. However, these materials are also associated with a number of potential post-operative complications, particularly when screws are used to attach the plates, with the incidence of post-operative infections likely to be the same as that found for metallic plates.

Over twenty years ago a number of research groups began to investigate the utility of adhesives for attaching fracture plates, particularly adhesives based around difunctional methacrylate monomers similar to those used in dentistry to attached polymer-based restorative materials to dentine [8-14]. The attractiveness of these adhesives for attaching materials to bone lies in the compositional and structural similarities between dentine and cortical bone. Satisfactory bond strengths have been reported in these studies between cortical bone and a variety different methacrylate-based materials. There is considerable dental literature showing that methacrylate-based materials and metal oxides can be chemically adhered to dentine using methacrylate adhesives. There is no inherent chemical interaction between these resorbable polymers and methacrylate-based adhesives, which could preclude the use of adhesives to attach resorbable fracture plates in areas under high stress.

Consequently, the aim of the work reported in this paper was the development of a PLGA copolymer for use as a fracture plate which has been functionalised with methacrylate groups, using ROP polymerisation. This well controlled polymerisation route enabled us to produce PLGA copolymers with high molecular weights that had methacrylate end groups attached to the copolymer backbone, which could be subsequently exploited for adhesion. To establish the potential for use as an internal fracture plate we report here on the changes in flexural modulus and molecular weight over 6 weeks of storage in an aqueous environment. We also report the cytocompatibility and osteogenic potential of this material, with these properties compared to those of commercially available polymers of the same PLA:PGA ratios.

2 Materials and Methods

2.1 Materials

PLGA (*Mw* 180 kDa, (H-PLGA)), PLGA (*Mw* 75 kDa, (L-PLGA)), hydroxyethyl methacrylate (HEMA), inhibitor-remover (Al_2O_3) glycolide, and anhydrous toluene ($PhCH_3$) were purchased from Sigma Aldrich (Dorset, UK). DL-Lactide, and tin octanoate ($SnOct_2$) were purchased from Alfa Aesar (Lancashire, UK). Phosphate-buffered saline (PBS) was purchased from (Lonza, Slough, UK). Tetrahydrofuran (THF), and diethyl ether (Et_2O) were purchased from Fisher (Loughborough, UK). Lactide and glycolide were recrystallized from ethyl acetate (EtOAc) prior to use. HEMA was passed through an inhibitor removal column immediately before use. Unless otherwise stated all other chemicals were used as received.

2.2 Synthesis

HEMA-terminated PLGA (HT-PLGA) was synthesised by ring opening polymerisation of lactide and glycolide (Fig. 1). A stirred solution of lactide (59.09 g, 410 mmol) and glycolide (8.40 g, 72 mmol) in anhydrous $PhCH_3$ (80 mL) was heated to 100 °C, HEMA (61.12 mg, 0.46 mmol) and $SnOct_2$ (92.34 mg, 0.23 mmol) were added and the reaction allowed to stir at 100 °C for 16 h. The reaction mixture was allowed to cool to room temperature, dissolved in a minimum amount of THF and added dropwise into a large excess of ice-cold Et_2O. The polymer precipitate was isolated by vacuum filtration and further dried under high vacuum to afford HT-PLGA, a white powder (48.7 g).

FIGURE 1 HERE.

2.3 Characterisation

^1H NMR spectra were obtained in $CDCl_3$ using a Bruker Advance 300 or a Jeol 400 spectrometer and analysed using MestreNova software. Chemical shifts are recorded in ppm relative to residual $CHCl_3$. The degree of polymerisation was determined by comparing integration of the vinyl protons of the HEMA end group with the integration of the methine signal of the lactide block or the methylene signal of the glycolide block.

Copolymer molecular weight was determined by gel permeation chromatography (GPC) using *N,N*-dimethylformamide (DMF) as a solvent containing 1% LiBr at a flow rate of 0.6 mL min^{-1} on a Varian Prostar instrument (Varian inc.) equipped with a Varian 325 UV-vis dual wavelength detector (254 nm), a Dawn Heleos II multiangle laser light scattering detector (Wyatt Technology Corp.), a Viscotek 3850 differential RI detector and a pair of PL gel 5 µm Mixed D 300 x 7.5mm columns with guard column (Polymer Laboratories Inc.) in series. Near monodisperse polystyrene standards (Polymer Laboratories) were used for calibration. Data analysis was achieved with Galaxie software (Varian Inc.) and chromatograms characterised with the Cirrus software (Varian Inc.) and Astra software (Wyatt Technology Corp.).

ATR-FTIR spectra were recorded using a Spectrum One spectrometer (Perkin Elmer, Bucks. UK) at $4cm^{-1}$ resolution with 32 co-addition scans recorded between 600 and 4000 cm^{-1}.

2.4 *In vitro* degradation studies

Polymer plates (40 x 3 x 2 mm, n = 5) were produced *via* compression moulding of the polymer powder in a PTFE mould under a pressure of 0.18 MPa at 70 °C for 90 min. Polymer plates were incubated in PBS (8 mL, changed weekly) at 37°C, for one, two, and six weeks. Upon removal, mechanical properties were measured using a three-point bend test (1 mm/min cross-head speed, Instron 5567, Bucks, UK). Changes in surface morphology, related to incubation time, were monitored using SEM (VEGA LMU, TESCAN UK Ltd.). Representative three-point bend specimens of each copolymer were selected at each time point, sectioned and then coated with 15 nm gold (Polaron SEM Coating Unit, Quorum Technologies, UK) prior to SEM analysis.

