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# Optimisation of polymer scaffolds for RPE cell transplantation

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**Abstract**

**Aim**: To evaluate a variety of copolymers as suitable scaffolds to facilitate retinal pigment epithelium (RPE) transplantation.

**Methods**: Five blends of poly(L-lactic acid) (PLLA) with poly(D, L-lactic-glycolic acid) (PLGA) were manufactured by a solid-liquid phase separation technique. The blends were 10:90, 25:75, 50:50, 75:25, 90:10 (PLLA:PLGA). All blend ratios were validated by nuclear magnetic resonance spectroscopy. Samples of polymer blends were coated with laminin. Coated and uncoated blends were seeded with a human RPE cell line. Cell attachment, viability and retention of phenotype were assessed.

**Results**: As the lactide unit content increased pore size generally became smaller. The 25:75 PLLA:PLGA blend was the most porous (44%) and thinnest (134μm) scaffold produced. ARPE-19 cells survived with minimal cell death and maintained their normal phenotype for up to four weeks.. Cell density was maintained with only one of the fabricated ratios (25% PLLA:75% PLGA). There was a consistent decrease in apoptotic cell death with time on laminin coated samples of this blend. A decrease in polymer thickness concomitant with an increase in porosity characteristic of degradation was observed with all polymer blends.

**Conclusions**: This study demonstrates that a 25:75 copolymer blend of PLLA:PLGA is a potentially useful scaffold for ocular cell transplantation.

## Introduction

Biomaterial transplants offer a means of restoring function in biological structures which cannot otherwise be repaired [1]. One such ocular structure is the retinal pigment epithelium (RPE). RPE degeneration is fundamental to the progression of common causes of blindness such as age-related macular degeneration (AMD)[2]. Therefore RPE transplantation would be a novel treatment.

The principal challenges involved in cell transplantation include delivery, localisation and survival of cells. Cell delivery to the retina is typically achieved by injection of cell suspensions into the sub-retinal space. However there are a number of drawbacks associated with this method including cell death, reflux from the injection site and incorrectly localised or disorganised grafts [3;4]. Interestingly recent studies have demonstrated that growth of RPE and retinal progenitor cells on polymer scaffolds can facilitate transplantation of cells as an intact functioning monolayer which should overcome these undesirable sequela [5]. Delivery of cells as composite polymer grafts reduced cell death and improved overall survival by up to 10 fold [6]. However there have only been limited studies to identify the optimal polymer composition for such grafts [5-8].

Polymers are macromolecules composed of many subunits which form linear, branched, or cross-linked structures [9]. The poly($\alpha$-hydroxy esters) have been extensively investigated for tissue engineering applications. They allow controlled degradation and facilitate the attachment of anchorage-dependent cells, which require a supportive matrix for survival and growth [4]. Additional benefits of these polymers include good biocompatibility with the possibility of processing into desired configurations of controlled thickness [10]. Two promising polymers for consideration as ocular bio-scaffolds are poly(L-lactic acid) (PLLA) and poly(D,L-lactic-glycolic acid) (PLGA). They are biocompatible within the eye [11], and have been successfully employed for various biomedical applications including gene, growth factor and drug carriage [12-14]. Both have been utilised for culture and transplantation of retinal cells [5;6;15;16]. Previous studies have evaluated a (50 PLLA: 50 PLGA) copolymer blend for ocular cell attachment [5;6].

The purpose of the present study was to fabricate a range of novel polymer blends and to evaluate their suitability as a bio-scaffold. An RPE cell line was seeded onto samples of each polymer blend and maintained *in vitro* for up to 4 weeks.

## Materials and Methods

Poly(L-lactic acid) (PLLA) Resomer® L 207 S, inherent viscosity (i.v.) 1.5-2.0dL/g and poly(DL-lactide-co-glycolide) (PLGA) Resomer® RG 755 S, i.v. 0.5-0.7dL/g, with a lactic to glycolic ratio 75:25 were obtained from Boehringer Ingelheim GmbH (Ingelheim, Germany). All solvents were obtained from Fisher Scientific (Loughborough, UK). Polymers scaffolds were prepared as previously described [5]. Five blends of PLLA with PLGA were prepared in neat 1,4-dioxane (Fig 1B). 0.5ml of each copolymer solution was applied to the surface of an uncoated microscope slides and allowed to spread uniformly. Slides were then placed on a metal grid sitting on ice for 1 minute. A dry-ice cooled copper wire was touched to the surface of the solution to initiate solidification. Slides remained on ice for a further minute to solidify the dioxane before being transferred to a -20$^{o}$C freezer overnight. Prior to use, polymers were placed under high vacuum at room temperature to completely remove dioxane.

Inverse-gated $^{13}$C NMR spectra were obtained for samples of all copolymer blends using established methods [17]. $CDCl_3$ was used as the solvent for all samples, polymer concentration used was 0.02g/ml. $\delta$($^{13}$C{$^{1}$H} inverse gate decoupled NMR; 400MHz; $CDCl_3$, ppm): 16.6 (methyl group on lactide unit), 60.8 (methylene group in glycolide unit), 67.1 (residual dioxane solvent), 69.0 (methine group in lactide unit), 166.4 (carbonyl group in glycolide unit), 169.6 (carbonyl group in lactide unit).

Cell seeded and unseeded polymer samples maintained in culture for up to one month were fixed in 3% glutaraldehyde with 4% formaldehyde in 0.1M Piperazine-1,4-*bis*-(2-ethanesulfonic acid) (PIPES) buffer (pH 7.2), followed by dehydration through a series of graded ethanol steps to 100% ethanol. Samples were then critical-point dried with $CO_2$ using a critical point drier (Balzers, Liechtenstein) prior to mounting on stubs and sputter-coating with gold/palladium. SEM was performed on a Quanta 200 (FEI, Eindhoven, Netherlands) scanning electron microscope. Micrographs of four random areas of each scaffold were taken.

A RPE cell line (ARPE-19) was purchased from the American Tissue Culture Collection (ATCC) (Manassas, VA, USA). Cells were maintained at 37$^{o}$C in a humidified atmosphere with 5% $CO_2$ in culture medium comprising 1:1 (vol/vol) mixture of D-MEM:F12 (ATCC) supplemented with 1%

antibiotic-antimycotic solution (10,000 units/ml penicillin G, 10 mg/ml streptomycin sulphate, 25 µg/ml amphotericin B) (Sigma-Aldrich, Ayrshire, UK) and 10% fetal bovine serum (FBS) (ATCC).

