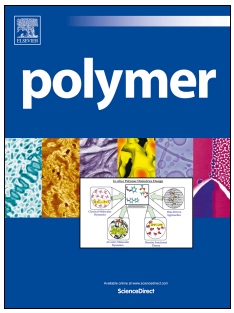
Accepted Manuscript



Cell morphology and growth observation studies on novel, chemically unmodified and patterned polymer surfaces for advanced tissue culture applications

C.H. Trease, M.R. Longman, A.T. Augousti, P.J.S. Foot, B. Pierscionek

PII: S0032-3861(16)31121-1

DOI: 10.1016/j.polymer.2016.12.032

Reference: JPOL 19268

To appear in: *Polymer*

Received Date: 8 October 2016

Revised Date: 25 November 2016

Accepted Date: 11 December 2016

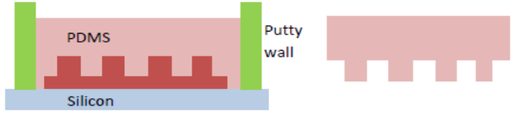
Please cite this article as: Trease CH, Longman MR, Augousti AT, Foot PJS, Pierscionek B, Cell morphology and growth observation studies on novel, chemically unmodified and patterned polymer surfaces for advanced tissue culture applications, *Polymer* (2017), doi: 10.1016/j.polymer.2016.12.032.

Electrohydrodynamic instability patterning

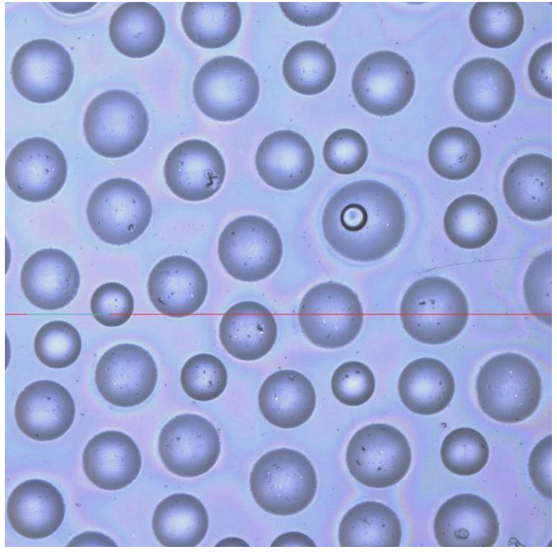
A

B

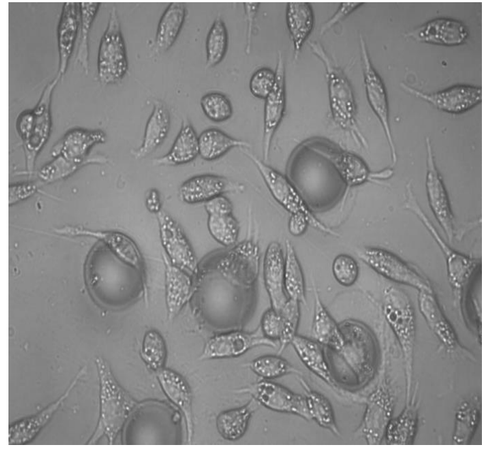
C



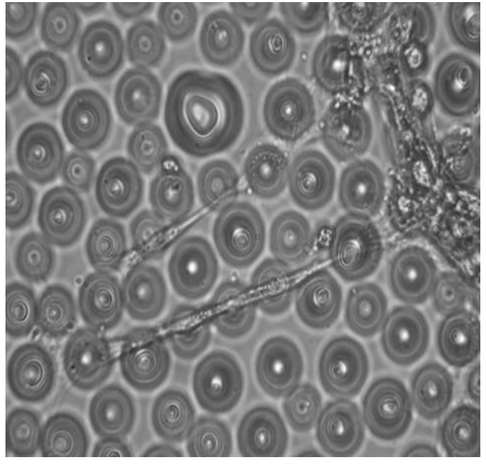
a b



Printed polymer surfaces



Cell Specific interactions.



Cell morphology and growth observation studies on novel, chemically unmodified and patterned polymer surfaces for advanced tissue culture applications

Trease, C.H.*[1]; Longman, M.R.*[1]; Augousti, A.T[1].; Foot, P.J.S[1] and Pierscionek, B.**[1]

[1]Faculty of Science, Engineering and Computing. Kingston University London, Penrhyn Road, Kingston-upon-Thames, KT1 2EE UK

*Joint first authors:

**Corresponding author

Abstract

Creation of more physiologically relevant cell models in tissue culture is a requisite for advancing medical research. It can involve complex substrates, expensive manufacturing and largely inaccessible methods of increasing surface energy and patterning of materials that may be unnecessary in many circumstances. An array of various different adherent cell lines (human, mammalian, healthy and disease states) were grown on simple sterilised but otherwise untreated thin film surfaces as well as on electro-hydrodynamically patterned surfaces to produce topographically patterned culture surfaces. Room temperature cure epoxy resin and unmodified poly(methyl methacrylate) (PMMA) thin film surfaces were used for cell growth and morphological observations. Differing responses in growth, morphology and adherence were observed in a surface- and cell-specific manner. With no complex and expensive modifications required, we demonstrate the application of novel, suitable and easily patterned materials for use in more advanced tissue culture applications for a variety of clinically relevant cell lines showing unique responses and potentially new and wide-reaching applications.

Key words *cell culture, novel material surfaces, human and mammalian lines*

1. Introduction

Alternative techniques to animal use for medical testing have been investigated since the 3 R's:- Refine, Reduce and Replace were first proposed over 50 years ago (1). The ethical issues associated with animal experimentation have been debated over the ensuing decades but a perceived lack of viable alternatives is most often cited as the reason that animals are still used (2) (3). Ethical issues notwithstanding, it has been recognised that animal testing is also expensive. Both these factors limit where research can be conducted and the type of experimentation that can take place. Furthermore, animal tests may not be as reproducible as better controlled alternatives, potentially inhibiting medical advances (2) (3) and animal models do not offer an adequate representation of the human body or disease, which results in wasted resources and expense in clinical trials (4). This will become particularly pertinent with the development of personalised medicine and as drugs become targeted towards individual human physiologies.

Improved cell and tissue culture models should more accurately replicate *in vivo* conditions (5) (6) (7) (8) (9) (10). Cell adhesion and cellular interaction with surfaces is highly complex and is of growing interest in academia (2) and in industry (11). Traditional cell culture methods utilise flat, chemically or plasma treated polystyrene (PS) surfaces in tissue culture dishes. This is a limiting factor in developing better *in vitro* cell models, as in many cases this does not represent *in vivo* conditions, nor encourage or allow cells to form more representative tissue structures (2). Improving tissue culture techniques on patterned surfaces and scaffolds may provide better *in vitro* models (3).

Further investigation of these surfaces and materials is needed (5) (6) (7) (8) (9) (10) (12). The manufacturing techniques and materials used to fabricate patterned cell culture surfaces and scaffolds can be expensive, time-consuming and require dedicated manufacturing environments such as clean room facilities (8) (13). If methods used to produce more complex tissue culture surfaces could be simplified and made available to every laboratory, this would lead to the advancement of new research opportunities and to the development of new protocols (14).

The materials used to fabricate the surfaces and scaffolds have many specific physical requirements. They should be bio-compatible with a variety of cell types, non-toxic and have no adverse effects on the biological tissue. Cells must be able to adhere to and to grow on the material which needs to be transparent to allow for the cells to be visualised. The materials must be amenable to specific (for particular cell types) and reproducible (for robust analysis) patterning at scales comparable to the cells. For large-scale uptake it is also crucial that any surface must require minimal pre-treatment and allow simple 'in-house' processing and manufacture. Materials that we have examined in this study were chosen for their ease of patterning and structuring. Curable resins can be patterned using modified nano-imprint techniques. Thermoplastics such as poly(methyl methacrylate) (PMMA) and polystyrene (PS) can be shaped by hot embossing methods where the material is softened by heating and then patterned by pressing a mould into the surface to shape the softened material (14). The great advantage of these kinds of techniques is that they require minimal and affordable equipment and can be manufactured in most laboratories.

Typically polymers used for cell culture such as PS, have their surface energy increased by treatments such as corona discharge, surface chemistry enhancement and protein conjugation in order to improve cell adhesion (2) (15) (16). Surface treated PS is routinely used in tissue culture as the plate material and this and other surface-treated thermoplastics such as PMMA have previously been used with many cell lines (7) (8) (17). These kinds of surface treatments can be equipment-heavy and can limit the ability for the surface to be further modified or patterned. PS and PMMA can be solution-coated onto a glass slide and easily patterned via the hot embossing method mentioned previously. There has been little investigation of the use of the native PMMA polymer surface, with previous studies all using additional coatings or modifications (14) (17) (18) (19) (20).

This work has additionally considered the use of a two-part epoxy resin that offers prospects as a novel material. While the use of a room temperature curable resin has not been studied before in a tissue culture system these materials have some desirable properties that make them biocompatible. Epoxy resin has been used for tissue embedding in microscopy as there is no discernible distortion of fixed samples once the epoxy has cured, and has value for tissue embedding as it creates a hard

composite with adjacent materials of a uniform consistency, allowing for even ultra-thin sectioning. The ability of epoxy to form an inert, hard surface that is water insoluble (so will not be effected by tissue culture media), the ease of shaping and of moulding when in liquid state and of cold-curing means this resin offers a range of applications as a novel tissue culture surface.

Different types of epoxy resins have been used to produce super-elastic hydrogels (21), some have been UV-cured to create hydrogels with acrylamide or alginates (22) others thermo-cured and combined with polyamindoamine linkers (23) to provide structures with varying properties suitable for bioengineering (22) (24). Hydrogels have a number of applications, notably for mechanical stress sensing in cells where deformable surfaces are required. Simple, elegant methods of producing micropatterned hydrogels for regulating the size of cell adhesions and traction forces have been described (25) and have also been applied to study cell migration (26). Novel means of generating grooved surfaces in hydrogels have been used to measure cell alignment and polarisation (27). Whilst hydrogels offer advantages such as permitting nutrient diffusion to cells as they are not stable at temperatures above 37^{o}C, crosslinking agents are required and care must be taken to avoid toxicity to cells (27). Swelling of gel substrates and the effects of varying concentrations of crosslinking agents have been investigated (28).

