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Haosmc Growth on Various Sustainable Nano/Micropatterned Surface Topographies Utilising Reusable Surface Templating

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Abstract

The aim of controlling/regulating cellular processes by surface patterning of biomaterials is a crucial concept in emerging research fields, where the precise fabrication of micro-nano surface topographies is required at manufacturing scales to meet consumer demand. Photolithography is typically employed for surface patterning; however, the high cost, process complexity, and lack of environmentally friendly and sustainable methodologies represent steep barriers for many researchers. Soft lithographic methods have been utilised extensively as a potential alternative for traditional photolithography, yet a photolithographic step is still required for initial template fabrication. Here, we demonstrate the use of repurposed optical consumer items such as DVDs, diffraction glasses, and gratings that would otherwise be thrown away after their useful lives, being used here as a sustainable approach as templates for the formation of complex nano-patterns. By replicating these templates in polydimethylsiloxane (PDMS) and polylactic acid (PLA), we show the varied interactions of human aortic smooth muscle cells (HAoSMC) to these surfaces. Notably, it was demonstrated that, in comparison to nonpatterned substrates, HAoSMC interaction (adhesion, spreading, and viability) was higher on DVD patterned surfaces compared to diffraction glass and grating patterned surfaces according to the results from cell metabolic activity (MTT and Alamar Blue), and proliferation (total DNA) assays. We demonstrate that a simple, reusable, inexpensive, yet robust technology could be a powerful tool in improving our understanding of cell-surface interactions and for the potential development of medical devices with micro- and nanoscale low or high adhesion features.

Keywords: Topography; Re-Purposing, Sustainable Technology; Smooth Muscle Cells; Cell Adhesion

Abbreviations: PLA poly lactic acid; PDMS polydimethylsiloxane; HAoSMC human aortic smooth muscle cells; DVD digital versatile discs; dH2O deionized water; PBS phosphate buffered saline.

Introduction

Inducing surface topography on materials has found use across numerous scientific disciplines such as biology, [1] chemistry,[2,3] physics,[3] engineering,[4] and materials science [5]. Due to their ability to affect a variety of cellular activities, including adhesion, morphological alterations, cell survival, differentiation, and proliferation, biomaterials with well-defined patterns at the micro- and nanoscale have been shown to be effective tools in numerous therapeutic applications [6-8]. Controlling cell function *in vitro* by using patterns and structuring to mimic the inherent structures present in native tissues has been a prominent motivation [9-11]. Controlling cellular processes using tailored surfaces has received a lot of attention in areas of drug delivery, stem cell research, diagnostics and therapeutics, tissue engineering, regenerative medicine, and the creation of *in vitro* models of human diseases. A plethora of nanofabrication techniques have been developed to create well-defined patterns on biomaterial surfaces and these techniques have been utilised successfully for controlling stem cell differentiation,[12] lowering platelet reactivity,[13] and bacterial biofilms [14] among others.

It is widely established that the surface characteristics of a biomaterial have a huge influence on its biocompatibility [15]. The extracellular matrix (ECM) presents topographical and bioadhesive characteristics that range from the nano to microscale impacting on cell attachment, differentiation and proliferation [16]. Regulating cell adhesion therefore holds great importance for the design and development of biomedical implants, sensors and regenerative medicine strategies [17]. An effective approach for regulating the relevant cell responses is the patterning of a surface with well-defined topography [18]. Photolithography, which has been used in microelectronics since the early 1960s, is a widely popular approach to surface patterning due to the precision and relevant size of feature fabrication [19].

Conventional lithographic methods (particularly those used in nanofabrication) such as photolithography and electron-beam lithography, require expensive equipment, have long and complex fabrication times, and often suffer from the lack of environmentally friendly, sustainable/recyclable production methods [20-22]. Soft lithography is an alternative technique to traditional lithographic methods [23]. Soft lithographic techniques are generally simple to employ, associated with low-cost, highly reproducible, ecologically friendly [24-26]. Reusing surfaces that have been fabricated to present nano-scale features offers the potential to develop more sustainable methodologies for surface patterning, whilst also enabling a much wider adoption of surface patterning methods.