Scaffolds were cut into $10mm^2$ sections and sterilised overnight in 70% ethanol. Under aseptic conditions scaffolds were rinsed 3 times in phosphate buffered saline (PBS) and then transferred to 6-well plates (Fisher Scientific). Half of the polymers prepared for each blend were coated with 1ml Laminin (0.5µg/ml) (Invitrogen, Paisley, UK) for 60 minutes at $37^oC$, the other half remained uncoated. Scaffolds were rinsed 3 times with PBS, all samples were then flooded with culture medium and incubated for 30 minutes at $37^oC$. Cells were seeded at a density of $4x10^4$ per 100µl. Cells were allowed to attach for 30 minutes, 4mls of culture medium was then added to each well. Cell seeded polymers were maintained for 4 days, polymer/cell constructs were then seeded at the same density on the opposite side of the polymer. Culture medium was changed every 48 hours, samples of polymer were taken 1, 2 and 4 weeks post-seeding.

Terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) was used to detect DNA fragmentation resulting from apoptosis. Samples of seeded polymer were cryosectioned (15µm) and fixed in 4 % paraformaldehyde (PFA) 1, 2 and 4 weeks post-seeding. Evaluation of apoptotic cell death was performed using a commercially available kit (Promega, Madison, WI, USA) in accordance with manufacturers' instructions. DNase I treatment was used as a positive control and exclusion of rTNT from the reaction buffer was used as the negative control. Cell nuclei were visualised with propidium iodide (PI) (Invitrogen). PI positive and TUNEL positive cell areas were evaluated using the density slice function on Improvision Volocity software (Improvision, Coventry Lexington, UK).

At 1, 2 and 4 weeks scaffolds were cryosectioned at 15µm and fixed in 4% PFA. Sections were stained with antisera directed against Proliferating Cellular Nuclear Antigen (PCNA) (1:6000 Sigma-Aldrich), Cytokeratin 18 (1:250 Abcam, Cambridge, UK), Cellular Retinaldehyde-Binding Protein (CRALBP) (1:250 Abcam) and Retinal Pigment Epithelium-specific protein 65kDa (RPE65) (1:250 Abcam). Samples were incubated overnight at $4^oC$ with primary antibody. Samples were then incubated for 2 hours at room temperature with an Alexa Fluor 488-conjugate (1:1000

Invitrogen). Cell nuclei were visualised with 4’,6’-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich). Samples were imaged using a Leica DM IRB microscope (Leica Microsystems UK Ltd, Milton Keynes, UK). Cryosections were also used to determine cell area by nuclear labelling with PI, polymer thickness and porosity. Ten fields were imaged per condition at each time point. Cell area was measured in relation to total polymer area, with analysis carried out using Volocity software (Improvision).

All data were analyzed by either Student’s t-test or one way analysis of variance (ANOVA) using GraphPad Prism Software (GraphPad Software Inc., San Diego, CA, USA). Post-hoc comparisons were made using the Tukey-Kramer Multiple Comparisons Test when the p-value was significant ($p<0.05$).

## Results

Scaffolds were fabricated using a solid-liquid phase separation technique which led to the formation of a reproducible pore structure, which was a direct result of the freezing process. As the lactide unit content was increased through the blends, pores generally became smaller. The 10:90 blend gave an open macroporous structure with pore size ranging from 20-70μm (Fig. 1A(i)). While the 90:10 blend had surface pores (10-20μm) with smaller micropores below the surface, approximately ten fold smaller (Fig. 1A(v)). Surface porosity was quantified using the density slice function of Volocity software (Improvision). Average porosity prior to seeding was 33.2% ± 2.3, the 25:75 PLLA:PLGA blend was the most porous scaffold with an average porosity of 44.4% ± 0.1, while the 50:50 blend was the least porous (25.4% ± 0.2). Polymer thickness was determined using Volocity software. The 50 PLLA:50 PLGA polymer blend was the thickest produced while the thinnest was the 25:75 blend. Average overall thickness at week 1, 2 and 4 post seeding was 186.3 μm ± 3.7, 172.7 μm ± 3.4 and 143.9 μm ± 3.9 respectively (Table 1).

| | **Thickness ($\mu m^2$)** | **SEM** | **Porosity (%)** | **SEM** | **Total Area ($\mu m^2$)** | **SEM** | **Cell area ($\mu m^2$)** | **SEM** |
|---|---|---|---|---|---|---|---|---|
| **10:90** | | | | | | | | |
| week 1 | 196.8 | 4.1 | 39.7 | 3.3 | 67617 | 2552 | 23939 | 1835 |
| week 2 | 170.5 | 4.9 | 45.7 | 0.7 | 52843 | 2597 | 25138 | 1362 |
| week 4 | 107.3 | 1.9 | 55.0 | 2.8 | 47820 | 1500 | 16463 | 1284 |
| **25:75** | | | | | | | | |
| week 1 | 133.1 | 3.5 | 42.9 | 3.8 | 56634 | 2979 | 33337 | 3706 |
| week 2 | 131.5 | 2.3 | 45.1 | 5.8 | 51423 | 1577 | 22149 | 1104 |
| week 4 | 103.5 | 1.2 | 64.9 | 2.5 | 40546 | 2758 | 40304 | 2513 |
| **50:50** | | | | | | | | |
| week 1 | 229.1 | 3.6 | 26.2 | 2.2 | 110885 | 25042 | 10477 | 938 |
| week 2 | 200.4 | 1.5 | 34.0 | 3.3 | 100264 | 12897 | 28041 | 3029 |
| week 4 | 183.9 | 8.8 | 37.9 | 1.2 | 70951 | 9061 | 17913 | 887 |
| **75:25** | | | | | | | | |
| week 1 | 197.2 | 4.3 | 32.6 | 1.8 | 70234 | 852 | 17543 | 1599 |
| week 2 | 190.3 | 12.4 | 37.4 | 7.8 | 68119 | 3014 | 27923 | 2103 |
| week 4 | 160.6 | 4.3 | 38.4 | 1.9 | 66783 | 9649 | 17265 | 1045 |
| **90:10** | | | | | | | | |
| week 1 | 177.9 | 10.2 | 34.7 | 7.2 | 63533 | 1057 | 15975 | 2169 |
| week 2 | 177.0 | 2.7 | 38.2 | 1.8 | 60265 | 6517 | 24168 | 1288 |
| week 4 | 155.5 | 3.5 | 49.1 | 3.0 | 58611 | 1100 | 11955 | 784 |