This study has explored the viability of using the unmodified surface of PMMA and a two part room temperature curable epoxy resin with a variety of cell lines using tissue culture treated PS as a control surface. These unmodified polymers were subsequently used to create patterned surfaces for tissue culture. Whilst surfaces patterned to scales that are comparable to cells have been used previously (29), these required substantial modification using expensive techniques.

2. **Methods**

2.1 Materials

The polymers used to create the moulds and surfaces were poly (dimethylsiloxane) (PDMS) Sylgard 184 (Dow Corning), epoxy Evo-Stik Epoxy Control resin (Bostik Ltd., Stafford, UK; Product code 808508) and polystyrene (PS) MW 280,000 (Sigma-Aldrich cat-182427). Toluene (VWR cat-VWRC28676.366), acetone (VWR cat-VWRC20066.330) AND industrial methylated spirit (IMS)

(VWR cat-VWRC23684.360) were used as solvents. The electrodes were made of made of silicon wafer P-type <100> (Sigma-Aldrich cat-647764), and Indian Tin Oxide (ITO) coated glass slide 70-100ohms 25x25mm (Diamond Coatings). Cells used were all commercial cell lines obtained from ATCC: human eye lens epithelial cells B-3(ATCC cat-CRL-11421), human cervical epithelial cells (Adenocarcinoma) (HeLa ATCC cat-CCL-2), human eye retinal pigmented epithelium. Htert RPE (ATCC cat-CRL-4000), RAT embryonic heart myoblast cells. H9C2 (ATCC cat-CRL-1446), human liver epithelial cells (hepatocellular carcinoma). HepG2 (ATCC cat-HB-8065).

2.2 Preparation of surfaces

A two-part room temperature cure epoxy resin polymer, Evo-Stik Epoxy Control resin, was chosen as this does not create the plate distortion that occurs with a heat cured resin. The polymer is based on bisphenol A-epichlorhydrin resin (Mn<700), bisphenol F epoxy resin and trimethylolpropane triglycidyl ether; the hardener was Evo-Stik Epoxy Control Hardener (Bostik Ltd.; Product code 808518) based on triethylenetetramine.

Very thin layers of epoxy 10 – 20µm in depth (calculated by laser confocal scanning, using a LEXT microscope) were applied, as the resin remained clear at this thickness, allowing the cells to be observed using an inverted transmission microscope. The epoxy layers were deposited into tissue culture plate wells via a printing technique. For the printing technique epoxy was spin-coated onto a flat poly(dimethylsiloxane) (PDMS) disk from a solution of two parts dissolved in a mixture of toluene and acetone, at 1000-3500 rpm for 30seconds creating a thin layer of uncured resin with the thickness dependent on spin speed. The PDMS disks were either 30mm or 15mm in diameter, depending on the size of the well. The coated PDMS disks were placed epoxy side down into standard tissue culture plate surfaces (6 and 12-well plates, Nunclon^{TM} Delta surface, Thermoscientific) and a force of approximately 11kN was applied to the disk to ensure contact between the disk and the plate whilst the epoxy was curing. Once the epoxy had cured, the PDMS disk was removed leaving a thin layer of epoxy coating the well surface.

Prior to seeding cells onto the epoxy surface, wells were soaked or washed with cell growth media (of the cell line to be used) to remove any residue or chemicals that could potentially leach into the media and affect the cells. The surfaces were sterilised using UV light by placing them in a tissue culture laminar flow hood (Msc Advantage laminar flow hood, Thermo Scientific) and activating the decontamination cycle.

Surfaces made of PMMA (MW 180 000) were prepared by spin coating the polymer from a solution 15% by weight in toluene directly onto glass microscope slides. The spin speed was 3500 rpm and the subsequent layers were 5-20µm in thickness. These were washed or soaked in media to remove any residual solvent material. To sterilize these surfaces, they were dipped into 70% industrial methylated spirit (IMS) v/v in de-ionised water (dH_2O) followed by UV sterilisation as above.

2.3 Electrohydrodynamic instability patterning

The electrohydrodynamic (EHD) instability patterning technique was used to structure a thin layer of room temperature curable epoxy resin. This technique uses the electric field that exists between two non-contacting electrodes when a voltage is applied across them, to pattern a liquid polymer which is then solidified, making the pattern permanent (30)(31). A silicon wafer and an indium tin oxide coated glass slide (both approximately 20mm x 20mm) were employed as electrodes. During the application of the voltage they were separated with insulating spacer rails. These were fabricated from a PS layer, spin coated onto the silicon wafer ranging from 1 to 10 µm thick (Figure 1A) again achieving the desired thickness by altering the spin speed. On the bottom electrode the area between the spacer rails was spin coated with a liquid epoxy resin layer, thinner than the spacers. This left an air gap between the resin film and the top electrode when the two were placed together.

When a voltage was applied across the electrodes, an electric field was produced in both the resin layer and the air. Differences in their dielectric constants created a mismatch between the two fields. This formed an electrostatic instability at the resin/air interface, causing waves which grew in amplitude and eventually reached the top electrode to become columns. When the resin hardened,

after around four hours, and the patterns were fixed in place, the voltage and top electrode were removed. Once the pillars have formed, separation of electrodes before the resin is cured, causes the pillars to assume regular domed rather than the flat topped structures (32).

The tunability of the patterns produced was dependent on a number of controllable parameters including the magnitude of the applied voltage, the electrode spacing and the initial thickness of the resin layer as described by Goldberg-Oppenheimer et al (33). The EHD process provides precise control over all the experimental parameters (31). Higher electric fields between the electrodes, which are the result of using a larger applied voltage and/or thinner spacer rails and epoxy layers, produce smaller scale patterns. The thickness of the spacer rails and the epoxy thickness was controlled by altering the spin speed during coating, higher speed producing thinner layers. Representative EHD prepared surfaces are shown in Figures 1B and 1C. The surface in Figure 1B was fabricated using a spacer layer of 4.9 µm, a resin layer 1 µm thick and an applied voltage of 80V; the surface shown in Figure 1C was fabricated using a spacer layer of 3.7 µm, a resin layer of 1.8 µm and 162V so with a much higher voltage resulting in smaller structures. The EHD technique is known to be highly reproducible, using set fabrication parameters i.e. voltages, electrode spacing and polymer thickness to create repeatable surface topographies; spacing between the pillars can be easily tuned and highly controlled by changing various experimental parameters (30). Limitations on reproducibility arise from maintaining uniformity of the surface; whilst height of pillars is regular, controlling the shape of pillar bases depends on polymer type, thickness and applied electric field. Some variability may be beneficial as it replicates a substrate that is more akin to biological situations.

Moulds were then taken from the EHD fabricated structures (either flat topped or doomed pillar arrays) by surrounding them with a wall of reusable putty and covering them with a layer of PDMS (Sylgard 184) in a 10:1 base to curing agent mix. This is shown schematically in Figure 1D. When the PDMS had cured, it was separated from the EHD structured surface, leaving a negative imprint of the surface in the PDMS (Figure 1D). The mould was then used to fabricate surfaces for tissue culture in PS and PMMA via hot embossing and in epoxy via the modified printing technique described previously used to prepare the flat epoxy surfaces in the tissue culture wells. The hot embossed

PMMA and PS surfaces were manufactured by coating a layer of the polymer from solution in toluene, 5-20 µm thick onto a microscope slide. The mould was placed in contact with the PMMA and PS using a force of approximately 11kN, and the slide was then heated to 120°C. Once softened, the mould was impressed into the polymer. After 1 hour, the slide was left to cool and the mould was removed, leaving the EHD pattern imprinted into the polymer surface. Figure 1E shows a laser confocal scan of the surface with topographical dimensions along a representative line and a magnified section of the hot embossed replica of the surface shown in Figure 1B. The PDMS master moulds taken from each of the EHD surfaces can be used to produce multiple identical replicates of the EHD original (34) and the epoxy EHD original can be used to produce multiple PDMS master moulds (35).

2.4 Tissue Culture

A range of cell lines obtained from ATCC were handled in an aseptic manner according to provided guidelines. The HeLa human cervical cancer cell line has been widely studied and was the first human immortal cell line. The H9C2 rat neonatal cardiomyocyte cell line was chosen as it is a healthy non-human cell model. The HepG2 human liver carcinoma cell line is clinically significant and grows with atypical morphology compared to other adherent cell lines. The human lens epithelial and human retinal epithelial cell lines were also investigated in this study because of their importance in vision research. The specific cells lines culture conditions are shown in Table 1. Briefly, purchased cryopreserved stocks of these cell lines were rapidly thawed to 37^{O}C then added to pre-warmed growth media in T-75 culture flasks (Nunclon^{TM}Delta surface, Thermoscientific). Cells were allowed to achieve a desired confluence and were then dispersed by trypsinisation using 0.25% w/v trypsin 0.2% w/v EDTA in phosphate-buffered saline (PBS) (Sigma-Aldrich). Following this the trypsin was inactivated by re-suspension in growth media containing serum. Cells were then counted and seeded out at specific seeding densities on the specialised plates, modified from standard tissue cultureware (Nunclon^{TM} Delta surface, Thermoscientific) or on unmodified wells (tissue culture treated PS control surface). Cells were seeded out at approximately $1x10^{5}$ cells per well (in 12-well plates for epoxy

studies) or $2x10^5$ cells per well (for 6-well plates containing PS or PMMA surfaces) in relevant ATCC-recommended medium per cell line (Table 1).

Cells were allowed to establish for 24 hours, a typical duration that allows cells to sink to the surface, spread and adhere and then were assessed under an optical light inverted transmission microscope (Nikon) (using x4 or x10 objective) and photographed using a MDX501 PIXIT series camera from Lanoptik, using I Works EX software. Alternatively cells were placed inside an Incucyte ZOOM automated incubator microscope (Essen biosciences). Media was refreshed every 48 hours. At 24 hour time-points, cells were monitored for confluency and morphological changes. Cell growth was assessed by confluency rather than by cell counting to maintain consistency between ongoing time points on an individual surface. Where applicable, confluency was calculated by use of the Incucyte ZOOM software. Images of equivalent confluency from the surfaces were used to assess differences in morphology, looking specifically at shape changes relating to size difference and elongation. Experiments were all performed in triplicate and conducted for a duration of 192 hours.