Although several copies of a master template can be manufactured using soft lithographic processes, the creation of the initial template still necessitates the employment of highly specialised photolithographic or electron beam apparatus. Similar methods have been demonstrated for the inverse replication of plant structures to fabricate non-wettable [27] or bioresponsive surfaces,[28] or optical storage devices (such as digital versatile discs, DVDs)[29] to produce structured ceramics [30]. In this study, soft lithography was used as a straightforward but efficient fabrication method to transfer large-scale micro- and nano-patterns with complicated geometries, using commercially available consumer products, with DVDs presenting nano-dots and diffraction gratings/ glasses presenting regular line protrusions from their surface. This study demonstrates a sustainable route for surface patterning at the nanoscale; notably, our research also shows that some patterned substrates have been shown to cause a significant control over cell adhesion, spreading and viability, opening avenues for the easy fabrication of surface implants with directed cell adhesive and anti--fouling properties. More importantly, from a clinical point of view, the concept of repurposing low-cost consumer products that already present complex topography might be of interest to scientists focused on surface patterning of biomaterials who do not have access to the infrastructure required for nanofabrication [31].

Experimental Section

Chemicals

Poly (dimethyl siloxane) (PDMS) was purchased as a 2-part Sylgard 184 Silicone Elastomer Kit from Dow Corning (Product:

000000840559). Blank DVDs (single layer), diffraction grating (300 lines/mm), and diffraction glasses (Rainbow Geometric) were purchased from an online store (GloFX). Poly (lactic acid) (PLA) 3D printing filament (1.75 mm, MW=16,000) was purchased from Ooznest Materials. All other chemicals were purchased from Sigma Aldrich unless specified otherwise and used without further modification or purification.

Master Preparation from Blank Dvds

Commercial optical DVDs consist of two polycarbonate (PC) layers that are coated with a metallic layer and an organic dye. The PC layers were first separated carefully to prevent damage to the metallic layer which contains the nanopattern [32]. The metallic layer was washed thoroughly in ethanol to remove any organic dye, followed by drying in a stream of nitrogen. The metallic layer was cut into ~5 X 5 cm square sections ready for soft lithography replication.

Master Preparation from Holographic Diffraction Gratings and Diffraction Glasses

Diffraction gratings and glasses were first cut from the edges, gently peeled off and separated from their cardboard mounts to obtain only the patterned films. Diffraction gratings (~5 X 5 cm) and diffraction glasses (~4 X 4 cm) were washed thoroughly with ethanol and cut into the desired shape to fit into petri dishes.

Fabrication of Polydimethylsiloxane (PDMS, Sylgard 184) Templates

Sylgard 184 was prepared as a 10:1 (wt / wt) PDMS: curing agent mixture. The master template was submerged, and air bubbles removed by placing under reduced pressure (~40 min) until all the bubbles disappeared. The PDMS was then heated in an oven at 80° C for ~3 hours, before detaching the PDMS from the template. These templates with inverted patterns were used as masters to generate new PDMS templates having the same pattern as the original master template. The '*as prepared*' PDMS master template was replicated using the same PDMS process for curing for 20 minute and separating the sample from the template whilst warm. The sample was heated for a further 2 hours and 40 minutes to achieve complete curing.

Fabrication of Polylactic Acid Templates by Solvent Casting

Commercially available PLA was dissolved in chloroform at concentrations of 10 % (w / v), being spin coat onto the PDMS template (SCK-300 Digital Spin Coater Kit (500 - 6,000 rpm, 10 mins)). Samples were left overnight to ensure solvent loss was complete. Patterns were successfully transferred over areas up to 5 cm^2, although most samples were kept to an area of ~ 1 cm^2.

Scanning Electron Microscopy (SEM)

Samples were sputter coated with Au/Pd (~100 nm) and analysed on a JOEL JSM 7100F at 5 kV. The SEM analysis was performed at the materials characterization centre at Loughborough University.

Preparation of Substrates for Cell Culture

Patterned substrates were cleaned in 70 % ethanol in dH_2O (18.2 MΩ). Substrates were placed upward facing in a 24 well plate, being sterilised with 70 % ethanol. Filter sterilised and autoclaved phosphate buffered saline (PBS) was used in 3 sequential rinses immediately prior to cell seeding.