Table 1

The polymers used in this paper are commercially available, however it was important to validate the blends produced. Inverse-gated $^{13}C$ NMR was used to determine the ratio of lactide to

glycolide units within the copolymer blends. Initially an inverse-gated $^{13}C$ spectrum was obtained for PLGA. This spectrum was used to check the veracity of the lactide to glycolide units composition supplied by the manufacturer. The spectrum showed close agreement to the anticipated 75:25 ratio. Most spectra showed the presence of slightly more lactide units than expected (Table 2). For the 90:10 PLLA:PLGA blend, a peak for the methylene group in the glycolide unit was not observed above spectral noise due to the relatively small percentage of glycolide units present in this blend (2.5% predicted).

| **Polymer blend PLLA:PLGA** | **Ratio PLLA:PLGA** | **Lactide unit calculation (%)** | **Glycolide unit calculation (%)** |
|---|---|---|---|
| **10:90** | **77.50:22.50** | **(0.75 x 90) + 10 = 77.50** | **0.25 x 90 = 22.50** |
| | 78.50:21.50 | $\frac{0.84}{0.84+0.23}$ x 100 = 78.50 | $\frac{0.23}{0.84+0.23}$ x 100 = 21.50 |
| **25:75** | **81.25:18.75** | **(0.75 x 75) + 25 = 81.25** | **0.25 x 75 = 18.75** |
| | 82.64:17.36 | $\frac{1}{1+0.21}$ x 100 = 82.64 | $\frac{0.21}{1+0.21}$ x 100 = 17.36 |
| **50:50** | **87.50:12.50** | **(0.75 x 50) + 50 = 87.50** | **0.25 x 50 = 12.50** |
| | 88.24:11.76 | $\frac{0.75}{0.75+0.10}$ x 100 = 88.24 | $\frac{0.10}{0.75+0.10}$ x 100 = 11.76 |
| **75:25** | **93.75:6.25** | **(0.75 x 25) + 75 = 93.75** | **0.25 x 25 = 6.25** |
| | 93.55:6.45 | $\frac{0.87}{0.87+0.06}$ x 100 = 93.55 | $\frac{0.06}{0.87+0.06}$ x 100 = 6.45 |
| **90:10** | **97.50:2.50** | **(0.75 x 10) + 90 =97.50** | **0.25 x 10 = 25.00** |
| | 100:0.00 | $\frac{0.86}{0.86}$ x 100 = 100 | Glycolic peak missing. |

Table 2

The pore architecture of the manufactured polymer blends allowed for cell attachment which is important for proliferation and maintenance of function for anchorage dependent cells [18]. Cells were noted on all polymer blends and were also seen to populate the interior of the scaffolds as visualised by transmission electron microscopy (data not shown). Laminin coating did not appear to affect cell attachment.

There was significantly less cell death ($p<0.001$) at all time points when compared to positive control (cells treated with DNase I) (Fig. 2 A;B). Minimal cell death was noted 1, 2 and 4 weeks post-seeding, average overall cell death was 6.6% ± 0.8, 9.1% ± 0.8 and 10.4% ± 0.9 respectively

(Fig. 2C). Average overall cell death on uncoated polymer blends was 9.7% ± 0.8, while on laminin coated polymers cell death was 9.2% ± 0.5, this difference was not significant (p=0.597) (Fig. 2D). Cell death was however reduced at all time points on the laminin coated 50:50 polymer blend. This effect did not reach significance. Cell death consistently decreased over the investigation period on the coated 25:75 polymer blend only. Cell death at week 1 (11.0% ± 2.6) decreased to 5.4% ± 1.5 by week 4 (p= 0.056) (Supplementary Table 1).

Quantification of cell density revealed that cells covered an average of 37.5%, 38.2% and 36.5% of the total polymer area at 1, 2 and 4 weeks post seeding. Average porosity was quantified as 35.2% ± 2.1 (week 1), 40.8% ± 2.1 (week 2) and 46.8% ± 1.6 (week 4) (Table 1). Only the 25:75 ratio maintained ARPE-19 cell proliferation/attachment throughout the investigation period. Morphology of ARPE-19 cells was unaffected by polymer ratio when compared to cells grown on tissue culture plastic. Scanning electron micrographs taken 7 days post seeding revealed numerous apical microvilli present on ARPE-19 cells (Fig. 1C, Supplementary Fig. 1). ARPE-19 cells also retained a characteristic cuboidal morphology with centrally located nuclei on all polymer blends. Staining was carried out for the non-squamous epithelial cell marker cytokeratin 18 and characteristic phenotypic RPE cell markers (RPE65 and CRALBP) (Fig. 3, Supplementary Fig. 2).

Gradual hydrolysis of the polymer component was seen over the investigation period. There was an average decrease in polymer thickness of 23.7% ± 5.7. Variations in pore size within the different polymer blends did not significantly affect the degradation process[1]. However degradation was observed as an increase in pore size. Large channels were noted 2 and 4 weeks post seeding in all polymers. Cross sectional porosity was quantified as a percentage of total polymer area ((Fig. 1D (i-iii) 42.9 % ± 3.8, 45.1% ± 5.8 and 64.9% ± 2.5). Laminin coating did not affect the rate of degradation of the polymer blends studied.

## Discussion

*In vitro* models offer a controlled environment in which the direct effect of polymer composition on cell adhesion, proliferation, and viability can be investigated [19]. In the present study we addressed the questions of cell attachment, proliferation and maintenance of phenotypic characteristics in a range of copolymer blends of PLLA:PLGA. A well-characterized human RPE cell line (ARPE-19) was used to investigate cell attachment and potential toxicity in blends of PLLA:PLGA (10:90-90:10). We verified the copolymer blend ratios by inverse-gated NMR Spectra produced by this method were in good agreement with those predicted.

Primarily we investigated cell adherence to the polymer blends. Samples of polymer/cell constructs were examined 1, 2 and 4 weeks post seeding. Microscopic analysis showed no obvious differences in cellular morphology between the polymer blends. Cells readily adhered to and proliferated on all materials tested. Minimal cell death was noted over the investigation period. Coating with laminin, an ECM component which plays a crucial role in cell migration and differentiation [20], did not alter cell attachment or survival. Chemical bonding of laminin or fragments of the laminin protein may produce a more significant effect [21]. ARPE-19 cells may transdifferentiate to a fibroblast-like state *in vitro* [22]. However in this study analysis using characteristic RPE cell markers, demonstrated retention of phenotype. Importantly, for cell transplantation to the sub-retinal space, ultra-structural inspection of cellular morphology with SEM showed monolayers of ARPE-19 cells with no apparent multilayering. Numerous apical microvilli were present on the surface of ARPE-19 cells. Microvilli normally interdigitate with the outer segments of photoreceptors and mediate phagocytosis of rod outer segments [23], thus the presence of microvilli on the surface of cultured ARPE-19 cells is indicative that these cells had retained functional integrity *in vitro*.