2.5 MTT cell viability assay

MTT cell viability assay was performed as described previously (36). Briefly, lens epithelial or HepG2 cells were prepared and seeded out at approximately $1x10^5$, $2x10^5$ or $3x10^5$ cells/well into 12-well plates (Nunc, Netherland) with either a flat epoxy surface or unmodified PS tissue culture treated control surface in cell line specific medium containing the required supplements (see Table 1) .Cells were allowed to establish for 48 hours. Media was removed and the cells washed twice with 37oC sterile PBS. Cells were then incubated with 1000µl per well of 0.5mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution in 37^{o}C cell line specific medium with no additions for 3 hours at 37^{o}C. After incubation, the MTT solution was carefully removed and the wells washed twice with sterile PBS. Finally, 1000µl of dimethylsulfoxide (DMSO) was added to each well to lyse the cells. The cells were then gently agitated to mix the samples and analyzed on a TECAN Infinite M200 pro plate reader at a wavelength of 540 nm. An epoxy surfaced well and an unmodified PS

tissue culture treated control surface that was left unseeded were used as negative controls. Experiments were performed in triplicate.

3. Results

3.1.Morphology of cells on unmodified flat PMMA surfaces

Under standard laboratory culture conditions using tissue culture plasticware (tissue culture treated PS control surface) lens cells exhibited typical epithelial cell morphology, as shown in Figure 2A. These cells were slightly angular with several sharp protrusions and adhered to the culture surface, with an average size of 50µm. Lens cells on PMMA surfaces adhered and grew with their usual morphology conserved, and although they took longer to establish they reached confluency during the experimental time course (Figure 2B).

The H9c2 Rat cardiomyocyte cells showed normal morphology and grew to a regular monolayer on the unmodified PMMA akin to the PS control surface (Figures 2C and D). HepG2 cells were able to adhere and grow on PMMA surfaces with their usual morphology conserved and reached confluency during the experimental time period, albeit with a slower rate of early establishment. Retinal cells adhered and grew on unmodified PMMA surfaces with their usual morphology conserved (Figures 2E and F), at approximately the same rate as cells on PS control surfaces. HeLa cells on PS control surfaces showed no observable difference in morphology or growth rate of control cells and cells seeded onto PMMA surfaces. All cell lines studied had little or no morphological differences when grown on PMMA compared to standard PS tissue culture surfaces.

3.2.Morphology and viability of cells on unmodified flat epoxy surfaces

During the experimental period there was no significant difference in lens cell size or morphology between cells grown on either the PS control or epoxy surfaces (Figures 3A and B respectively). However, it was noted that approximately 5% of the lens cells on the epoxy surface were slightly elongated, similar to primary lens cells in culture. Cells seeded onto the epoxy surface grew at a slower rate and did not reach full confluency. Control conditions for H9c2 cells are shown in Figure

2C. If left to reach confluency they formed long multinucleated cell structures. The H9c2 cultures seeded onto the epoxy surface had a different morphology with thinner cells compared to PS control surfaces. Cells adhered to the epoxy surface, but growth rate was slower compared to H9c2 cells growing on the control PS surface.

HepG2 cells are a small, round cell type that commonly grow in isolated clusters or 'islands' (Figure 3C). These cells seeded on the epoxy layer showed a very different phenotype; they were slightly larger, more angular, more individually spread out and grew as a single monolayer (Figure 3D). Retinal epithelial cells under traditional tissue culture conditions are commonly slightly larger, wider and more angular than lens epithelial cells. These cells also differed from their control pattern of growth in how they reached confluency, with retinal cells appearing as localised 'wave like' patterning and not like the disordered monolayer of lens epithelial cells at confluency. Although well-adherent and demonstrating a typical morphology, 5-10% of retinal cells exposed to an epoxy substrate showed a much more spheroidal shape. HeLa cells on PS control surfaces exhibit traditional morphology and there was no observable difference in growth rate of control cells and cells seeded onto epoxy. Cells studied showed some cell-specific morphology on epoxy surfaces, most differently in the HepG2 cells.

MTT cell viability assays on lens epithelial and HepG2 cells indicated no significant difference on cell viability between HepG2 cells grown on the epoxy surface compared to the same cell type grown on the PS tissue culture control surface. There was a slight loss in cell viability with the lens cells (roughly 15%) grown on the epoxy surfaces, compared to the PS control surfaces, which could be caused by cell specific initial adherence, likely influenced by the different surface energy of the materials.

3.3. Comparative growth rates of cells on flat surfaces

Cells studied had differing growth rates naturally and varied depending on surfaces used. Lens cells grown on the PS control surface reached full confluency in approximately 6 days, as expected with the seeding density used (Figure 4A). However, they were unable to reach confluency on epoxy in the

time allocated for the experiment, but did so on PMMA. H9C2 cells grew at a similar rate on PMMA to PS control surface but were unable to reach confluency on epoxy (Figure 4B). The HepG2 cells on the PS control surface or PMMA took approximately 7 days to colonise the entire surface, but cells seeded on the epoxy layer reached confluency in half of this time, around 72 hours (Figure 4C). Retinal cells on PS control surfaces or PMMA grew relatively fast and reached confluency in approximately 6 days, but on epoxy cells were unable to reach confluency during the experimental time period (Figure 4D). HeLa cells on PS control surfaces exhibited traditional morphology and there was no observable difference between their morphology or growth rate of control cells and cells seeded onto epoxy or PMMA surfaces (Figure 4E).

3.4.Cell interactions with structured surfaces

Structured surfaces using moulds of EHD instability patterned surfaces and fabricated in PMMA and epoxy were seeded with lens epithelial cells and HepG2 cells. The EHD surfaces have an overall pattern of columns that are highly ordered at a localised scale. Moulds of surfaces that were composed of features with three different sizes were used. The patterned surfaces used for the lens cells were mainly fabricated from PMMA since the cells showed better growth on this surface, the HepG2 cells were investigated with mainly epoxy surfaces since they seemed to show a preference for this material. Human lens epithelial cells are generally about 50 µm in width and when grown on surfaces that contained protrusions (either flat-topped pillars or round-topped 'bumps') manifested an interaction with the surface that was specific to the scale of the topography. On the surface with protrusions that were much smaller in scale than cells (less than 1% of cell area size), lens epithelial cells were able to adhere and grow to confluence with no obvious difference in morphology to cells grown on traditional flat surfaces. This surface contained the smallest features with protrusions spaced on average 5 µm apart and ranging from 0.4 to 1.5 µm in height with a round-topped pillar profile.

Where the surface consisted of flat-topped pillars that were approximately 10% of cell surface area, lens epithelial cells initially adhered to the pillars but adhesion did not last. On the flat areas of the PMMA surface, surrounding the pillars, cells were only able to grow up to the edges of the structures

but not over them (Figure 5A). The pillars had a cross sectional diameter of average 5 µm, spaced at roughly 7 µm centre-to-centre with a pillar height of 4 µm. Surfaces tested with the largest topography, 'bumps' of an approximate scale to the lens cells (60-80% of cell surface area), such as the surfaces shown in Figure 5B and C, showed a unique interaction. In addition to the PMMA, this surface was also fabricated in PS to observe the interaction of the cells with the untreated but structured PS surface. Cells attached, grew and elongated from the apex of each individual structure to the next on both materials. The structured PS surface, compared to unstructured PS, showed lens epithelial cells initially adhering to the tops of the convexities and eventually colonising the entire surface whereas they were unable to adhere or grow on unstructured PS. The bumps were up to 4 µm in height with an average diameter of approximately 30 µm although, as can be seen in Figure 5B, some were half this size and others were considerably larger. Their centre-to-centre spacing was around 40 µm, although this also varied widely.

The surface that consisted of flat-topped pillars that were approximately 10% of cell surface area described previously (Figure 5A) was also fabricated from epoxy resin. The lens cells behaved in a similar way on the epoxy as they did on the same PMMA surface. They were able to adhere to the flat areas of epoxy but unable to attach and grow on the patterned areas. On a PMMA surface that consisted of features (roughly 30µm in diameter described previously) which were comparable to the size of the cells, the HepG2 cells appeared to interact with the surface features and to arrange themselves around the protrusions (Figure 6A).

The surface with columns roughly 5 µm in diameter with a 7µm centre-to-centre spacing was fabricated in epoxy and seeded with HepG2 cells (Figure 6B). Similarly to the lens cells, the HepG2 cells did not appear able to attach to this surface but were able to adhere to the adjacent flat epoxy and can be seen curled up on the patterned areas of the surface. The larger scale surface of bumps with an average diameter of around 30 µm was also fabricated in epoxy and seeded with HepG2 cells (Figure 6C). The cells can be seen clustered around the circumference of the bumps and appear to be interacting with the surface features.

4 Discussion

The ability of the cell lines studied to grow on PMMA that had not been surface modified, combined with its natural ability to be shaped was not found reported in the literature. This work has shown that surfaces patterned at the microscale by hot embossing without the use of clean room techniques could provide greater accessibility to advanced tissue culture experiments without the need for using surface energy modification equipment or expertise in photo-lithography patterning. Hot embossing of PMMA in this way is highly reproducible and can be readily available in most laboratories hence benefitting many fields of biological research including biofilm formation and tissue regeneration.

Though some surfaces such as PMMA and PS have been used as tissue culture substrates previously, these, amongst others have, until now, either been surface modified by UV, ozone or plasma deposition (7) (17) or else coated with collagen or fibronectin. Such processes are costly, laborious or complex, are not readily available (5) (13) (37) (38) and not always reproducible. This paper demonstrates that unmodified surfaces can easily be created and moulded or patterned without the need for costly techniques and with little or no morphological change in the cells under ‘normal’ conditions.

HepG2 cells have been grown on PMMA following significant surface treatments. However, there is a paucity of literature about HepG2 cells on unmodified PMMA (39) and no studies on epoxy resin as a tissue culture surface, though it has been used in the production of structured surfaces in PDMS, where epoxy was used as a mould master, providing improvement over the more commonly used etched silicon in terms of durability as shown by Kamande et al (35). PMMA is widely used as a material for intraocular lenses, especially in the developing world (40) and studies have been carried out on cell adhesion to intraocular lens materials (41). Human lens cells have been grown on pristine PMMA and PS surfaces but with significant modifications (42). No reports of lens cells grown on epoxy were found in the literature.