GPC was used to measure molecular weight changes in the polymers as a function of incubation time, and the pH of the storage medium was measured at each time point (Hanna checker, SLS, UK).

2.5 *In vitro* cytocompatibility

For the *in vitro* assays, disc specimens (8 mm diameter) of the polymers were compression moulded using the same conditions described for the three-point bend test specimens. Bone marrow-derived stromal cells (BMSCs) were isolated from adult male Sprague–Dawley

rats as described previously [15] and used at passages 3 to 5. The experiment was approved by the Research Ethics Committee of Karolinska University Huddinge Hospital in accordance with the policy on human care and use of laboratory animals. For all the *in vitro* assays, the BMSCs were seeded in triplicate for each material into 48-well plates (Corning) on the top of the PLGA discs (10,000 cells/disc/0.5 ml culture media/well) in a humidified atmosphere of 5% CO_2 at 37°C. Tissue culture plastic was used as a control. The experiments were repeated twice.

Alkaline phosphate (ALP) activity was used as a marker for the osteogenic differentiation of the BMSCs in the presence of the PLGA discs. For the ALP assay, the normal medium was replaced with commercially available osteogenic medium (Osteogenic Differentiation BulletKit™, Lonza) containing ascorbic acid, beta-glycerophosphate, and dexamethasone. After 7 and 14 days of the direct contact osteogenic culture, the BMSCs were washed with phosphate buffer, then lysed with 0.2% Triton X-100 (Sigma-Aldrich), and sonicated. ALP activity was quantified by measuring the rate of formation of p-nitrophenol (pNP) produced by hydrolysis of p-nitrophenylphosphate (Sigma-Aldrich) in 1M diethanolamine solution, buffered to pH 9.8 at 37°C for 30 min using a UV/Vis spectrometer, measuring at 405 nm (Labsystems Multiskan MS). The ALP activity was normalized to the cell number correlated to lactate dehydrogenase (LDH) values as reported previously [16] using LDH activity kit (CytoTox 96® Non-Radioactive Cytotoxicity Assay kit, Promega Corporation) according to the manufacturer's instructions. The final ALP data were expressed as µM pNP/1000 cells. The BMSCs cultured alone on tissue culture plastic were used as controls.

The cytocompatibility was assessed by 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide colorimetric assay according to manufacturer's instructions (Cell Proliferation Kit I (MTT), Roche Diagnostics) after 24h or 72h direct contact culture in alpha-minimum essential medium supplemented with 10% fetal bovine serum and antibiotics/antimycotics (all from Life Technologies, Inc.). The cell viability was normalized to the L-PLGA results.

3 Results

3.1 Polymer Characterisation

^{1}H NMR spectra of HT-PLGA, L-PLGA and H-PLGA are presented in figure 2. ^{1}H NMR: (400 MHz, $CDCl_3$): 6.10 (s, *a*, 1H), 5.59 (bs, *b*, 1H), 5.29-5.04 (bm, *e*, 1H), 4.93-4.58 (bm, g, 2H), 4.34 (bm, d, 3H), 1.80-1.29 (bm, f, 3H), 0.85 (bs, *c*, 3H). The degree of polymerisation was found to be 1900 (86%) and 290 (14%) for the lactide and glycolide units respectively. ^{1}H NMR spectra of commercially sourced L-PLGA and H-PLGA are comparable to the synthesised HT-PLGA, except for the extra resonances present for the HEMA, (Fig 2. a, b, d). FT-IR spectra of HT-PLGA H-PLGA and L-PLGA can be observed in figure 2. The spectra of the three polymers are identical revealing strong bands at 1760-1750 cm^{-1} and 1300-1150 cm^{-1} that can be attributed to the ester groups and bands between 3000-2800 cm^{-1} and 1500-1300 cm^{-1} associated with alkane groups. The absence of a visible band between 1700-1650 cm^{-1} indicates there are no visible alkene groups.

FIGURE 2 HERE

3.2 *In vitro* degradation study

The change in flexural modulus was different for the different polymers as time of exposure to an aqueous environment increased, figure 3a. Both the type of polymer and the time of testing significantly affected the modulus ($P<0.001$, two-way ANOVA, SigmaPlot 12.5, Systat Software Inc. UK). Initially, both the HT-PLGA and H-PLGA specimens exhibited significantly higher average flexural moduli than the L-PLGA specimens ($P<0.05$, two-way ANOVA with Holm-Sidak post-hoc analysis). After 1 week storage in PBS both HT-PLGA and H-PLGA specimens exhibited a decrease in average modulus ($P<0.05$), while the L-PLGA group average modulus remained approximately constant ($P>0.05$). As time in storage increased the HT-PLGA and H-PLGA average moduli showed no further decrease maintaining a value in excess of 4 GPa at all times, while the L-PLGA average modulus reduced significantly at both further time points eventually reducing to 0.6 GPa by week 6.