Polymer environment, monomer ratio, and scaffold thickness have a major influence on the kinetics of degradation [24]. Degradation rate is governed by the ratio of hydrophilic polyglycolic acid to hydrophobic polylactic acid [7]. A high degree of hydrophilicity facilitates the absorption and diffusion of water leading to hydrolysis, accordingly the 10:90 and 25:75 PLLA:PLGA polymer blends appeared to have degraded more when compared to the other more lactide rich blends. Degradation time is fundamental for successful biocompatibility and functionality [1]. It is important for polymers to act as a substrate for a sufficient time for cells to become confluent and establish essential cell-cell interactions. Excessively long degradation times may however induce chronic

inflammatory reactions *in vivo* [25]. PLLA alone has a degradation time of 1 to 2 years while PLGA may degrade within weeks. Thus neither polymer alone would be ideal for transplantation. This prompted our evaluation of these polymers as composite copolymers.

Only the 25:75 (PLLA:PLGA) ratio blend enabled maintenance of cell proliferation and attachment throughout the study. This blend was the thinnest produced, and also the most porous. The balance between lactide units and thickness ensured that the polymer scaffolds did not fully degrade over the investigation period thus affording structural support to cells throughout. These findings are indicative that for RPE cell growth the 25:75 (PLLA:PLGA) ratio produced is the most efficacious. Preliminary work such as this is required prior to assessment in animal models of disease. Such research is necessary to develop innovative treatments for common causes of blindness such as age related macular degeneration.

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**Competing Interests-** None

Reference List


1. **Barbanti SH,** Santos AR, Jr., Zavaglia CA *et al*. Porous and dense poly(L-lactic acid) and poly(D,L-lactic acid-co-glycolic acid) scaffolds: in vitro degradation in culture medium and osteoblasts culture. *J.Mater.Sci.Mater.Med.* 2004;**15**:1315-21.

2. **Lotery A,** Trump D. Progress in defining the molecular biology of age related macular degeneration. *Hum.Genet.* 2007;**122**:219-36.

3. **Klassen H,** Ng TF, Kurimoto Y *et al*. Multipotent retinal progenitors express developmental markers, differentiate into retinal neurons, and preserve light-mediated behavior. *Invest Ophthalmol.Vis.Sci.* 2004;**45**:4167-73.

4. **Lu L,** Yaszemski MJ, Mikos AG. Retinal pigment epithelium engineering using synthetic biodegradable polymers. *Biomaterials* 2001;**22**:3345-55.

5. **Lavik EB,** Klassen H, Warfvinge K *et al*. Fabrication of degradable polymer scaffolds to direct the integration and differentiation of retinal progenitors. *Biomaterials* 2005;**26**:3187-96.

6. **Tomita M,** Lavik E, Klassen H *et al*. Biodegradable polymer composite grafts promote the survival and differentiation of retinal progenitor cells. *Stem Cells* 2005;**23**:1579-88.

7. **Lu L,** Peter SJ, Lyman MD *et al*. In vitro and in vivo degradation of porous poly(DL-lactic-co-glycolic acid) foams. *Biomaterials* 2000;**21**:1837-45.

8. **Ng TF,** Lavik E, Keino H *et al*. Creating an immune-privileged site using retinal progenitor cells and biodegradable polymers. *Stem Cells* 2007;**25**:1552-9.

9. **Hasirci V,** Lewandrowski K, Gresser JD *et al*. Versatility of biodegradable biopolymers: degradability and an in vivo application. *J.Biotechnol.* 2001;**86**:135-50.

10. **Lu L,** Garcia CA, Mikos AG. Retinal pigment epithelium cell culture on thin biodegradable poly(DL-lactic-co-glycolic acid) films. *J.Biomater.Sci.Polym.Ed* 1998;**9**:1187-205.

11. **Sakurai E,** Nozaki M, Okabe K *et al*. Scleral plug of biodegradable polymers containing tacrolimus (FK506) for experimental uveitis. *Invest Ophthalmol.Vis.Sci.* 2003;**44**:4845-52.

12. **Guosen H,** Min F, Xin L *et al*. Design, synthesis and in vitro evaluation of a novel "stealth" polymeric gene vector. *Int.J.Pharm.* 2008;**350**:344-50.

13. **Dong Y,** Zhang Z, Feng SS. d-alpha-Tocopheryl polyethylene glycol 1000 succinate (TPGS) modified poly(l-lactide) (PLLA) films for localized delivery of paclitaxel. *Int.J.Pharm.* 2008;**350**:166-71.

14. **El-Ayoubi R,** Eliopoulos N, Diraddo R *et al*. Design and fabrication of 3D porous scaffolds to facilitate cell-based gene therapy. *Tissue Eng Part A* 2008;**14**:1037-48.

15. **Hadlock T,** Singh S, Vacanti JP *et al*. Ocular cell monolayers cultured on biodegradable substrates. *Tissue Eng* 1999;**5**:187-96.

16. **Thomson RC,** Giordano GG, Collier JH *et al*. Manufacture and characterization of poly(alpha-hydroxy ester) thin films as temporary substrates for retinal pigment epithelium cells. *Biomaterials* 1996;**17**:321-7.

17. **Hausberger AG,** DeLuca PP. Characterization of biodegradable poly(D,L-lactide-co-glycolide) polymers and microspheres. *J.Pharm.Biomed.Anal.* 1995;**13**:747-60.

18. **Giordano GG,** Thomson RC, Ishaug SL *et al*. Retinal pigment epithelium cells cultured on synthetic biodegradable polymers. *J.Biomed.Mater.Res.* 1997;**34**:87-93.

19. **Huhtala A,** Pohjonen T, Salminen L *et al.* In vitro biocompatibility of degradable biopolymers in cell line cultures from various ocular tissues: direct contact studies. *J.Biomed.Mater.Res.A* 2007;**83**:407-13.

20. **Huang YC,** Huang CC, Huang YY *et al*. Surface modification and characterization of chitosan or PLGA membrane with laminin by chemical and oxygen plasma treatment for neural regeneration. *J.Biomed.Mater.Res.A* 2007;**82**:842-51.