Cell Culture of Haosmc

HAoSMC (Promocell C-12533, p 3-5) were cultured in medium kit (Promocell C-22062) consisting of a 500 mL smooth muscle cell growth medium mL that contains fetal calf serum 0.5 %, epidermal growth factor (recombinant human) 0.05 %, basic fibroblast growth factor (recombinant human) 0.02 %, insulin (recombinant human) 0.5% supplemented with 1 % penicillin-streptomycin, maintained within an incubated environment at 37° C and 5% CO_2. Cultures were passaged on reaching 80-90 % conflu-

ency (3-4 days) using trypsin/EDTA (Gibco, 11538876). Sampled were seeded at a density of 15k cells/cm^2 in 24 well plates.

Metabolic Activity Assays (MTT), Total DNA (T-DNA), and Alamar Blue (AB)

MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma-Aldrich) was carried out on day 7 of culture, following manufacturers protocol. Cell media was removed from each well, and the cells were washed three times with sterile PBS. MTT solution (0.5 mg/mL) was added to each well and incubated at 37° C and 5 % CO_2 for 1 hr. After incubation, 0.5 mL dimethyl sulfoxide (DMSO) was added to each well to dissolve the granules and returned for incubation for further 5 mins. Following the addition of 100 µL of the incubated MTT solution to a transparent 96-well plate, absorbance data was obtained at 570 nm using a Biostack 4 (Agilent Technologies, Inc, CA, USA) plate reader. The relative activities observed per nanopatterned substrate were compared to tissue culture plasticware controls.

DNA concentration was also measured on day 7 of culture using a fluorescent assay (t-DNA assay) (Figure S1). Hoechst 33258 t-DNA kit (Sigma-Aldrich) was used following manufacturers protocol. Culture media was removed and replaced with deionised water (~18MΩ) (200 µL) in each well of a 24 well plate. Cells were subjected to 6x rapid freeze-thaw cycles (freeze in -80°C for 30 mins and thawed for 5 minutes in an oven at 70°C) to encourage membrane fracture. Dye solution was incubated in each well for 10 minutes before 100 µL was removed into separate wells of a black 96-well plate. These were read using a Biostack 4 (Agilent Technologies, Inc, CA, USA) plate reader with Ex/Em 360 / 460 nm.

Alamar Blue assay was carried out to further characterise cell metabolic activity at culture days 1 and 4. Samples were PBS-washed before being incubated for 4 hours under standard culture conditions with a 1:10 dilution of Alamar Blue solution (0.75 mg resazurin (Sigma-Aldrich,

62758-13-8) in 10 mL PBS). Controls were also taken for sample normalisation, being comprised of dye in media only. Fluorescence of 100 ⊠L of each of the samples was measured in black 96 well plates on a CLARIO star Plus microplate reader Ex/Em 544 / 590 nm.

Cell Fixing and Immunofluorescent Staining

Cells were fixed with cold absolute methanol (Fisher Scientific, 10365710) for 30 mins before 0.1% methylene blue (Fisher Scientific, 10455081) in deionized water was added to each well for 5 mins. Each substrate was rinsed 3 times with deionized water and air dried. For fluorescence staining of the cells on different surface samples, cells were fixed with 4% paraformaldehyde (PFA) (Sigma Aldrich, 252549) for 30 mins. Each substrate was rinsed 3 times with PBS and the samples stained with DAPI (1 µL/mL, Sigma Aldrich, D9542) and rhodamine tagged phalloidin (1 µL/mL, Abcam, ab235138) for 30 mins. Another sequential wash has been conducted again with PBS and images captured on a Nikon Eclipse Ti2 fluorescent microscope.

Substrate Topography Characterisation

Samples were interrogated either by brightfield (Nikon Ti) or SEM. Patterned surfaces were analysed using SEM to identify the efficiency of the lithographic method used. Therefore, all the nano- and micro-features were measured and compared to the ones from the original template that were used as master. Cell-surface interaction was assessed from 5 random spots from each substrate to calculate the average and the standard deviation of cell-surface coverage, orientation, cell surface area and aspect ratio, again using ImageJ for various measures using standard analysis plugins. The determination of the orientation of the cells was achieved by using the "Directionality" plugin (http://fiji.sc/User:JeanYvesTinevez). Directionality is used to estimate the orientation or direction of structures present in an image as well as generating quantification data for the directionality parameters to assess any changes of cell orientation in response to underlying surface structure.

Water Contact Angle Measurement

Measurements were performed according to the sessile drop technique on an Attension Theta Lite optical tensiometer. Droplets of deionized water (5 μL) were positioned on substrates, with contact angles recorded over a period of 10 secs after droplet detachment from the syringe as shown in Figure S2-3 for PLA and PDMS, respectively. Droplets were measured in triplicate using 2 tangents from each to give an average.