Prior to placement in an aqueous environment all three copolymers exhibited similar surface morphology, when inspected using an SEM, figure 3b. In general the surfaces were irregular with some perturbations most likely resulting from the surface of the compression

mould. As time in an aqueous environment increased the HT-PLGA and H-PLGA specimens began to become less rough with less evidence of the perturbations. In contrast, the L-PLGA specimens exhibited similar surface morphology at all points up to week 2. However, some surface fissures did become apparent on the week 2 L-PLGA specimens. Unfortunately, by week 6 the L-PLGA specimens became so weak that it was not possible to prepare SEM specimens from at this time point.

FIGURE 3 HERE

The molecular weight of all the polymers decreased with increasing time as seen in the shifting to shorter retention times in GPC chromatograms, (Fig. 4 c, b, d). Initially the HT-PLGA specimens exhibited a Mn of 169,000 which was between that found for the low and high molecular weight commercially available polymers, Table 1. The reduction in Mn was greatest in L-PLGA, which by week six had an Mn of only 19% the original value. In contrast, by week 6, the HT-PLGA was 52% the original value and H-PLGA 77% the original value. The polydispersity index (PDI, *Mw/Mn*) for all materials increased, in general, as time in aqueous storage increased but no consistent trend was observed.

FIGURE 4 HERE

TABLE 1 HERE.

The pH of the PBS media in contact with HT-PLGA and H-PLGA exhibited an approximately constant pH over the six week period with mean values of approximately 7.35 at all measurement times, figure 5. In contrast, the PBS in which the low Mw commercially available PLGA specimens were stored exhibited initially a higher pH at time 0 followed by a reduction in pH to eventually reaching a value of pH 7.2 by week 6, which was significantly different when compared to either H-PLGA or HT-PLGA specimens at all times ($P<0.01$, two-way ANOVA with Holm-Sidak post hoc analysis).

FIGURE 5 HERE

3.3 *In vitro* cell viability and ALP activity

All PLGA discs were tolerated well by the BMSCs, figure 6(a). The cell viability was 91% after both 24h and 72h in culture for the HT-PLGA. Cells cultured on the H-PLGA had higher cell

viabilities at both time periods, 100% and 108% respectively, relative to the L-PLGA specimens.

The lowest ALP activity was in the presence of the H-PLGA both after 7 days and 14 days in culture. The highest ALP activity was for L-PLGA, figure 6(b)

Figure 6 HERE

4 Discussion

The value of using resorbable polymers as internal fracture plates relies upon their capability to adequately support the healing craniomaxillofacial bones and subsequently be completely resorbed, thus erasing the need for surgical removal [17]. To this end, we synthesised biodegradable HT-PLGA *via* ring opening polymerisation using the FDA approved catalyst stannous octonoate ($SnOct_2$) [18]. ^{1}H NMR spectroscopic analysis (Fig 2. a, b, d) revealed the HT-PLGA copolymer matched the monomer composition and feed ratio, with signals corresponding to vinyl protons suggesting the presence of the vinyl group of HEMA, ^{1}H NMR spectra of L-PLGA and H-PLGA are comparable to the synthesised HT-PLGA and FT-IR spectroscopy showed no obvious differences between the synthesised HT-PLGA and commercially-available H-PLGA and L-PLGA. The absence of a visible band in the region 1700-1650 cm^{-1} expected for alkene groups suggests that the bulk of the HT-PLGA masked any vibration bands attributable to the HEMA end groups. GPC analysis of HT-PLGA (Fig 4, c - Time 0, 169 kDa) confirmed monomodal distributions and low PDIs suggesting the polymerisation proceeded with a high degree of control. Taken together, results from ^{1}H NMR and FTIR spectroscopies together with GPC analysis indicated successful synthesis of HEMA-teminated PLGA.

In order to support healing it is vital that fracture plates have sufficient strength and stiffness to protect the broken bones during healing, a period that typically takes six weeks [19], progressively allowing the transferal of load to the healing bones [20]. PLGA-based plates are both considerably weaker and less rigid than metallic plates, which has limited their use to bones that are relatively non-load bearing. In the craniomaxillofacial region, this means that PLGA-based plates are most suitable for use with mid-facial and upper facial fractures [6]. Bone is an anisotropic material demonstrating a complex elastic behaviour. Resonant ultrasound spectroscopic analysis of a human femur specimen identified that the Young's modulus varies between 12 and 20 GPa within the three principal axes [21]. There are no clear experimental data regarding what the modulus of the mandible is, although FEA analysis has suggested that the modulus is approximately 5.5 GPa [22]. Both the HT-PLGA and H-PLGA specimens had flexural moduli in excess of 5.5 GPa when first measured. Although both materials exhibited a reduction in modulus after aqueous storage both had moduli not significantly lower than 5.5 GPa over the 6 weeks.

The rate of degradation of PLGA copolymers is multifactorial, with increases in copolymer molecular weight [23] and greater concentrations of lactic acid relative to glycolic acid [24] in particular leading to reductions in the rate of degradation [7]. There is a range of PLGA copolymers currently used as craniomaxillofacial fracture plates [25]. We chose to develop our copolymer at a ratio of 85:15 LA:GA and with a high molecular weight to reduce the rate of degradation to a sufficient level that the mechanical properties of the copolymer would remain approximately stable over the six weeks during which fracture healing typically occurs.