21. **Hersel U,** Dahmen C, Kessler H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. *Biomaterials* 2003;**24**:4385-415.

22. **Opas M.** Substratum mechanics and cell differentiation. *Int.Rev.Cytol.* 1994;**150**:119-37.

23. **Lee CJ,** Fishman HA, Bent SF. Spatial cues for the enhancement of retinal pigment epithelial cell function in potential transplants. *Biomaterials* 2007;**28**:2192-201.

24. **Spenlehauer G,** Vert M, Benoit JP *et al*. In vitro and in vivo degradation of poly(D,L lactide/glycolide) type microspheres made by solvent evaporation method. *Biomaterials* 1989;**10**:557-63.

25. **Ronneberger B,** Kao WJ, Anderson JM *et al*. In vivo biocompatibility study of ABA triblock copolymers consisting of poly(L-lactic-co-glycolic acid) A blocks attached to central poly(oxyethylene) B blocks. *J.Biomed.Mater.Res.* 1996;**30**:31-40.

**Legends to Figures**

**Fig. 1A** Scanning electron micrographs of the range of PLLA:PLGA polymer scaffold blends prepared using the solid-liquid separation technique. **i-**10:90, **ii-** 25:75, **iii-** 50:50, **iv-** 75:25 and **v-** 90:10**.** Scale bar- 10µm. Original magnification 3000 x. **Fig. 1B** Polymer blend formulations. All blends formulated in neat 1,4-Dioxane with Poly(L-lactic acid) (PLLA) Resomer® L 207 S and poly(D, L-lactide-co-glycolide) (PLGA) Resomer® RG 755 S with a lactic to glycolic ratio 75:25, both obtained from Boehringer Ingelheim GmbH (Ingelheim, Germany). **Fig. 1C** Morphological characterisation of ARPE-19 cells cultured on 25:75 blend PLLA:PLGA polymers for one week. Apical microvilli (black arrows) noted on the surface of ARPE-19 cells. Scale bar-10µm. Original magnification 3000 x. **Fig. 1D** Light micrographs of uncoated 25:75 PLLA:PLGA polymer blend. **i-** 1 week, **ii-** 2 weeks and **iii-** 4 weeks after initial seeding with ARPE-19 cells. Scale bar-26µm

**Fig. 2 A;B** Histograms depicting percentage ARPE-19 cell death assessed by TUNEL assay. Percentage apoptosis was quantified using the density slice function on Volocity software (Improvision). Results are mean ± SEM (n=10). Significant difference * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to cells cultured on tissue culture plastic at corresponding time points by ANOVA followed by the Tukey-Kramer multiple comparison test. **2C-** Total ARPE-19 cell death grouped by time ** $P<0.01$ compared to week 1. **2D-** Total ARPE-19 cell death on uncoated versus coated polymer blends.

**Fig. 3** Immunocytochemical characterization of ARPE-19 cells cultured for 1 week on laminin coated porous (PLLA:PLGA 25:75) scaffold using antibodies directed against: **A-** the retinal pigment epithelium marker-retinal pigment epithelium-specific protein-65kDa (RPE 65), **D-** the non-squamous epithelial marker cytokeratin 18, **G-** Anti-cellular retinaldehyde binding protein (CRALBP), **J-** the cell cycle marker- proliferating cell nuclear antigen (PCNA) and **M-** negative control (omission of primary antibody). Labelled in **green** (**A, D, G, J, M**). Cell nuclei are labelled in **blue** with 4',6-diamidino-2-phenylindole DAPI (**B, E, H, K, N**). Merged images (**C, F, I, L, O**). Scale bar- 13µm

**Table 1** Table depicting average scaffold thickness, porosity, polymer area for cell attachment and cell area at 1, 2 and 4 weeks post seeding with ARPE-19 cells. Ten fields were imaged per

condition at each time point. Porosity and cell area were measured in relation to total polymer area. Measurements and analysis carried out using Volocity software (Improvision). Results are mean ± SEM (n=10) per condition.

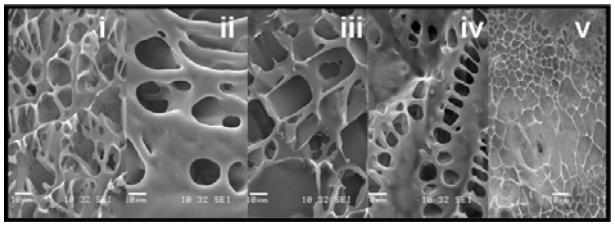
**Table 2** $^{13}$C Nuclear Magnetic Resonance Spectroscopy ratios for each polymer blend. Table showing NMR predicted and spectral ratios for each polymer blend, with lactide and glycolide unit calculations for the predicted (in bold) and spectral values.

**Supplementary Table 1**- Table comparing ARPE-19 cell death on laminin coated polymer blends with uncoated scaffolds. Measurements and analysis carried out using Volocity software (Improvision). Results are mean ± SEM (n=10) per condition. Data were analyzed by ANOVA.

**Supplementary Fig. 1**- Morphological characterisation of ARPE-19 cells cultured on blended PLLA:PLGA copolymers for 1 week. Apical microvilli (black and white arrow heads) noted on the surface of ARPE-19 cells. **A**- 10:90 blend coated with laminin, scale bar 50µm and **B**- same image taken at a higher magnification, scale bar 5 µm, **C**- uncoated 75:25 blend scale bar 10µm, **D**- coated 75:25 blend, scale bar 10µm.

**Supplementary Fig. 2**- Immunocytochemical characterization of laminin coated porous (PLLA:PLGA 25:75) copolymer scaffolds maintained in cultured for 1 week. Control incubation of polymers with antibodies directed against: **B-** the retinal pigment epithelium marker-retinal pigment epithelium-specific protein-65kDa (RPE 65), **D-** the non-squamous epithelial marker cytokeratin 18, **F-** Anti-cellular retinaldehyde binding protein (CRALBP), **H-** the cell cycle marker- proliferating cell nuclear antigen (PCNA) and **J-** negative control (omission of primary antibody). Corresponding light microscopy images (**A,C,E,G,I**). Scale bar- 13µm.