In the case of HeLa cells, the results show that epoxy resin can be used without affecting cell morphology and it offers potential as a material that is easy to pattern for investigating the effects of

surface topography on cell growth, alignment and differentiation studies. The protein interaction between the epoxy and the extracellular matrix (41)(43), and the reduced levels of adhesion and growth rates in lens and retinal cultures are also worthy of further study.

Results from the experiments in which lens cells were cultured on surfaces covered with different size structures, showed changes in the cell morphology (notably an increase in the length of the cells) and adherence (which interestingly also appeared to be replicated in the HepG2 cells). The difference in microscale structures with flat compared to round tops at an identical scale, also warrants further investigation as adherence and focal adhesion formation could be affected by the relative curvature (or lack thereof). From a biological perspective, interaction or non-adherence with specific sized topographies has implications for intraocular lenses, and the need to avoid irregular cell growth that can cause light scatter after cataract surgery. There is also a need to understand cell colonisation and potentially infectious biofilm formation on medical implants (44). The variations in cellular interactions with the surfaces are multifactorial and could include differences in cytoskeletal rigidity (45) varying cellular responses to non-flat topographies and formation of certain attachment sites that may influence the length of exploration filopodia (46).

The epoxy surface altered the growth and pattern of adherence of HepG2 cells to resemble characteristics that are akin to monolayers seen in other cell lines, such as the lens epithelial cells. The usual ‘order’ of in vitro cell culture consists of cell attachment to the substratum, radial growth of filopodia, cytoplasmic webbing, and flattening of the cell mass progressing in a sequential fashion (47), is not usually observed in HepG2 cells on treated polystyrene surfaces, as these cells more commonly attach to one another in preference to the surface. On the epoxy surfaces produced in this work, these cells altered from their normal behaviour and exhibited the usual ‘order’ of growth seen in other cells that form monolayers. Interestingly this morphology was also observed following complex and expensive modifications of surfaces by NH_3 plasma treating and galactosamine grafting (48) rather than the unmodified flat epoxy surface presented in this paper.

The surface energy of epoxy is known to be fairly low, and when tested by water contact angle in air using a sessile drop machine (alongside the other surfaces used in this study) it showed the lowest surface energy of all substrates used here. This indicates that the HepG2s cells prefer to grow on a low surface energy material, in contrast to the vast majority of cell lines. This concurs with previous work on HepG2 cells and other cell lines for which a low or limited range of surface energy is preferable for growth (49)(50). The spread of the HepG2 cells on the epoxy patterned surfaces could mean that these cells were able to interact with the surface features individually which potentially offers further methods of control. It is likely the drastic change in morphology observed on flat epoxy is due to a difference between cell-cell interactions and cell-surface interactions. On tissue-culture treated PS surfaces HepG2 cells show a great deal of affinity for one another forming clumps but on epoxy surfaces they spread out and hence indicate a greater attraction for the surface than for one another.

This kind of morphology may have multiple uses. It could be more applicable to studies of toxicity, siRNA knockdown and penetrance or for investigation of genes involved in cell-cell interactions in HepG2 cells and liver cancer research. There is a clinical shortage of liver tissue, and the unlimited proliferative capacity of the HepG2 cell model offers a good candidate for liver tissue engineering if a greater degree of control on assembly could be reached (allowing for co-cultures with other cell types and so on) (51). Tissue engineering is thought to require a scaffold or support structure to establish cell growth such as acinar cells for replacement salivary glands (52), and epoxy might serve as this scaffold for liver cells, or other secretory cell types.

The objective of this study was to manufacture and process micro scale surfaces using simple widely-available techniques and materials so that they could be investigated for use in tissue culture to determine different phenotypes and behaviours of cells (with an ultimate goal of creating surfaces that could grow more biologically relevant cell models). This required overcoming limitations in tissue culture systems as previously mentioned (53)(54). The surfaces tested in this study did not result in contamination, despite no antibiotics or anti-fungal agents having been added to the culture. Hot-embossing with a PDMS master positive, taken from an EHD instability patterned surface, can be performed with a simple hotplate for the materials described.

Traditional cell culture is a well-established method and a very common research tool in biology and medicine (55)(56) but as novel techniques are advancing and expanding the breadth of applications in cell biology and medicine (57)(58), fundamental understanding of tissue formation and generation is still required. One of these challenges is the large discrepancy between cell kinetics in vivo and in vitro and the difficulty in reproducing an artificial microenvironment in which cells behave as they would in their natural physiological environment (59). Synthetically nano and micropatterned topographies can control a range of cellular behaviours and processes, such as alignment differentiation and cytoskeletal organization and offer the potential for gaining greater insights into complex cellular processes and functions. (59).

With tissue regeneration as the ultimate goal in clinical medicine, some procedures have reached clinical trials, such as injecting of single cell suspensions of mesynchmal autologous stem cells intra-myochardially. Nonetheless, the results have been controversial and concerns have been raised about the technology (60)(61). New methods to improve cell retention are being developed; pre-cultured cells in hydrogels (62) and functionalized hydrogels (containing paracrine signaling elements)(63). Development of entire or microtissue implants requires more research into cell systems (64). While cutting edge techniques such as bio-printing are showing great promise in regenerative medicine (65), these techniques are not available to all researchers, and have limitations such as cost, access to equipment, chemical or UV surface modification and technical expertise (66)(67)(68). This paper describes a means of improving standard tissue culture conditions by a production of surfaces that need no further modifications and are hence readily available and accessible for a range of cell lines and applications.

The fabrication method described in this study utlises aspects of established techniques. Electrohydrodynamic patterning (30) has been used successfully in a number of applications such as microlens arrays (32), surfaced-enhanced Raman scattering (33), and as a direct tool for fabrication of a single surface topography to study cell migration (29). The method has been extended in this work to produce a variety of controlled surface topographies eliminating the need for more expensive equipment required in techniques such as photo or electron beam lithography (69)(70). Even simpler

lithographic fabrication models require equipment that may be available in all laboratories and have been used in experiments that investigate protein adsorption, cellular adhesion and migration (71)(72). Combining the EHD technique with PDMS to produce multiple molds of an individual EHD surface and with the use of the modified hot embossing method allowed for rapid multiple identical replicates of a surface on which a variety of cell-lines could grow.

5 Conclusion

The work introduces further avenues of research showing viability of utilisation of small bespoke surfaces for cell culture. Controlling cellular ability to preferentially interact with surfaces would enhance understanding of cell growth and differentiation and potentially tissue formation, by controlling cell-cell interactions and studying related gene expression changes. This could lead to development of more advanced and complex tissue culture surfaces and improved in vitro models and eventual reduction of animal models.

Acknowledgements
CT is funded by a studentship from Kingston University London.

Author contributions
All authors contributed to the experimental design and writing of the manuscript; CT prepared the surfaces, ML conducted tissue culture experiments.

Disclosures
There are no known conflicts of interest.

References

[1] W. Russell and R. Burch, The Principles of Humane Experimental Technique, London, Methuen, 1959.

[2] G. Hickman, D. Boocock, G. Pockley and C. Perry, "The Importance and Clinical Relevance of Surfaces in Tissue Culture," ACS Biomaterials Science and Engineering, 2 (2015)152-164.

[3] D. Antoni, H. Burckel, E. Josset and G. Noel, "Three Dimensional Cell Culture: A Breakthrough in Vivo," International Journal of Molecular Sciences, 16 (2015)5517-5527.

[4] K. Archibald, A. Baxter, K. Berube, D. Bunton, M. Clotworthy, B. Coleman, C. Foster, C. Hillier, M. McFarlane, A. Patel, B. Pierscionek, J. Root, G. Thomas, K. Tsaioun, M. Wilkinson, I. Wilmut and K. Wright, "Safety of medicine and the use of animals in research," The Lancet, (2011) 378 e2.

[5] M. Kim, M. Kino-oka and M. Taya, "Designing culture surfaces based on cell anchoring mechanisms to regulate cell morphologies and functions," Biotechnology Advances 28, (2010)7-16.

[6] Y. Li, G. Huang, X. Zhang, L. Wang, T. J. Lu and F. Xu, "Engineering cell alignment in vitro," Biotechnology Advances 32 (2014)347-365.

[7]A. Welle and E. Gottwald, "UV-Based patterning of polymeric substrates for cell culture applications," Biomedical Microdevices 4 (2002) 33-41.

[8] A. Ross, Z. B. M. Jiang and J. Lahann, "Physical aspects of cell culture substrates: topography, roughness and elastcity," Small 8, (2012) 336-355.

[9] M. Ventre, F. Causa and P. Netti, "Determinants of cell-material crosstalk at the interface: towards engineering of cell instructive materials," Journal of the Royal Society Interface, 9 (2012)2017-2032.

[10] D. Falconnet, G. Csucs, M. Grandin and M. Textor, "Surface engineering approaches ro micrpattern surfaces for cell-based assays," Biomaterials, 27(2006) 3044-3063.

[11] Becton, Dickinson & Company, "Defined cell culturing surfaces and methods of use," Becton, Dickinson & Company, US, (2010).

[12] Y. Ito, "Surface micropatterning to regulate cell functions," Biomaterials, 20 (1999)2333-2342.

[13] I. Tsai, A. Crosby and T. Russell, "Surface Patterning," Methods in Cell Biology, 83 (2007)67-87.

[14] A. Brown, G. Burke and B. Meenan, "Patterned cell culture substrates created by hot embossing of tissue culture treated polystyrene," Journal of Materials Science Materials in Medicine 12(2013) 2797-2807.

[15] S. Shukula, S. Singh, A. Pandey and A. Mishra, "Review on production and medical applications of sigma-polylysine," English Journal of Biochemistry,65 (2012)70-81.

[16] W. S. Ramsey, W. Hertl, E. D. Nowlan and N. J. Binkowski, "Surface treatments and cell attachment," in vitro, 20 (1984)802-808.