Despite the multifactorial nature, the degradation of all PLGA copolymers can be described as consisting of following three key stages [26-28]. During the first stage, termed the quasi-stable stage or stage I, some degradation may occur either by erosion or bulk degradation but with no significant mass change (termed stage I-1) followed by the beginning of a decrease in molecular weight but with all other properties remaining constant (termed stage I-2). Next, during stage II, the mechanical properties begin to decrease as low molecular weight degradation products begin to evolve from the polymer. These degradation products lead to a broadening of the molecular weight distribution. There are, however, no other significant changes in the mass or dimensions of the polymer during this stage. Finally, once the polymer molecular weight has dropped below a critical value, the degradation enters stage III, during which significant mass loss, dimensional change and decrease in mechanical properties are found. It is also during this stage that a significant decrease in the pH of the surrounding medium are observed due to the large-scale release of the acidic degradation products [26-28].

The link between mechanical properties and polymer molecular weight is well established with higher molecular weight leading to superior mechanical properties. We chose to produce our HEMA-terminated PLGA via ROP, a method that enables the production of high molecular weight polymers with well-controlled architecture. For degradable amorphous polymers there is a further relationship between molecular weight and mechanical properties, in particular Young's modulus. The Young's modulus of an amorphous polymer remains constant so long as the molecular weight of the polymer allows for a sufficient

amount of entanglement between the chains [29]. Below a certain critical molecular weight the polymer chains are able to move past each other freely, resulting in a reduction in stiffness. A significant reduction in the flexural modulus of L-PLGA was observed over the six weeks, correlating with a diminution in molecular weight, suggesting that L-PLGA is at least at stage II of degradation at the final time point. Previously, the mechanical properties of a 77 kDa PLGA polymer (75:25 LA:GA), a similar molecular weight to the L-PLGA, was shown to degrade over six weeks when measured using dynamic mechanical analysis [30]. However, H-PLGA and HT-PLGA maintain their stiffness during the six week degradation study, demonstrating that the polymers were of sufficiently large molecular weight for inter-molecular chain entanglement to occur and that degradation of these polymers remained at stage I-2 throughout the six week study.

All resorbable polymers have been shown to degrade by either bulk erosion, in which the external dimensions remain largely the same with degradation occurring sub-surface, or by surface erosion, which leads to a reduction in the external dimensions [31]. At present there is no general consensus as to what leads to degradation following one or the other of these mechanisms. Some authors [32, 33] have suggested that there is a critical dimension above which materials lose the tendency to bulk erosion and become more liable to surface erosive degradation. Morphological analysis of our specimens after storage in PBS suggest that average molecular weight may also be a factor. The two high molecular weight copolymers exhibited some surface erosion as incubation time increased leading to a decrease in the roughness. In contrast, the lower molecular weight L-PLGA specimens exhibited generally similar surface morphology as incubation time increased, suggesting that for these specimens bulk degradation was occurring. Specimens of L-PLGA proved too weak to prepare for SEM analysis by week six, a problem previously reported by other authors for low molecular weight PLGA copolymers, for instance [34]. Unfortunately without data from this time point it is not possible to say whether the L-PLGA specimens would have begun surface erosion by week 6, but when the SEM and mechanical test data are considered in combination, it is clear that degradation had proceeded much faster in these specimens compared to the HT-PLGA and H-PLGA copolymers.

To further investigate the degradation of the polymers under physiologically relevant conditions, changes in their molecular weights were determined using GPC analysis (Fig. 4).

After six weeks incubation in PBS, H-PLGA, HT-PLGA and L-PLGA incurred reductions of 20, 53, and 80% of their molecular weight, respectively. These results show that the lower molecular weight PLGA plates degrade more significantly than higher molecular weight counterparts, a finding consistent with work by Kamei *et al*. [35] who found that *in vivo*, PLGA of molecular weight 10 kDa degraded twice as fast as PLGA of molecular weight 20 kDa. The molecular weight distributions of HT-PLGA and H-PLGA are mono-modal and remain consistent throughout the experiment. L-PLGA exhibits a substantial broadening of molecular weight distribution after six weeks, with a corresponding increase in polydispersity from 1.3-1.6. This broadening of the molecular weight distribution is consistent with the production of low molecular weight degradation products in stage II degradation [26-28]. PLGA is known to degrade *via* random hydrolysis of the polymer backbone [36] and our findings are consistent with Deng *et al.* [30] and Park *et al*. [24] who observed a broadening in GPC traces when they investigated the degradation of PLGA microspheres.