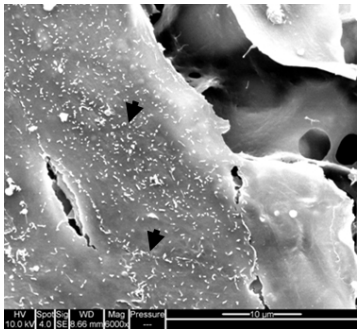
A



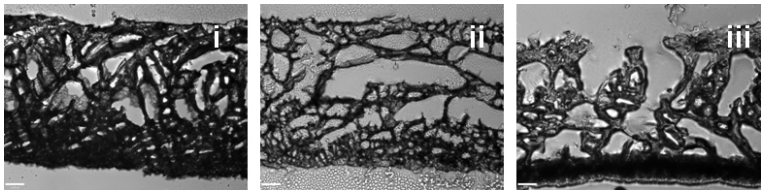

B

| Blend ratio | PLLA weight (g) | PLGA weight (g) |
|---|---|---|
| **i- 10:90** | 0.025 | 0.225 |
| **ii- 25:75** | 0.0625 | 0.1875 |
| **iii- 50:50** | 0.125 | 0.125 |
| **iv- 75:25** | 0.1875 | 0.0625 |
| **v- 90:10** | 0.225 | 0.025 |