[17] P. van Midwoud, A. Janse, M. Merema, G. Groothuis and E. Verpoorte, "Comparison of biocompatibility and adsorption properties of different plastics for advanced microfluidic cell and tissue culture models.," Analytical Chemistry, 84 (2012)3938-3944.

[18] F. Johansson, P. Carlberg, D. Nils, L. Montelius and M. Kanje, "Axonal outgrowth on nano-imprinted patterns," Biomaterials, 27 (2006)1251-1258.

[19]W. Ni, W. H. Tong, D. Choudhury, N. A. A. Rahim, C. Iliescu and H. Yu, "Cell Culture on MEMS Platforms: A Review," International Journal of Molecular Sciences, 10 (2009) 5411-544.

[20] A. Welle, E. Gottwald and K. Weibezahn, "Patterned polymer surfaces for cell culture applications.," Biomed Tech (Berl), 47 (2002)401-403.

[21] [21] M.Banister, D.McGrath “SUPER ELASTIC EPOXY HYDROGEL” Patent number EP2041214 A2 (2009).

[22] S.Bakarich, R.GorkinIII, M.Panhuis, G.Spinks. “Three-Dimensional Printing Fiber Reinforced Hydrogel Composites” ACS Appl. Mater. Interfaces, 6 (2014), pp 15998–16006.

[23] A.Ekenseair, K.Boere, S.Tzouanas, T.Vo, F.Kasper, A.Mikos. “Synthesis and characterization of thermally and chemically gelling injectable hydrogels for tissue engineering” Biomacromolecules 13 (2012) 1908-15.

[24] L.Koh, M.Islam, D.Mitra, C.Noel, K.Merrett, S.Odorcic, P. Fagerholm, W.Jackson, B.Liedberg, J.Phopase, M.Griffith. “Epoxy Cross-Linked Collagen and Collagen-Laminin Peptide Hydrogels as Corneal Substitutes” Journal of Functional Biomaterials 4 (2013) 162-177.

[25] A.Rape, W.Guo, Y.Wang. “The regulation of traction force in relation to cell shape and focal adhesions” Biomaterials 32 (2011) 2043-2051

[26] S.Chang, W.Guo, Y.Kim, Y.Wang. “Guidance of Cell Migration by Substrate Dimension” Biophysical Journal 104 (2013) 313-321.

[27] J.Soleas, T.Waddell, A.McGuigan. “Topographically grooved gel inserts for aligning epithelial cells during air–liquid-interface culture” Biomaterials Science 3 (2015) 121-133.

[28] J.Charest, J.Califano, S.Carey, C.Reinhart-King. “Fabrication of Substrates with Defined Mechanical Properties and Topographical Features for the Study of Cell Migration” Macromolecular Bioscience 12 (2012) 12-20.

[29] M.Frey, I.Tsai, T.Russell, S.Hanks, Y.Wang.”Cellular esponses to Substrate Topography: Role of Myosin II and Focal Adhesion Kinase” Biophysical Journal 90 (2006)3774-3782.

[30] N. Wu, W. Russel. “Micro- and nano-patterns created via electrohydrodynamic instabilites,” Nano Today 4 (2009)180-192.

[31] E. Schaffer, T. Thurn-Albrecht, T. Russell and U. Stiener, “Electrohydrodynamic instabilities in polymer films,” Europhysics letter 53 (2001) 518-524.

[32] Y.J. Lee, Y.W. Kim, Y. Kim, C. Yu, J.S. Gwag, and J. Kim, “Microlens array fabricated using electrohydrodynamic instability and surface properties,” Optics Express 19 (2011) 10673-10678.

[33] P. Goldberg-Oppenheimer, S. Mahajan and U. Steiner, “Hierarchical Electrohydrodynamic Structures for Surface-Enhanced Raman Scattering,” Advanced Optical Materials 24 (2012) 175-180

[34] M. Lima, V. Correlo, R. Reis ”Micro/nano replication and 3D assembling techniques for scaffold fabrication” Materials Science and Engineering C 42 (2014) 616-621.

[35] J. Kamande, Y. Wang and A. Taylor, “Cloning SU8 silicon masters using epoxy resins to increase feature replicability and production for cell culture devices”. Biomicrofluidics 9 (2015)

[36] H. Abdelkader, M. Longman, R. Alany and B. Pierscionek, “Phytosome-hyaluronic acid systems for ocular delivery of L-carnosine”, International Journal of Nanomedicine 11 (2016) 2815-2827.

[37] J. N. Lee, X. Jiang, D. Ryan and G. Whitesides, "Compatibility of mammalian cells on surfaces of poly(dimethylsiloxane)," Langmuir,26 (2004)11684-11691.

[38] P. Chandra, J. Kim and R. S. Woo, "Quantitative analysis of growth of cells on physiochemically modified surfaces," Bulletin of the Korean Chemical Society, 34 (2013) 524-530.

[39] H. Yamazaki, S. Gotou, I. Koju, S. Kohashi, Y. Goto, Y. Yoshiura, Y. H. Sakai Y, M. Shimomura and K. Nakazawa, "Micropatterned culture of HepG2 spheroids using microwell chip with honeycomb-patterned polymer film," Journal of Bioscience and Bioengineering, 118 (2014) 455-460.

[40] R. Bellucci, "An Introduction to Intraocular Lenses: Material, Optics, Haptics, Design and Aberration," Cataract, 3 (2013) 38-55.

[41] C. Cunanan, N. Tarbaux and P. Knight, "Surface properties of intraocular lens materials and their influence on in vitro cell adhesion," Journal of Cataract and Refractive Surgery, 17 (1991)767-773.

[42] R. D'Sa, G. Burke and B. Meenan, "Protein adhesion and cell response on atmospheric dielectric barrier discharge-modified polymer surfaces," Acta Biomaterialia, 6 (2010)2609-2620.

[43] F. Gattazzo, A. Urciuolo and P. Bonaldo, "Extracellular matrix: a dynamic microenvironment for stem cell niche," Biochimica et Biophysica Acta, general subjects, 1840 (2014)2506-2519.

[44] R. Donlan, “Biofilm Formation: A clinically relevant microbiological process” Clinical Infectious Diseases 33 (2001) 1387-1392.

[45] E. Fadeeva, S. Schlie, J. Koch, B. Chichkov,”Selective cell control by surface structuring for orthopaedic applications” Journal of adhesion science and technology 24 (2010) 2257-2270.

[46] M. Ventre, C. Natale, C. Rianna, P. Netti. “Topographic cell instructive patterns to control cell adhesion, polarization and migration” Journal of the Royal Society interface 11 (2014) 1-10.

[47] R. Rajaraman, D. Rounds, S. Yen, A. Rembaum. “A scanning electron microscope study of cell adhesion and spreading in vitro.” Experimental Cell Research 88 (1974) 327–339.

[48] L. Lopez, M. Belviso, R. Gristina, M. Nardulli, R. d’Agostino, P. Favia “Plasma-Treated Nitrogen-Containing Surfaces for Cell Adhesion: The Role of the Polymeric Substrate” Plasma Processes and Polymers 4 (2007) 402-405.

[49] J.-N. Lu and T.-H. Young, "Interactions of HepG2 Cells on Biomaterials with Various Surface Properties," in International Conference on Biomedical and Parmaceutical Engineering ICBPE, 1 (2006) 285-289.

[50] Y.-W. Wang, Q. Wu and C. Guo-Qiang, "Reduced mouse fibroblast cell growth by increased hydrophilicity of microbial polyhydroxyalkanoates via hyaluronan coating," Biomaterials,24 (2003)4621-4629.

[51] N. Pereira-Rodrigues, P. Poleni, D. Guimard, Y. Arakawa3, Y. Sakai, T. Fujii, ”Modulation of Hepatocarcinoma Cell Morphology and Activity by Parylene-C Coating on PDMS” PlosOne 5 (2010) 1-13.

[52] M. Chen, Y. Hsu, C. Lin, Y. Chen, T. Young “Interactions of acinar cells on biomaterials with various surface properties” Journal of Biomedical materials research part a 74 (2005) 254-262

[53] Kimlin, L. C., Casagrande, G. and Virador, V. M In vitro three-dimensional (3d) models in cancer research: an update. mol. Carcinog 52 (2013) 167-182.

[54] Gillet, J. P.; Calcagno, A. M.; Varma, S.; Marino, M.; Green, L. J.; Vora, M. I.; Patel, C.; Orina, J. N.; Eliseeva, T. A.; Singal, V.; Padmanadhan, R.; Davidson, B.; Ganapathi, R.; Sood, A. K.; Rueda, B. R.; Ambudkar, S. V.; Gottesman, M. M.Redefining the relevance of established cancer cell lines to the study of clinical anti-cancer drug resistance,Proc Natl Acad Sci U.S.A. 108 (2011) 18708-18713.

[55] S.Aoki, T.Takezawa, H.Sugihara, S.Toda. ” Progress in cell culture systems for pathological research” Pathology International 66 (2016) 554-562.

[56] R.Freshney. “Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications”. Hoboken, New Jersey: Wiley-Blackwell, 2016.

[57] H.Sugihara, N.Yonemitsu, S.Miyabara, K.Yun. “Primary cultures of unilocular fat cells: characteristics of growth in vitro and changes in differentiation properties”. Differentiation 31 (1986) 42–9.

[58] H.Green, J.Rheinwald, T.Sun. “Properties of an epithelial cell type in culture: the epidermal keratinocyte and its dependence on products of the fibroblast”. Progress in Clinicaland Biological Research 17 (1977) 493–500.

[59] C.Bettinger, R.Langer, J.Borenstein. et al. (2009). “Engineering substrate topography at the micro- and nanoscale to control cell function”. Angewandte Chemie (International ed. in English) 48 (2009) 1433-7851.

[60] A.Mallone, B.Weber, S.Hoerstrup. “Cardiovascular Regenerative Technologies: Update and Future Outlook” Transfusion medicine and hemotherapy 43 (2016) 291-296.

[61] M.Rota, A.Leri, P.Anversa. “Human heart failure: is cell therapy a valid option?” Biochemical Pharmacology 88 (2014) 129–138.