Statistical Analysis

All quantitative results are presented as sample mean ± standard deviation. A two-sample student's t-test assuming both unequal and equal variance was used for statistical analysis where indicated caried out in MS Excel. A significance level was set at $p<0.05$.

Results

Characterisation of Patterned Surfaces

PDMS substrates fabricated by soft lithographic templating of the DVD, diffraction gratings and diffraction glasses showed excellent fidelity of the micro- and nano-scale patterns, with features down to the nanoscale being well presented in replicated substrates as shown in Figure 1. Features showed good replicability: diffraction gratings presented an array of 3 μm squared holes with 1.5 μm spacings; diffraction glasses produced 3.2 μm square holes, the edge of each presenting a circular protrusion with diameter of 1.5 μm. Much smaller features were obtained from the unrecorded blank DVD template, with 400 nm regular grooves and ridges. Water contact angle measurements were performed on all the substrates to investigate any changes in the surface wettability and consequently the substrate surface energy due to patterning and compared to their corresponding unpatterned surfaces (Table 1). The surface wettability was found not to be significantly altered at both length scales on either PDMS or PLA, which was expected due to aspect ratios of features presented [33].

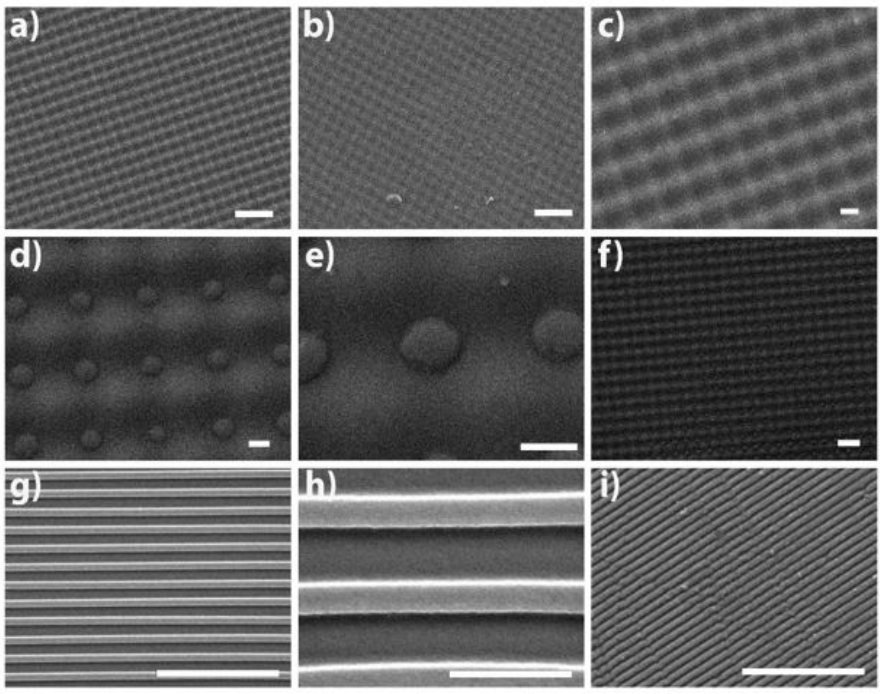

Figure 1: A, D, G) SEM image of the diffraction grating template, diffraction glasses and DVD respectively. B, E, H) Replications of the templates A, D, G fabricated in PDMS and C, F, I) fabricated in PLA. Intro scale bars A, B, F) 10 μm; C) 5 μm; D, E, G, H, I) 1 μm.

Table 1: Wettability measurements across all the patterned substrates ± St Dev n=3.

PDMS	PLA
DVD: 108.2o ± 3.19	DVD: 97.68o ± 3.31
Diffraction grating: 106.4 o ± 2.11	Diffraction grating: 102.1 o ± 3.68
Diffraction glasses: 104.17o ± 2.89	Diffraction glasses: 101.2o ± 3.15
Unpatterned: 96.14o ± 1.09	Unpatterned: 85.68o ± 1.85

The Effect of Topography on Haosmc's Attachment and Growth

The response of the HAoSMC cell to the PDMS and PLA patterned substrates are shown in Figures 2.A and 2.B, respectively. As it can be seen from both figures, induced topography has significant impact on cell behaviour compared to the unpatterned substrates. DVD patterning of the surfaces on both PDMS (Figure 2.A) and PLA (Figure 2.B) has more cell attachment compared to the control, whereas diffraction grafting, and diffraction glass surfaces have less cell adherence. Moreover, Table 2 highlights the average cell surface coverage results from the HAoSMC cells that were cultured on PDMS and PLA patterned substrates with different patterns. Cell surface coverage on DVD patterned PDMS and PLA is ~2 and ~5 times greater in comparison to un-patterned surfaces, respectively.