During the dissolution of PLGA polymers acidic degradation products are released, which affect the local pH around the material. This degradation is a bulk process, starting as soon as the material is placed in an aqueous environment [37]. Consequently, we monitored the pH of the storage medium choosing PBS, which has a starting pH of 7.4 similar to that found in the body. The storage medium for the HT-PLGA and H-PLGA specimens maintained a consistent pH of approximately 7.35 with no significant variation over the whole period, indicating that both the HT-PLGA and H-PLGA were in stage I of degradation up to week 6. In contrast, the L-PLGA specimens initially had a significantly higher pH than the other materials, which decreased significantly over the 6 weeks eventually falling to approximately pH 7.3. While this small reduction in pH for the L-PLGA would be unlikely to cause any significant problem it is indicative of the more advanced dissolution of the L-PLGA materials compared to the others, indicating that by week 6 these polymers were in either stage II of degradation, or the beginning of stage III. The pH drop of PLGA polymers has been previously shown to be linked to the molecular weight of the polymer, with significant reductions in pH occurring once the molecular weight is sufficiently low to enable the chains to diffuse from the material into the medium [37].

All of the different materials were found to be cytocompatible over both time periods. Fracture plates made using PLGA have already been approved for use by the FDA and the results from the MTT assay shows that the newly produced HEMA-terminated polymer is as well tolerated by BMSCs as the commercially available materials. The ALP assay showed that none of the materials exhibited significant osteogenic potential, in contrast to previous research which showed a 75:25 PLGA polymer to have increased ALP activity after 7 days [38]. While in many instances it is desirable for a synthetic implant to elicit osteogenesis, in the case of an internal fracture fixation plate the aim is to support fracture healing rather than to stimulate additional bone growth. Bone growth into the fracture plate would be undesirable and could potentially lead to post-operative complications. Consequently, the similarity in behaviour shown by the HT-PLGA specimens and the H-PLGA specimens suggests that the new material will behave similarly *in vivo*. Clearly, this behaviour will need to be qualified with *in vivo* studies.

The HT-PLGA copolymer developed here has been shown to perform comparably to a commercially available high molecular weight PLGA in terms of the mechanical properties, degradation rate and cytocompatibility. Having established this level of performance that next step is to measure how well this copolymer adheres to cortical bone when attached using methacrylate-based adhesives typical of those used in previous studies [8-14]. This will be the focus of a further paper.

5 Conclusion

In conclusion, we have synthesised HT-PLGA for use in craniomaxillofacial fixation plates and compared its properties to two commercially available PLGA polymers by conducting an *in vitro* degradation study. HEMA was incorporated into the polymer to potentially improve its interaction with bone adhesives with the aim to reduce the use of screws in mandible alignment surgery. The degradation characteristics of HT-PLGA, evaluated *in vitro*, and cytocompatibility were found to be comparable to commercially available high molecular weight PLGA

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Table 1. Molecular weights of each polymer at set time points of *in vitro* degradation study

Polymer	Time 0 M_n (PDI)	Time 1 M_n (PDI)	Time 2 M_n (PDI)	Time 6 M_n (PDI)
HT-PLGA	169,000 (1.5)	131,000 (1.5)	129, 000 (1.5)	81,000 (1.6)
H-PLGA	225,500 (1.3)	210, 500 (1.4)	197,000 (1.4)	182, 000 (1.4)
L- PLGA	116,500 (1.4)	111, 000 (1.3)	47, 800 (1.6)	26, 700 (1.4)

Figure Legends

Figure 1: Synthesis reaction schematic for the production of HT-PLGA

Figure 2: Left: ^{1}H NMR spectrum (400 MHz, $CDCL_3$) i) HT-PLGA, ii) H-PLGA and iii) L-PLGA. Right: Typical ATR-FTIR spectra of a) HT-PLGA, b) H-PLGA and c) L-PLGA, red - 1760-1750 cm^{-1} and 1300-1150 cm^{-1} ester groups green - 3000-2800 cm^{-1} and 1500-1300 cm^{-1} alkane groups.

Figure 3: (a)Flexural modulus of polymer plates at time points of *in vitro* degradation study. * = P<0.05, ** = P<0.01, all other combinations not significant. (b) Typical SEM micrographs for each polymer after incubation in PBS.

Figure 4: a) Mn (g mol^{-1}) in kDa as determined by gel permeation chromatography calibrated against near monodisperse poly(styrene) standards b) GPC chromatograms of H-PLGA, c) HT- PLGA, d) L-PLGA, tailing at time 6 on account of monomer released by hydrolysis.

Figure 5: pH of PBS during *in vitro* degradation study. A significant difference (P<0.01) was measured between the L-PLGA specimens both the H-LPGA and HT-PLGA specimens at week 1 and week 6. No other significant differences were measured.

Figure 6: (a) *In vitro* cell viability and (b) ALP activity.