[62] M.Sepantafar, R.Maheronnaghsh, H.Mohammadi, S.Rajabi-Zeleti, N.Annabi, N.Aghdami, H.Baharvand. “Stem cells and injectable hydrogels: synergistic therapeutics in myocardial repair”. Biotechnology Advances 34 (2016) 362–379.

[63] A.Hasan, R.Waters, B.Roula, R.Dana, S.Yara, T.Alexandre, A.Paul. “Engineered biomaterials to enhance stem cell-based cardiac tissue engineering and therapy” Macromolecular bioscience (2016).doi: 10.1002.

[64] M.Emmert, P.Wolint, N.Wickboldt, G.Gemayel, B.Weber, C.Brokopp, A.Boni, V.Falk, A.Bosman, M.Jaconi, S.Hoerstrup. “Human stem cell-based three-dimensional microtissues for advanced cardiac cell therapies”. Biomaterials 34 (2013) 6339–6354.

[65] A.Arslan-Yildiz, R.El Assal, P.Chen, S.Guven, F.Inci, U.Demirci. “Towards artificial tissue models: past, present, and future of 3D bioprinting” Biofabrications 8 (2016) 1-17.

[66] G.Villar, A.Graham, H.Bayley. “A tissue-like printed Material” Science 340 (2013) 48–52

[67] U.Gurkan, R.Assal, S.Yildiz, Y.Sung, A.Trachtenberg, W.Kuo, U.Demirci. “Engineering anisotropic biomimetic fibrocartilage microenvironment by bioprinting mesenchymal stem cells in nanoliter gel droplets” Molecular Pharmaceutics 11 (2014) 2151–9.

[68] S.Moon, S.Hasan, Y.Song, F.Xu, H.Keles, F.Manzur, S.Mikkilineni, J.Hong, J.Nagatomi, E.Haeggstrom, A.Khademhosseini, U.Demirci. “Layer by layer three-dimensional tissue epitaxy by cell-laden hydrogel droplets Tissue Engineering 16 (2009) 157–66.

[69] E. Menard , M. A. Meitl , Y. G. Sun , J. U. Park , D. J. L. Shir , Y. S. Nam , S. Jeon , J. A. Rogers “Micro-and nanopatterning techniques for organic electronic and optoelectronic systems” Chemical Reviews 107 (2007) 1117 – 1160 .

[70] M.Kahl , E.Voges , S.Kostrewa , C.Viets , W.Hill . “Periodically structured metallic substrates for SERS” Sensor and Actuators B: chemical 51 (1998) 285-291.

[71] P. Kim, D.Kim, B.Kim, S.Choi, S.Lee, A.Khademhosseini, R.Langer, K.Suh. “Fabrication of nanostructures of polyethylene glycol for applications to protein adsorption and cell adhesion” Nanotechnology 16 (2005) 2420-2426.

[72] D.Kim, C.Seo, K.Han, K.Kwon, A.Levchenko, K.Suh.“Guided Cell Migration on Microtextured Substrates with Variable Local Density and Anisotropy” Advanced Functional Materials 19 (2009) 1579-1586.

Figure captions

Figure 1. Surface preparation methods showing A) EHD patterning, blue-electrode, red-spacer material, yellow-patterned fluid (Small panel A= no applied voltage B= initial instability C= fully evolved structures); B) and C) micrographs of EHD patterned epoxy surfaces D) a PDMS moulding of an EHD patterned surface (red); b Completed mould, separated and trimmed E) Laser confocal scan of the polymer surface showing topographical dimensions along the red line across the surface.

Figure 2. Cell growth responses on control PS tissue culture surfaces and flat PMMA surfaces. Micrographs (x10 magnification) showing representative cell morphology of lens cells on A) PS control tissue culture treated surface B) poly methyl methacrylate) (PMMA). H9C2 cells on C) PS control tissue culture treated surface D) poly methyl methacrylate) (PMMA)retinal cells on E) PS control tissue culture treated surface F) poly methyl methacrylate) (PMMA).

Figure 3. Lens and HepG2 cell growth responses on control PS tissue culture surfaces and flat epoxy surfaces. Micrographs (x20 magnification) showing representative lens cell morphology on A) PS control tissue culture treated surface B) epoxy thin film surfaces and HEPG2 cells morphology on C) PS control tissue culture treated surface D) epoxy thin film surfaces.

Figure 4. Cell confluency curves on unmodified surfaces showing cell confluency changes over time for A) lens Cells, B) h9c2 cells, C) HepG2 cells, D) retinal cells and E) HeLa cells.

Figure 5. Morphology of human lens epithelial cells in response to topography of pillars and bumps in different materials. Lens epithelial cells grown on A) PMMA middle-sized pillared surfaces (x10 Magnification) B) lens epithelial cells grown large bumped PS surfaces (x20 Magnification) C) lens epithelial cells grown large bumped PMMA surfaces (x20 Magnification).

Figure 6. Morphology of HepG2 cells in response to topography of pillars and bumps in different materials. Micrographs (x20 Magnification) of HepG2 cells grown on surfaces of A) large bumped PMMA surface. B) epoxy middle-sized pillared surfaces. C) large bumped epoxy surfaces.

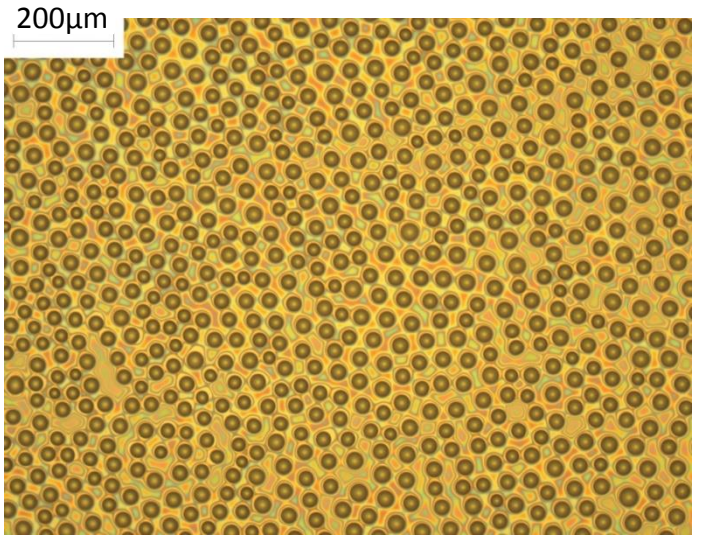
Table 1. Cell lines and culture conditions.

Cell line used	Catalogue number (ATCC)	Cell type	Growth medium	Growth conditions	Desired confluency for subculturing
B-3	ATCC CRL-11421	Human eye Lens epithelial cells	Eagles Minimum Essential Medium (EMEM), supplemented with 20% v/v Foetal bovine serum (FBS).	Air 95%, Carbon Dioxide (CO_2) 5% **37^{O}C**	80-100%
Hela	ATCC CCL-2	Human cervical epithelial cells (Adenocarcinoma)	EMEM supplemented with 10% v/v FBS	Air 95%, Carbon Dioxide (CO_2) 5% **37^{O}C**	90%
Htert RPE-1	ATCC CRL-4000	Human eye retinal pigmented epithelium.	Dulbeccos Minimum Essential Medium F12 (DMEM) supplemented with 10% v/v FBS and 0.01mg/ml hygromycin B.	Air 95%, Carbon Dioxide (CO_2) 5% **37^{O}C**	90%
H9c2	ATCC CRL-1446	RAT embryonic heart myoblast cells	DMEM supplemented with 10% v/v FBS	Air 95%, Carbon Dioxide (CO_2) 5% **37^{O}C**	100%
Hep G2	ATCC HB-8065	Human liver epithelial cells (hepatocellular carcinoma)	EMEM supplemented with 10% v/v FBS	Air 95%, Carbon Dioxide (CO_2) 5% **37^{O}C**	80%

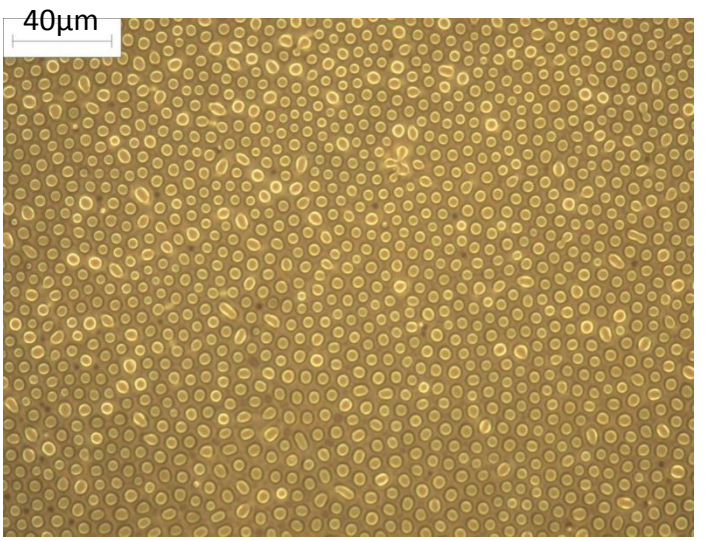
Figure 1. Surface preparation methods.

A.

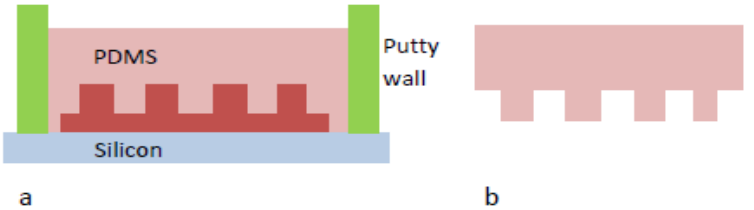
B.



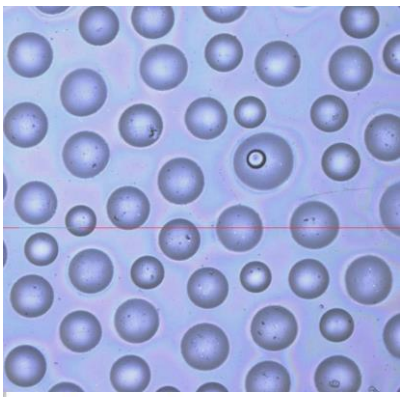
C.