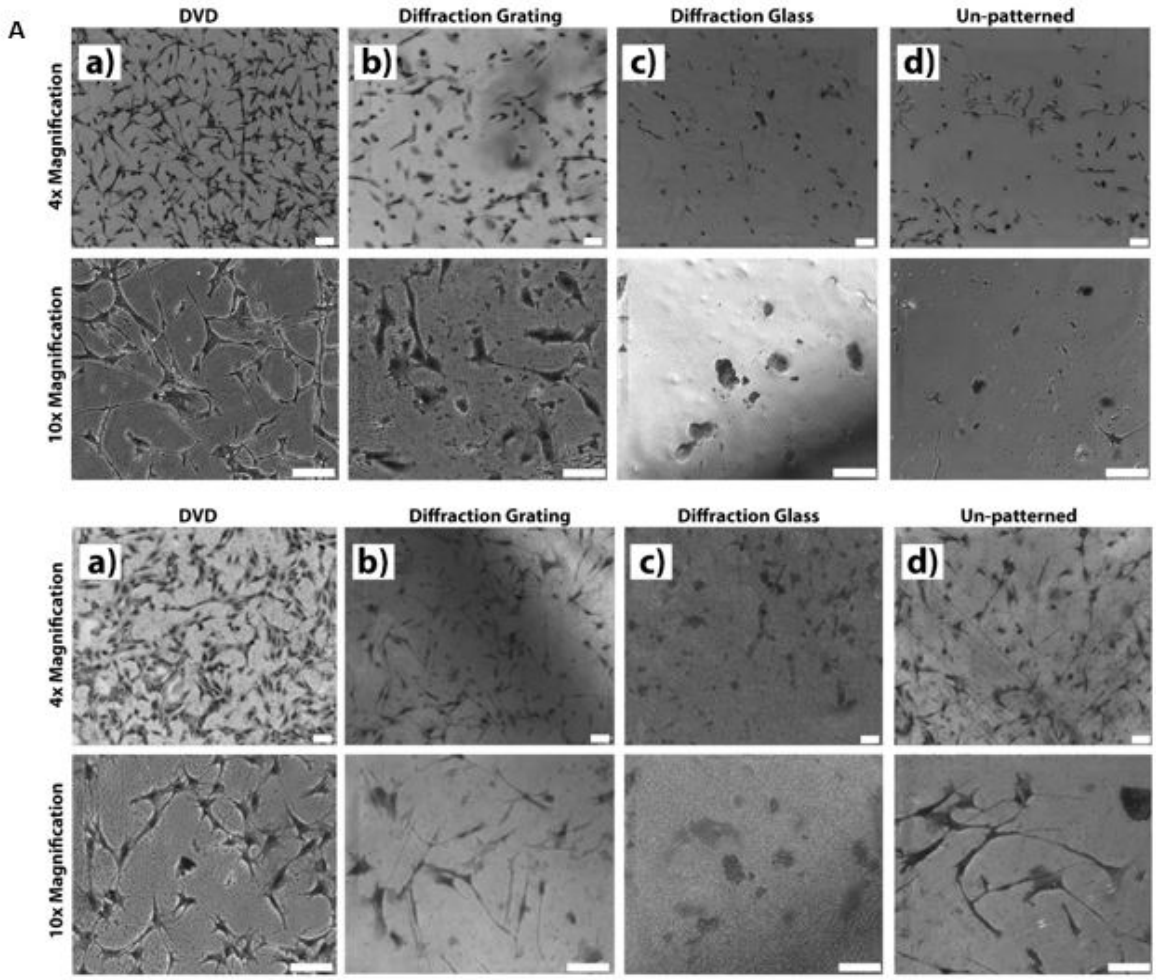

Figure 2: Brightfield imaging of HAoSMC on A.) PDMS, B.) PLA patterned substrates (DVD, Diffraction Grating and Diffraction Glasses) vs unpatterned flat controls. Cells stained with methylene blue at day 7. Scale bars: 10 μm.