Figure 1

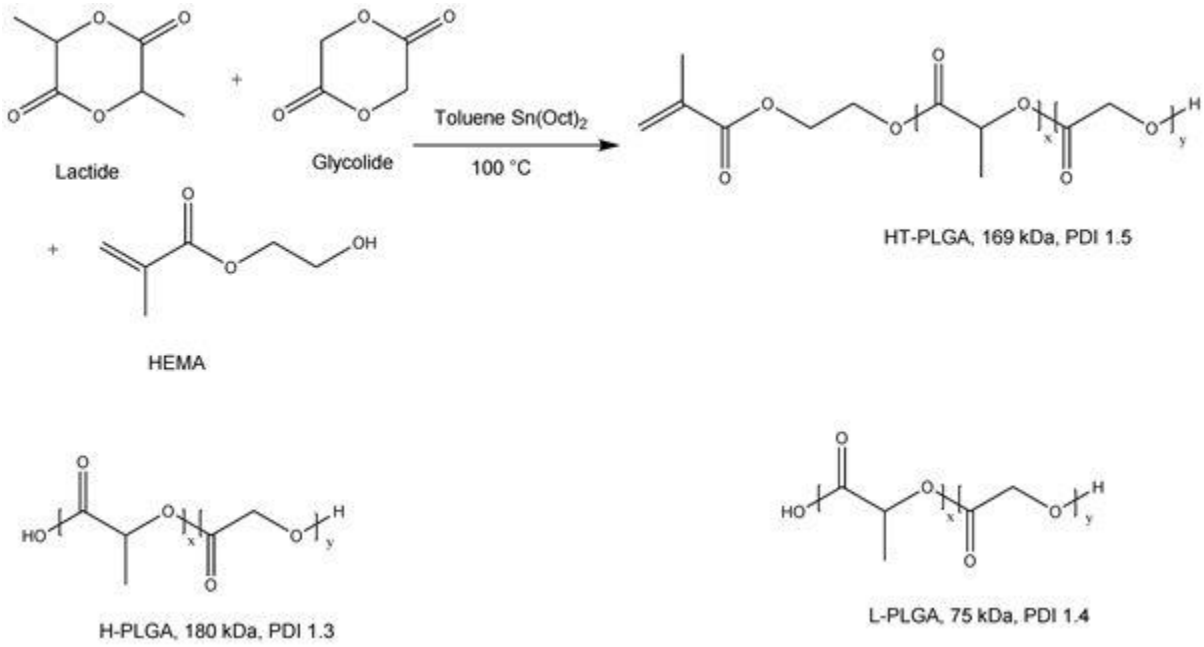

Lactide
Glycolide
Toluene Sn(Oct)2
100 °C
HT-PLGA, 169 kDa, PDI 1.5
HEMA
H-PLGA, 180 kDa, PDI 1.3
L-PLGA, 75 kDa, PDI 1.4

Figure 2

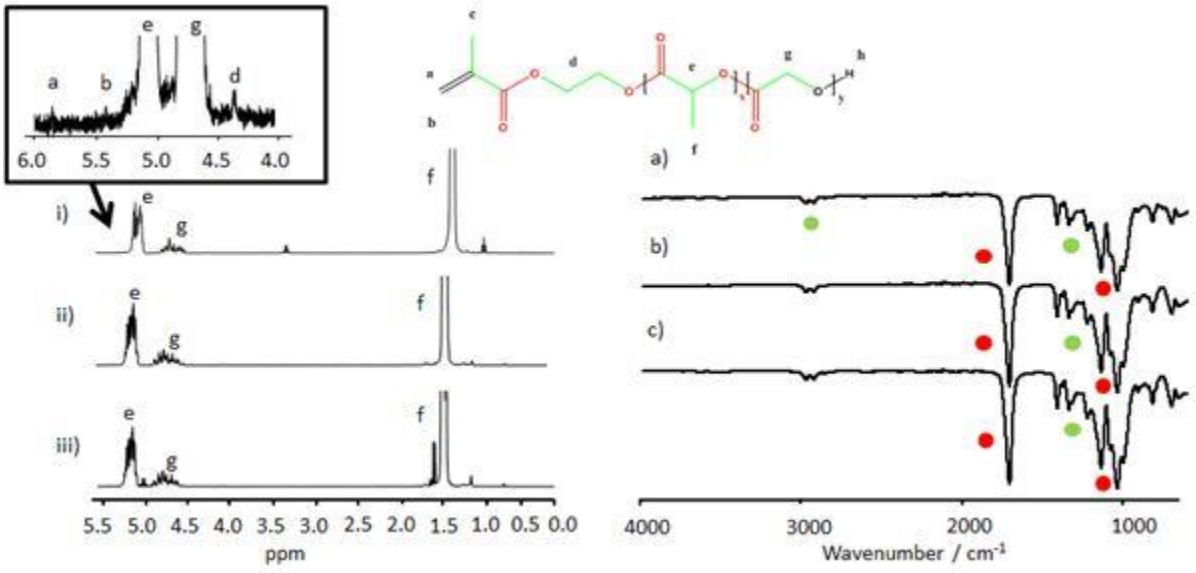

a
b
e
g
d
6.0
5.5
5.0
4.5
4.0
i)
ii)
iii)
f
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm
a)
b)
c)
4000
3000
2000
1000
Wavenumber / cm-1