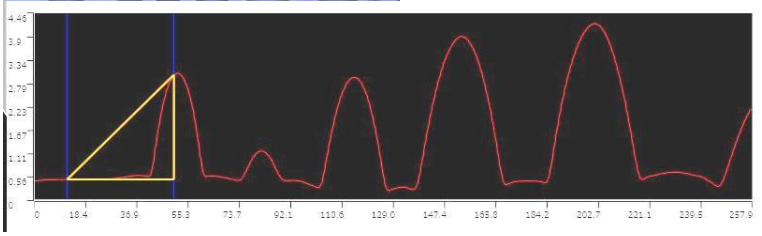


D.



E.

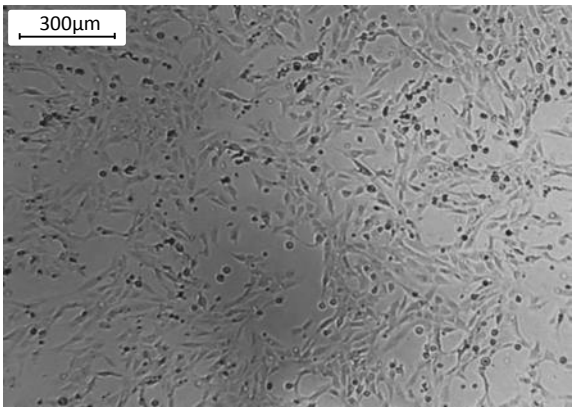




Width 38.541µm
Height 2.496µm
Length 38.622µm
Angle 3.705

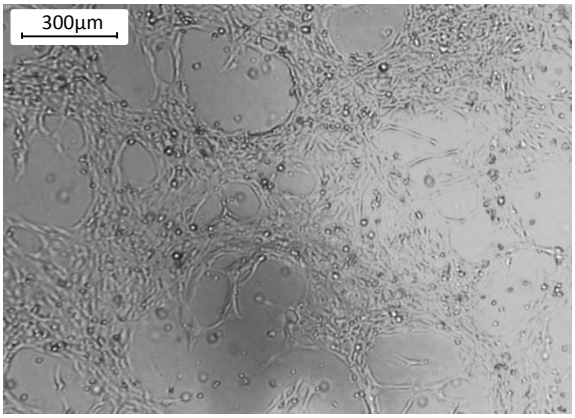
Figure 2. Cell growth responses on control PS tissue culture surfaces and flat PMMA surfaces.

A



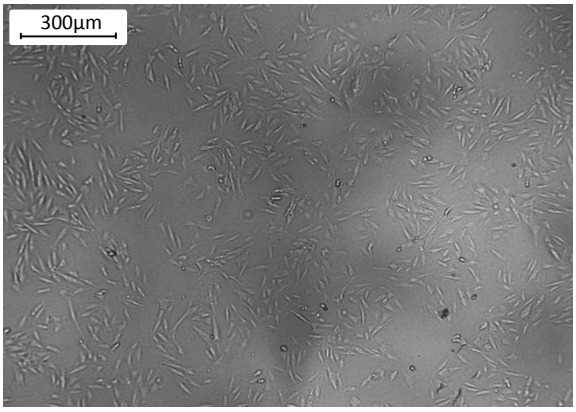
Lens Cells

B



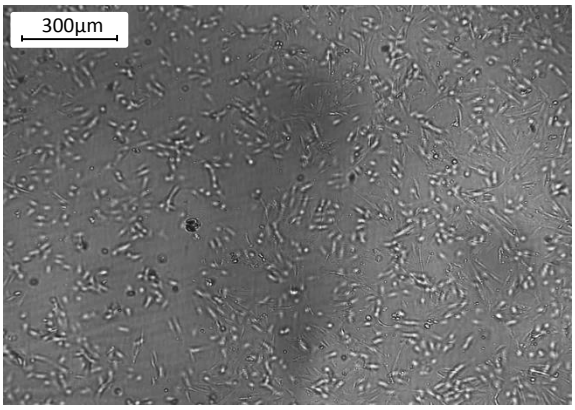
Lens Cells - PMMA

C



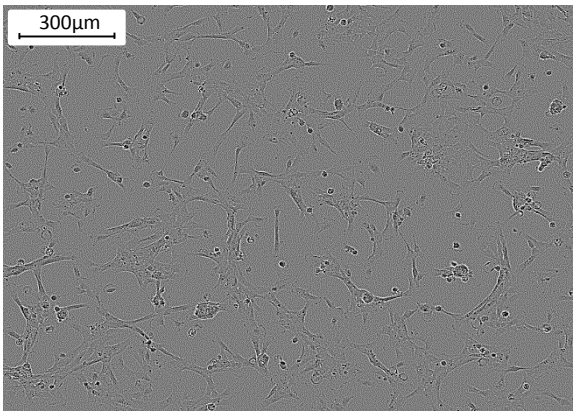
H9C2 Cells

D



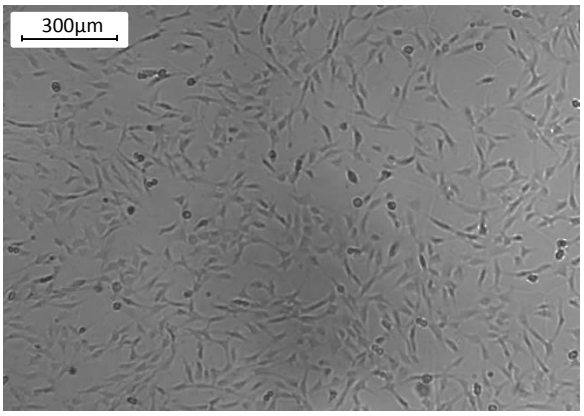
H9C2 Cells - PMMA

E



Retinal Cells

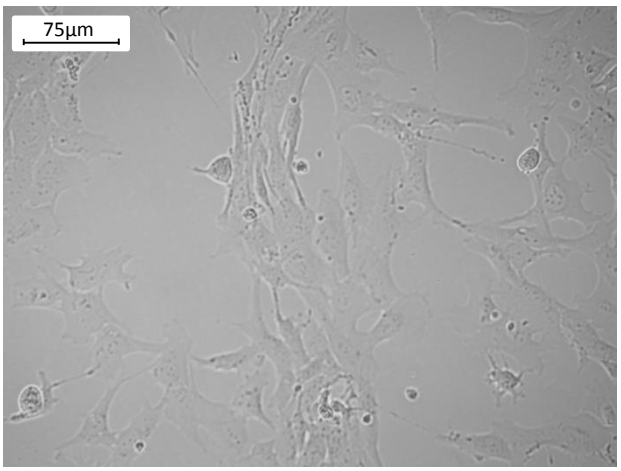
F



Retinal Cells - PMMA

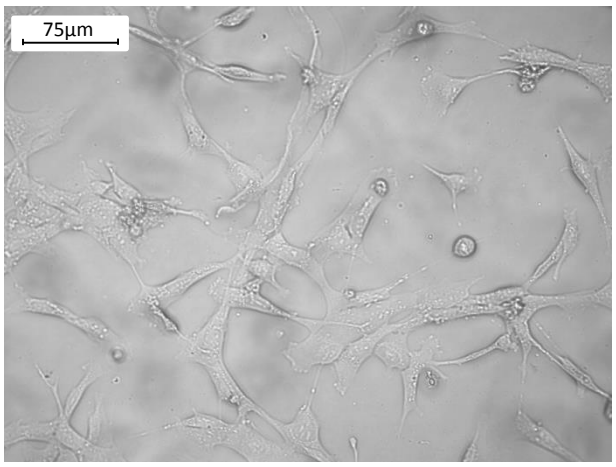
Figure 3. Lens and HepG2 cell growth responses on control PS tissue culture surfaces and flat epoxy surfaces.

A



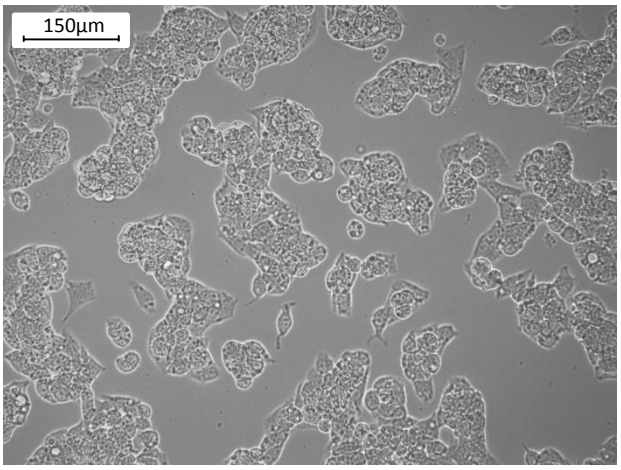
Lens Cells

B



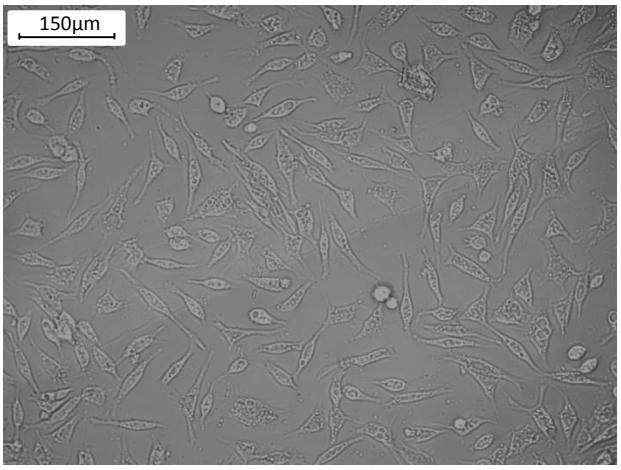
Lens Cells - Epoxy

C



HepG2 Cells

D



HepG2 Cells - Epoxy

Figure 4. Cell confluency curves on unmodified surfaces.