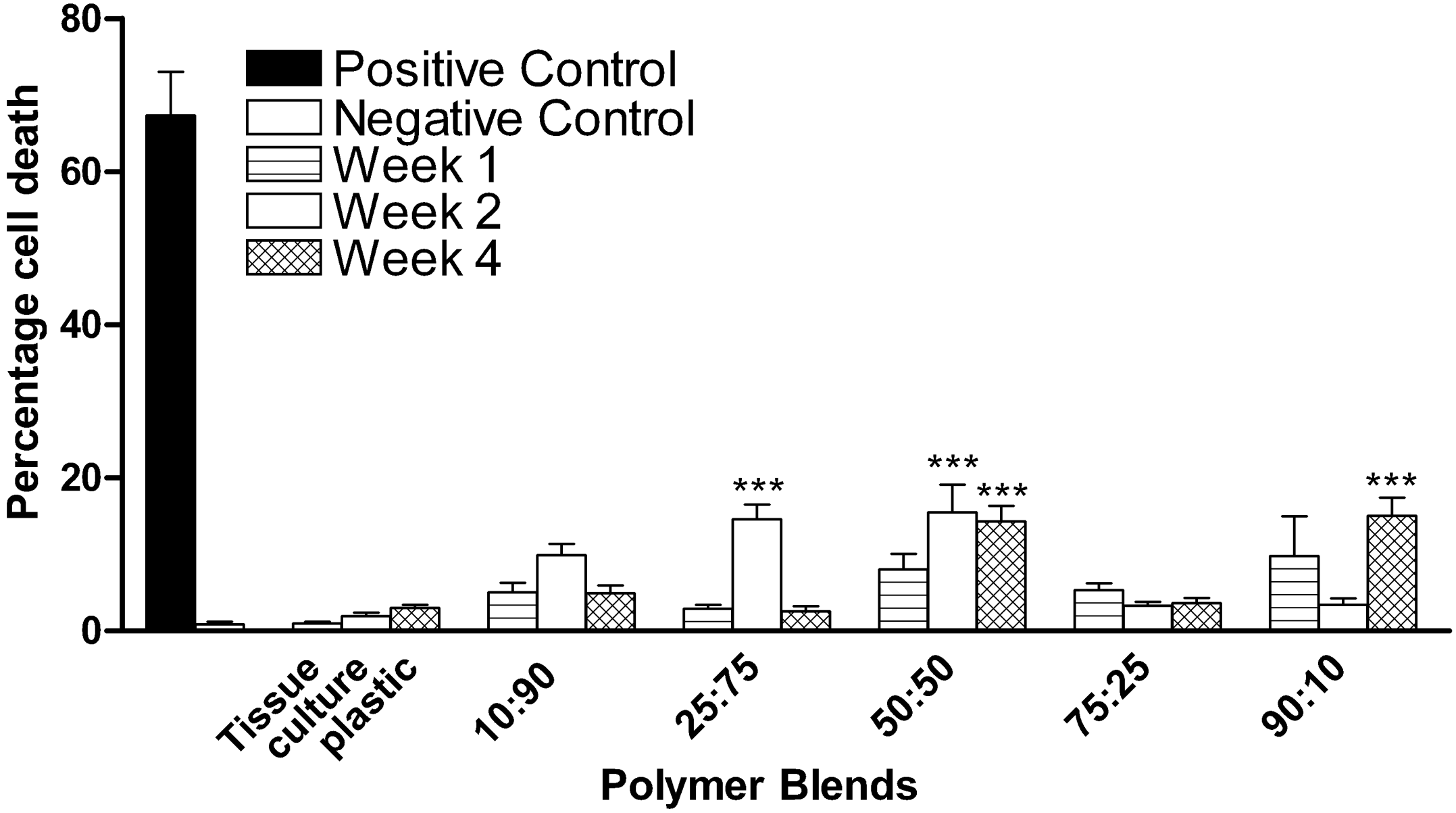
C



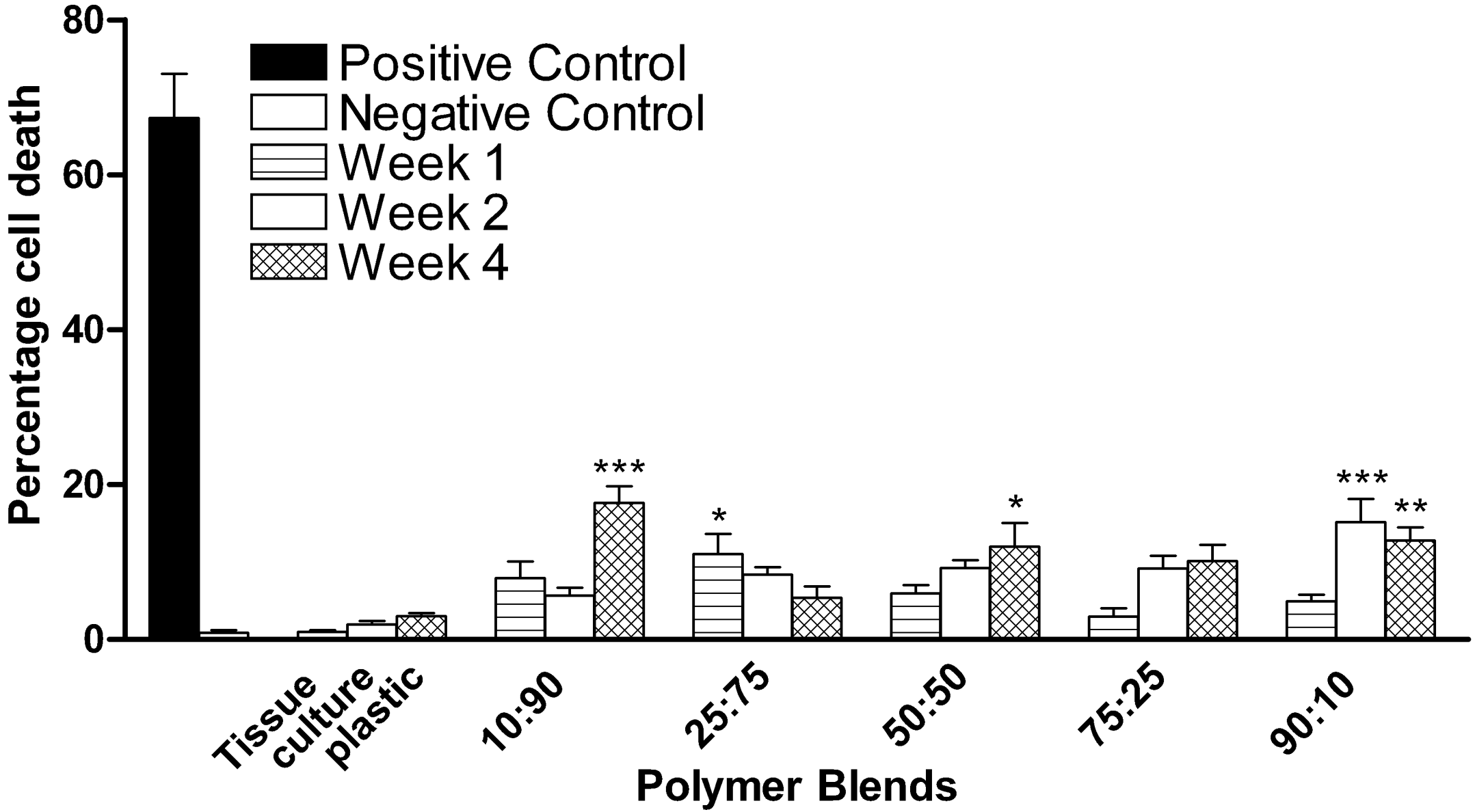
D



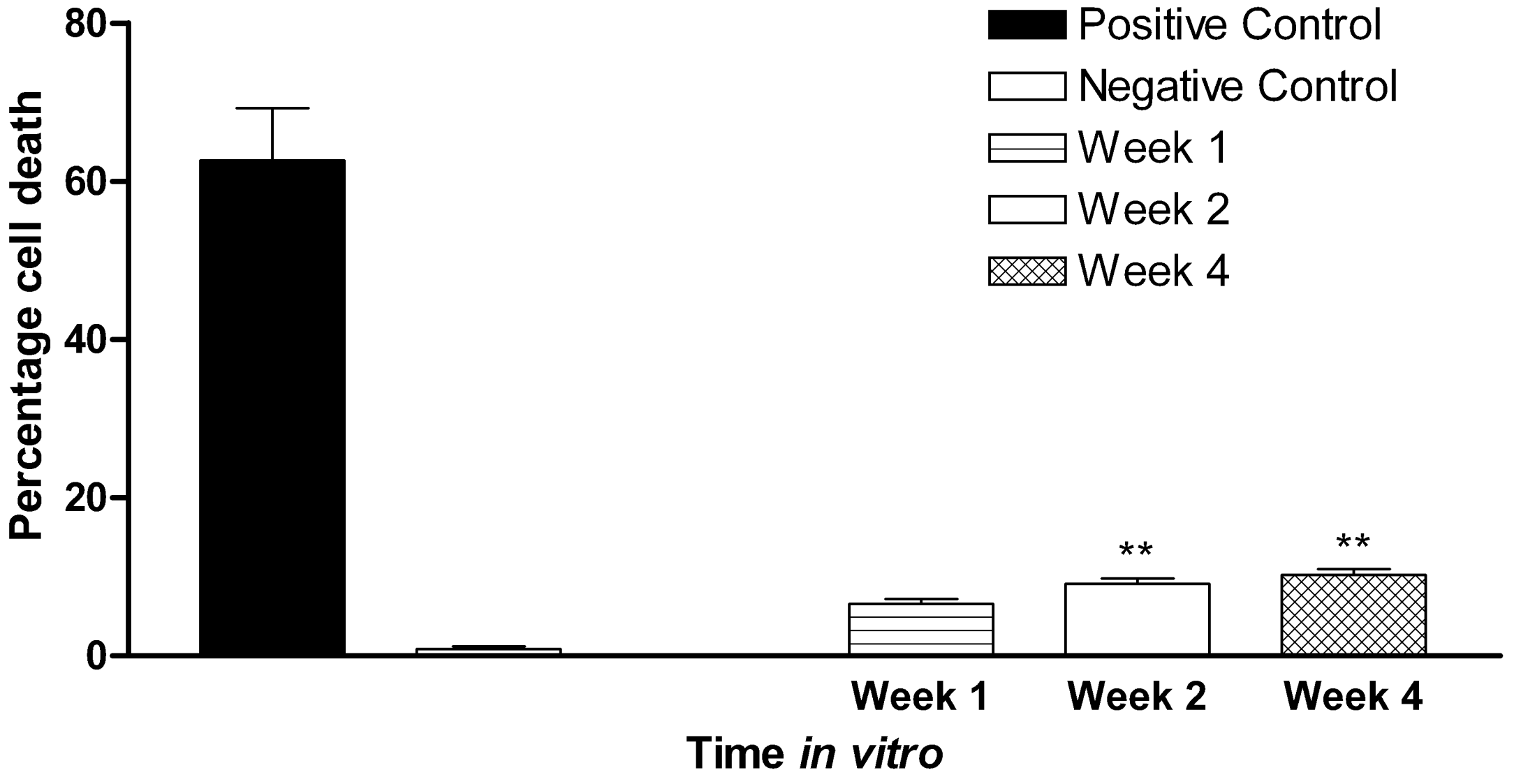
# Fig. 2A: Percentage ARPE-19 cell death on uncoated polymers 1, 2 and 4 weeks following seeding