Figure 3

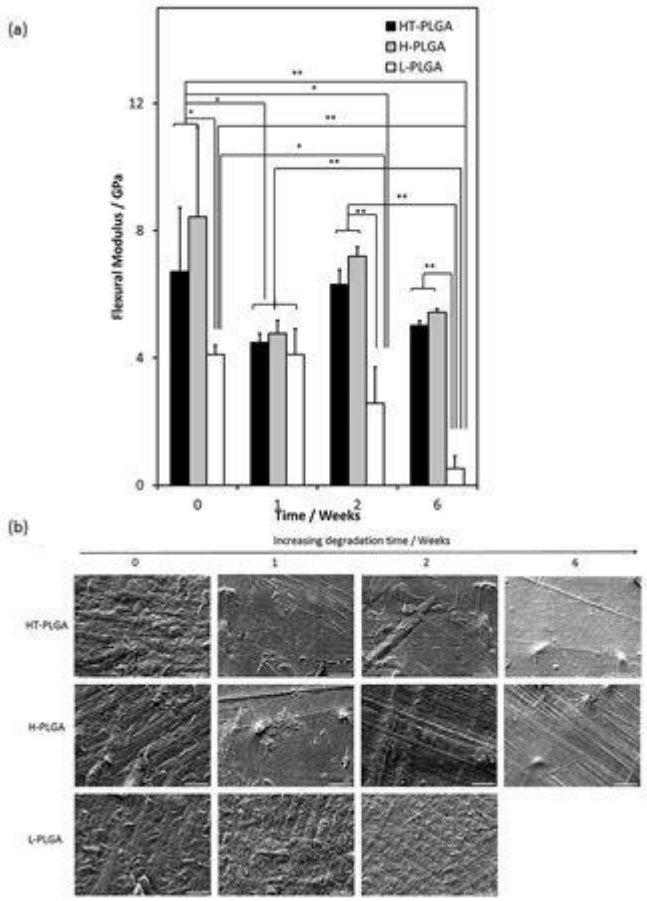

(a)
HT-PLGA
H-PLGA
L-PLGA
Flexural Modulus / GPa
Time / Weeks
(b)
Increasing degradation time / Weeks
HT-PLGA
H-PLGA
L-PLGA

Figure 4

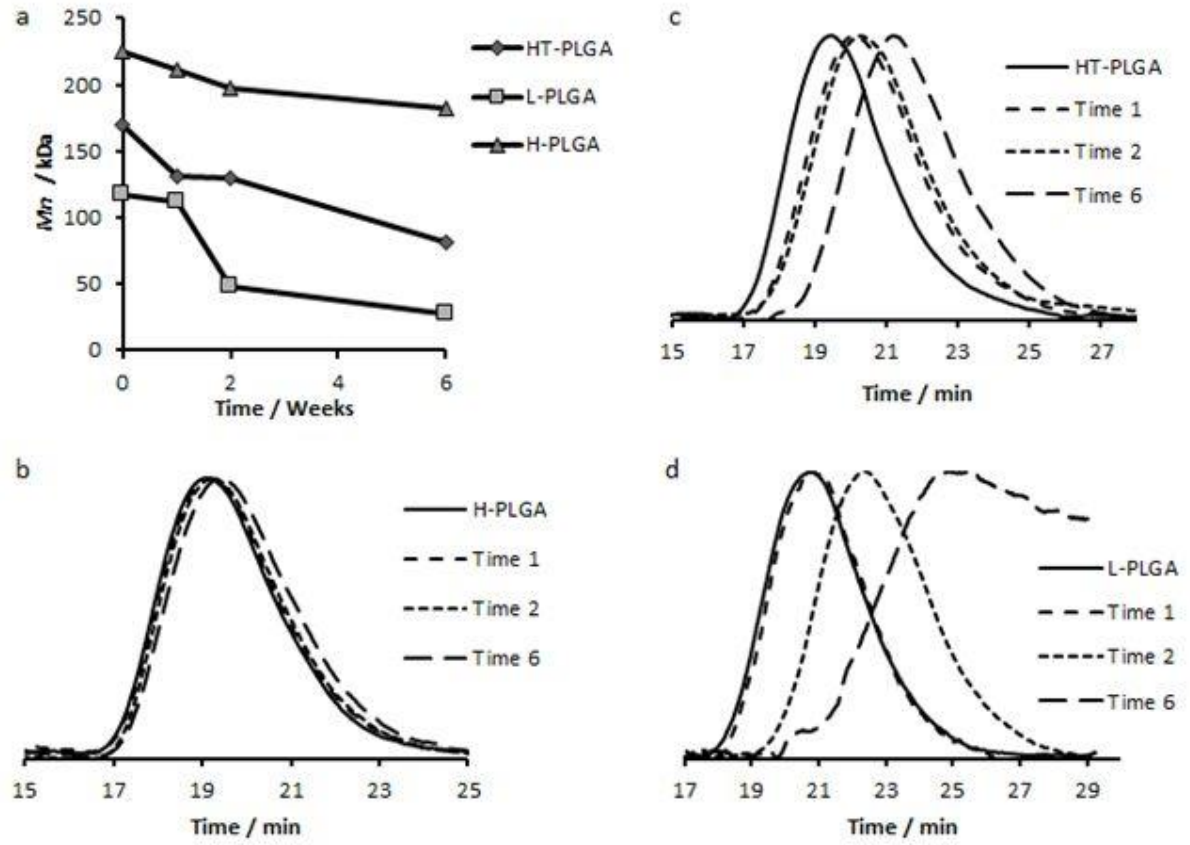

a
250
200
150
100
50
0
Mn / kDa
0
2
4
6
Time / Weeks
HT-PLGA
L-PLGA
H-PLGA
c
HT-PLGA
Time 1
Time 2
Time 6
15
17
19
21
23
25
27
Time / min
b
H-PLGA
Time 1
Time 2
Time 6
15
17
19
21
23
25
Time / min
d
L-PLGA
Time 1
Time 2
Time 6
17
19
21
23
25
27
29
Time / min