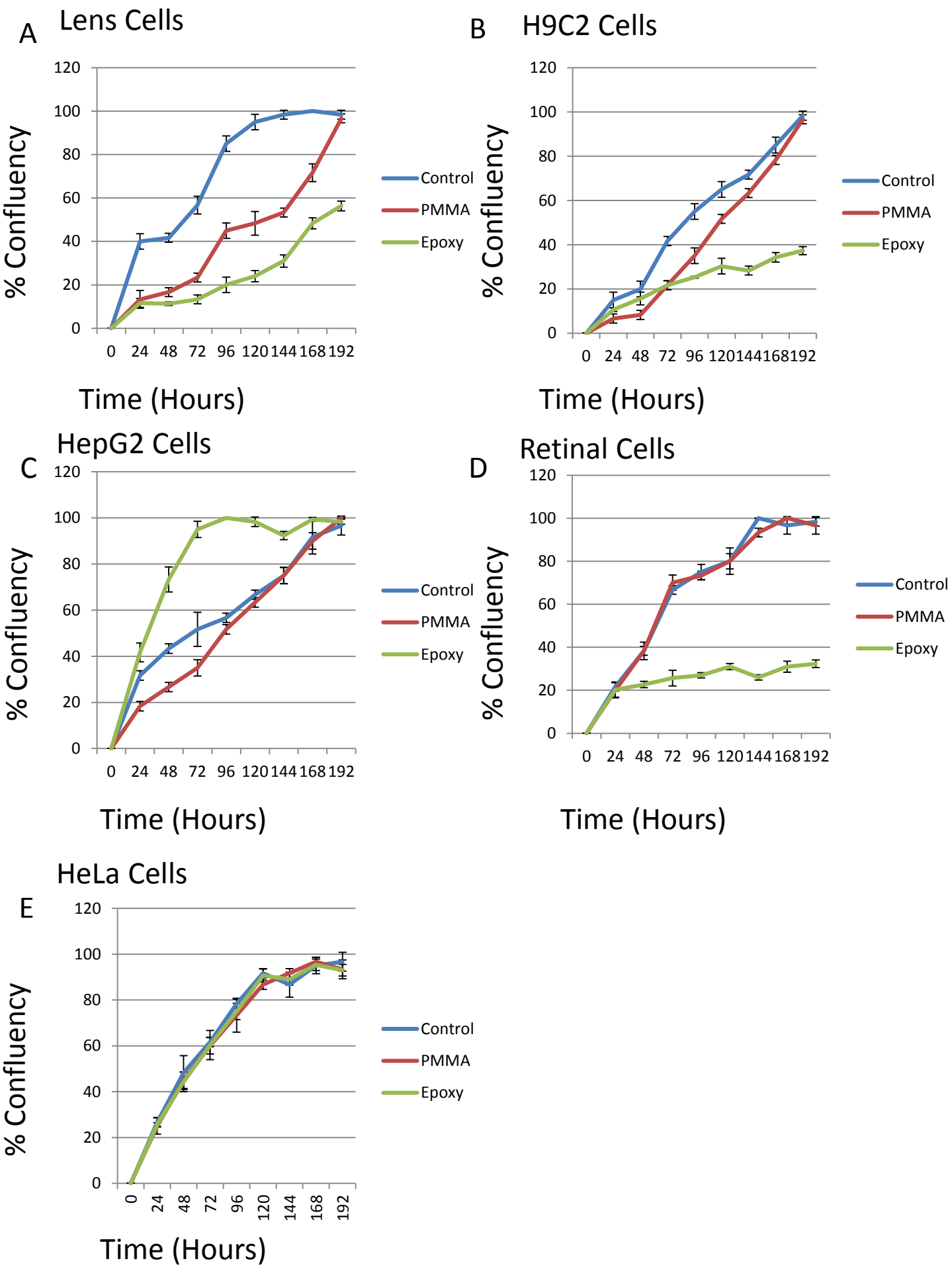
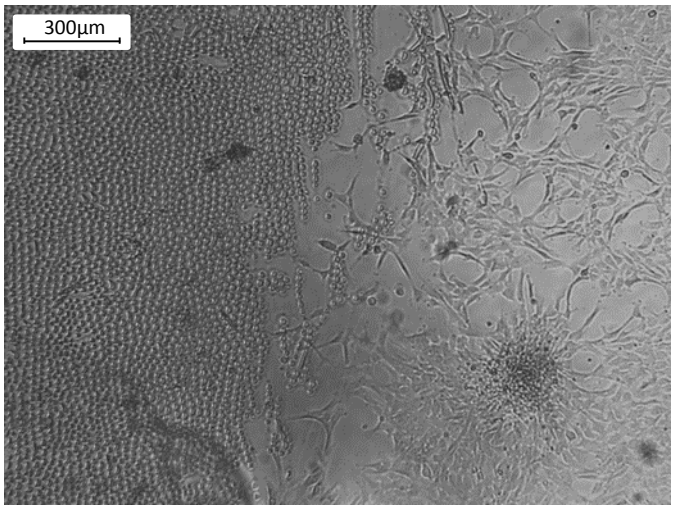


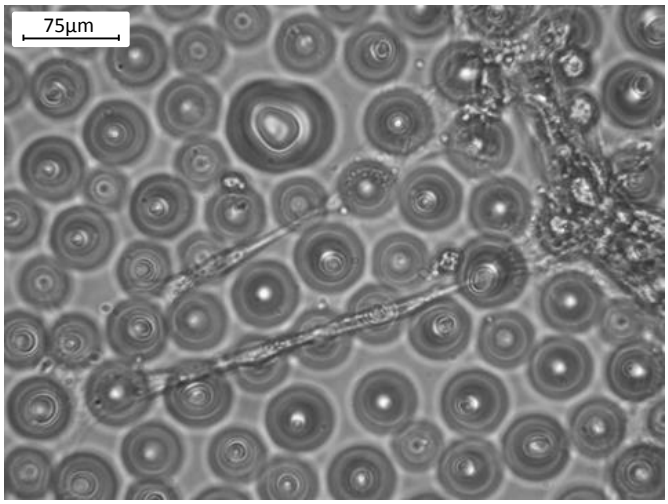
Figure 5. Morphology of human lens epithelial cells in response to topography of pillars and bumps in different materials.

A.



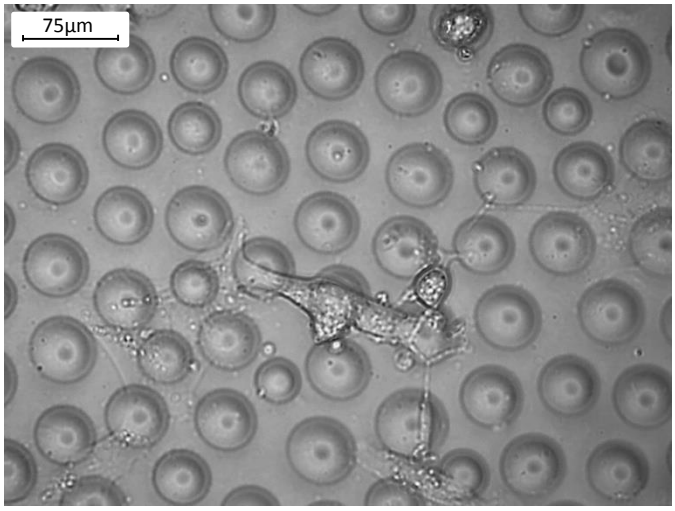
PMMA

B.



PS

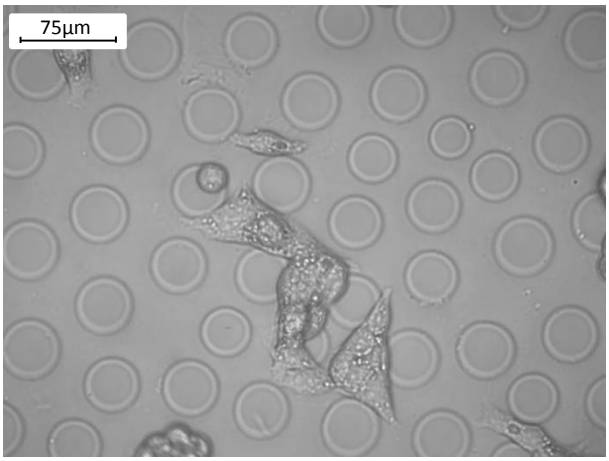
C.



PMMA

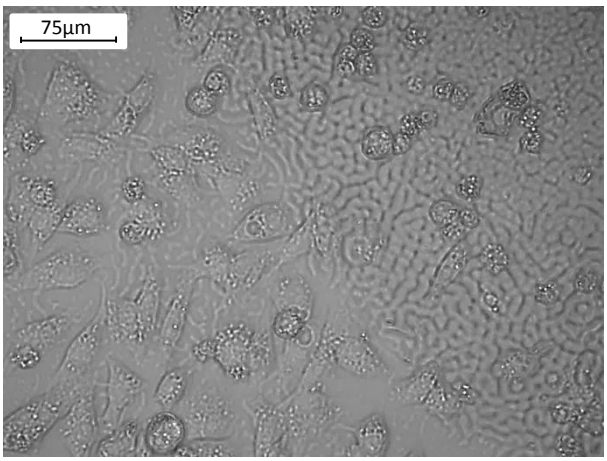
Figure 6. Morphology of HepG2 cells in response to topography of pillars and bumps in different materials.

A.



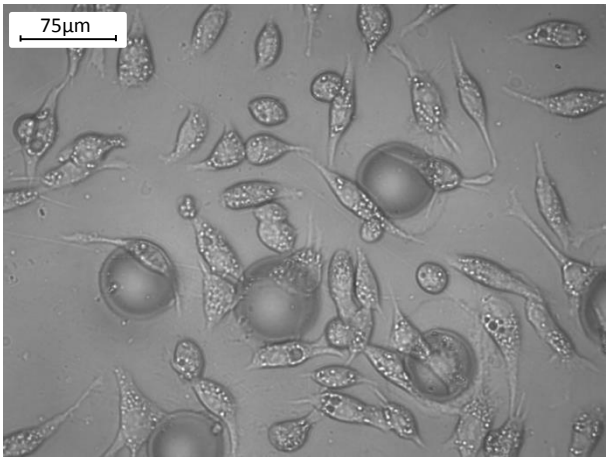
PMMA

B.



Epoxy

C.



Epoxy

Highlights

- Use of room temperature cure epoxy resin can be used for tissue culture
- Significantly different HepG2 morphology was observed
- Non-clean room required micro scale surface patterning techniques can be applied
- Surface topography and cell interactions based on specific size of features was observed