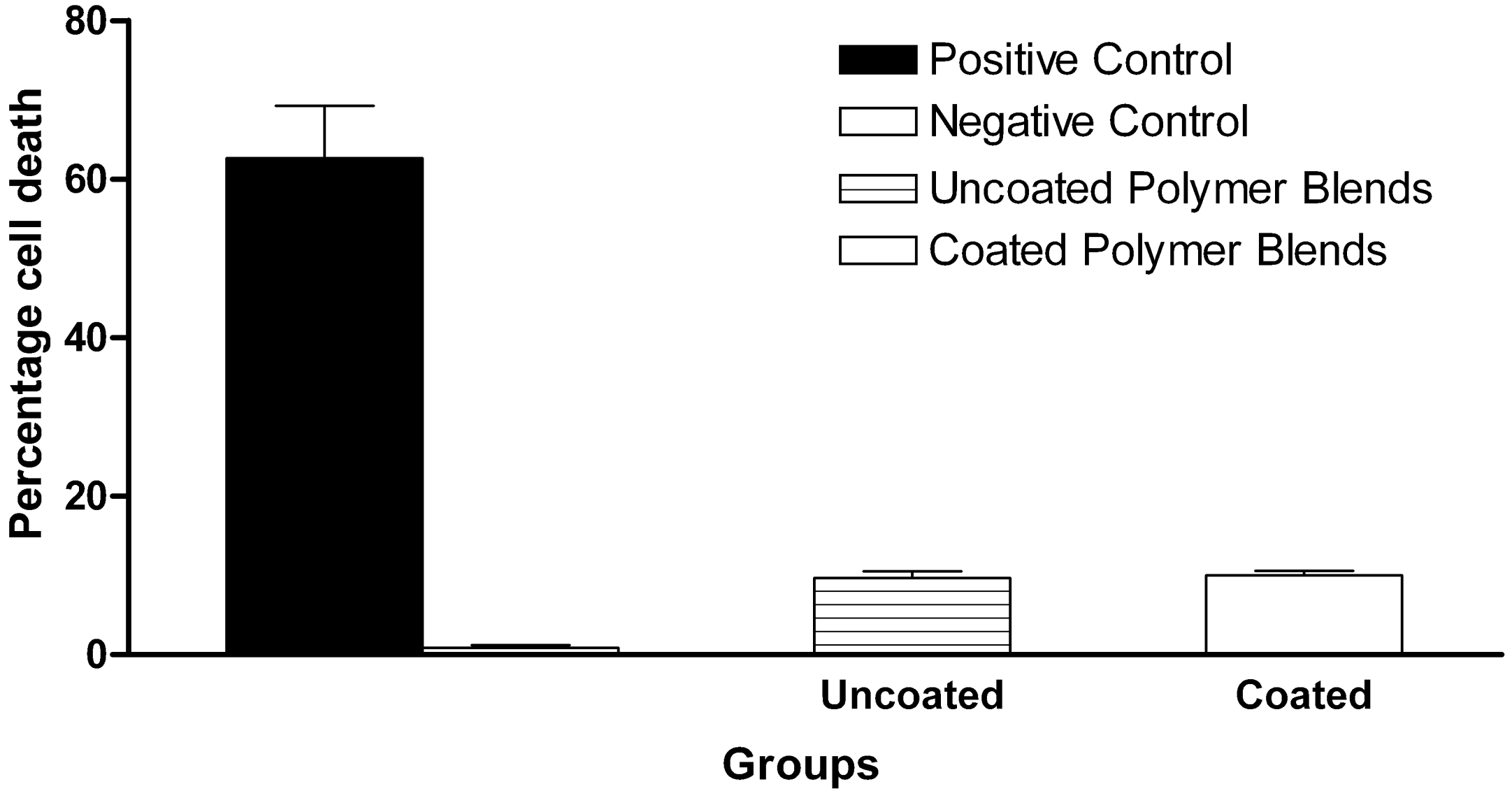
# Fig. 2B: Percentage ARPE-19 cell death on laminin coated polymers 1, 2 and 4 weeks following seeding

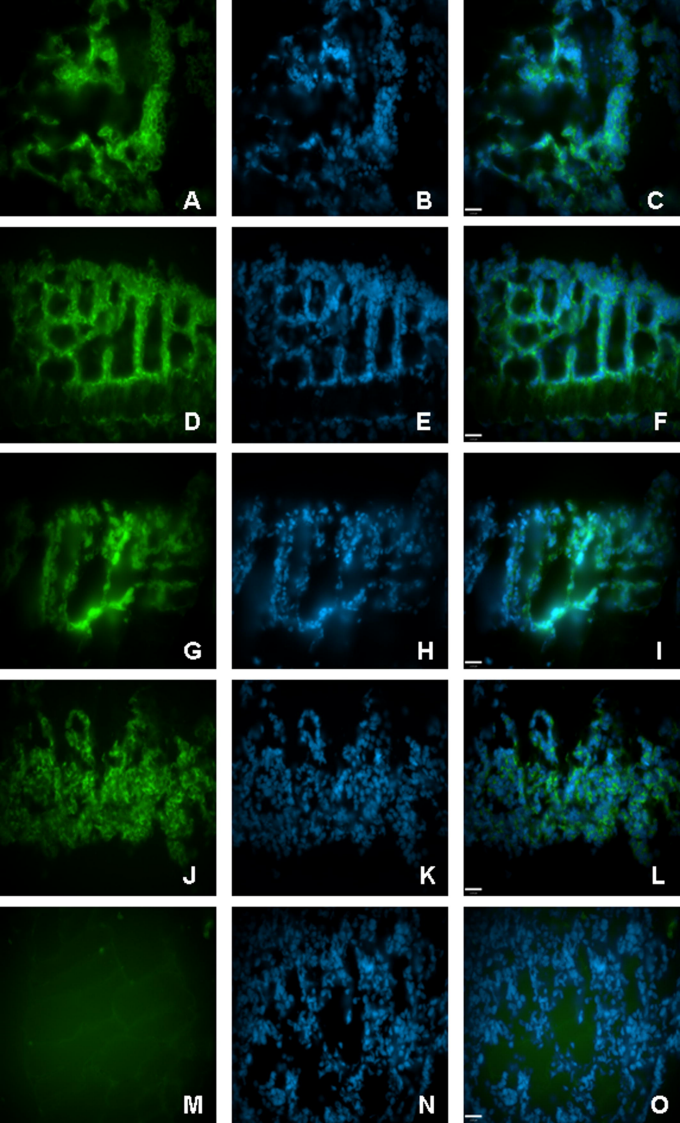


# Fig. 2C: Total ARPE-19 cell death on polymer blends 1, 2 and 4 weeks following seeding



# Fig. 2D: Total ARPE-19 cell death on uncoated and coated polymer blends 1, 2 and 4 weeks post seeding

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