Figure 5

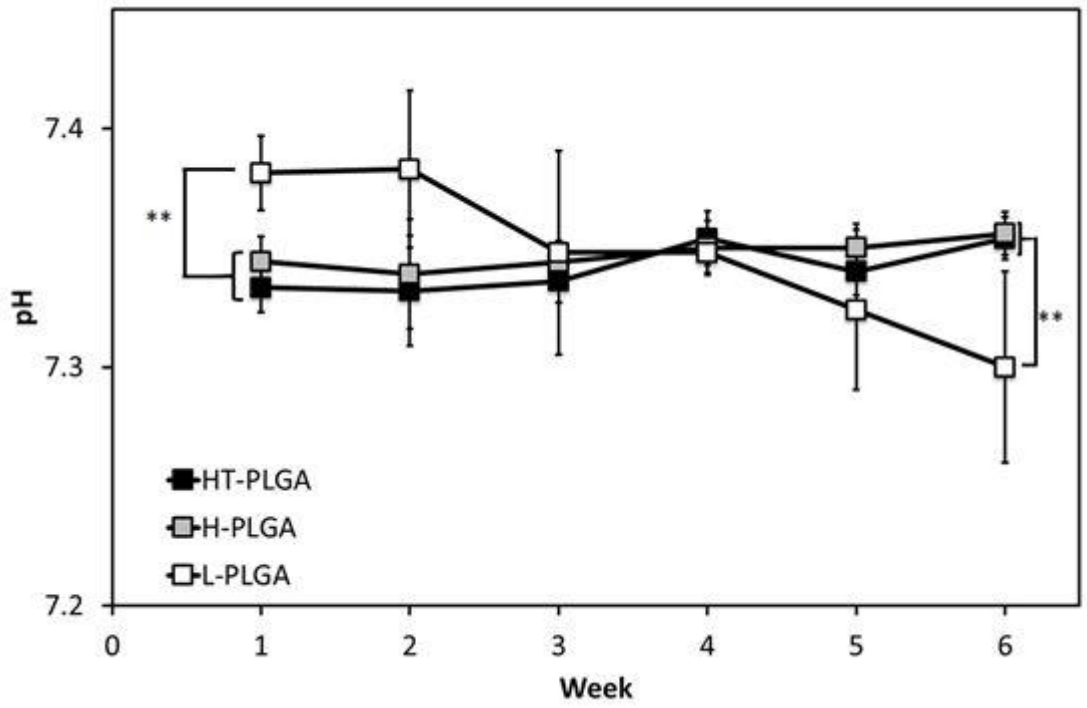
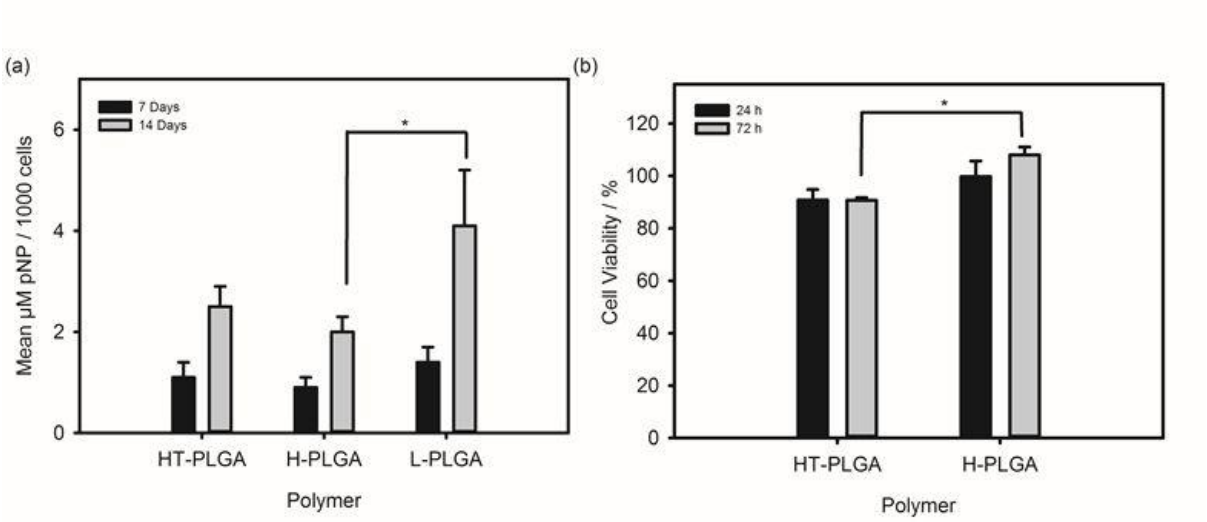


Figure 6


(a)
7 Days
14 Days
*
Mean µM pNP / 1000 cells
0
2
4
6
HT-PLGA
H-PLGA
L-PLGA
Polymer
(b)
24 h
72 h
*
Cell Viability / %
0
20
40
60
80
100
120
HT-PLGA
H-PLGA
Polymer