In contrast, diffraction grating, and diffraction glass surfaces showed reduced cell surface coverage area. Additionally, different - types of nano- and micro-features were shown to influence the orientation and level of cell attachment to the substrate surface at a different extent. Comparatively, simple patterned substrates with grooves and ridges of 400 nm (DVD) width were shown to have the highest orientation, as well as highest surface area coverage, when compared with the unpatterned and other patterned substrates. In contrast, the diffraction glasses patterned PLA samples showed the most profound impact on inhibiting cell attachment - to the surface and reduced proliferation capacity. Additionally, cells also showed the lowest orientation compared to DVD and unpatterned surfaces, with most cells presenting rounded morphologies. Moreover, as it can be followed up from Table 2, the surface area and aspect ratio results of the cells are the highest on DVD surfaces both with PLA and PDMS samples.

Table 2: Cell surface coverage, cell orientation, surface area and aspect ratio on patterned substrates for PDMS and PLA substrates. Cell coverage reported as % surface area; cell orientation reported as % alignment, surface area and aspect ratio are represented as pixel unit which was normalized to the scale bar of the examined cell image with underlying surface structure direction or pattern grooves/topographic array ± St Dev of n=5.

	PDMS	PLA
Cell surface coverage	DVD: 30.1% ± 3.08	DVD: 60.0% ± 2.67
	Diffraction glasses: 8.3% ± 2.95	Diffraction glasses: 3.4% ± 0.44
	Diffraction grating: 11.2% ± 4.66	Diffraction grating: 4.1% ± 0.88
	Unpatterned surface: 17.3% ± 10.39	Unpatterned surface: 12.3% ± 1.58
Orientation	DVD: 68.8% ± 7.19	DVD: 67.0% ± 6.08
	Diffraction grating: 11.2% ± 4.66	Diffraction grating: 2.0% ± 1.58
	Diffraction glasses: 8.3% ± 2.95	Diffraction glasses: 7.6% ± 8.96
	Unpatterned surface: 3.4% ± 3.36	Unpatterned surface: 8.0% ± 1.00
Cell Surface Area	DVD: 34.928 ± 7.504	DVD: 22.176 ± 3.699
	Diffraction glasses: 19.16 ± 8.279	Diffraction glasses: 11.42 ± 3.330
	Diffraction grating: 28.244 ± 14.206	Diffraction grating: 10.728 ± 5.226
	Unpatterned surface: 16.08 ± 2.491	Unpatterned surface: 14.132 ± 4.698
Aspect Ratio	DVD: 5.99 ± 1.35	DVD: 5.08 ± 2.17
	Diffraction glasses: 2.76 ± 1.21	Diffraction glasses: 3.33 ± 2.35
	Diffraction grating: 1.51 ± 0.19	Diffraction grating: 1.71 ± 0.26
	Unpatterned surface: 1.92 ± 0.51	Unpatterned surface: 5.82 ± 3.62

Metabolic Activity Assays – Mtt, T-Dna, and Alamar Blue

The results from the metabolic activity assay showed topography on PDMS and PLA substrates significantly altered the HAoSMC viability relative to control at day 7, (Figure 3a). MTT results showed that patterns based on DVD templates on PLA increase the metabolic activity of the cells (Figure 3a). PLA and PDMS patterned substrates with grooved nano-structured features (DVD) have shown their ability to enhance significantly ($p<0.05$) the cell viability compared to the unpatterned ones. Interestingly, both substrates patterned with features on the nanoscale from the diffraction glasses were shown to have a profound effect on reducing the metabolic activity of the HAoSMC ($p>0.05$) which indicates the ability of such patterns to inhibit the growth as well as proliferation of the cells.

Total DNA assay confirmed differences in cell number as a result of substrate patterning, being supported also by MTT and image analysis. Results suggested that all patterns affect the cell behaviour of the HAoSMC cell line. As it can be seen from Figure 3a-b, the metabolic activity, and DNA content was slightly higher in cell populations cultured on DVD patterned substrates for both PDMS and PLA polymers compared to all other micro- and nano-patterns; diffraction gratings and glasses showed less metabolic activity and total DNA content.

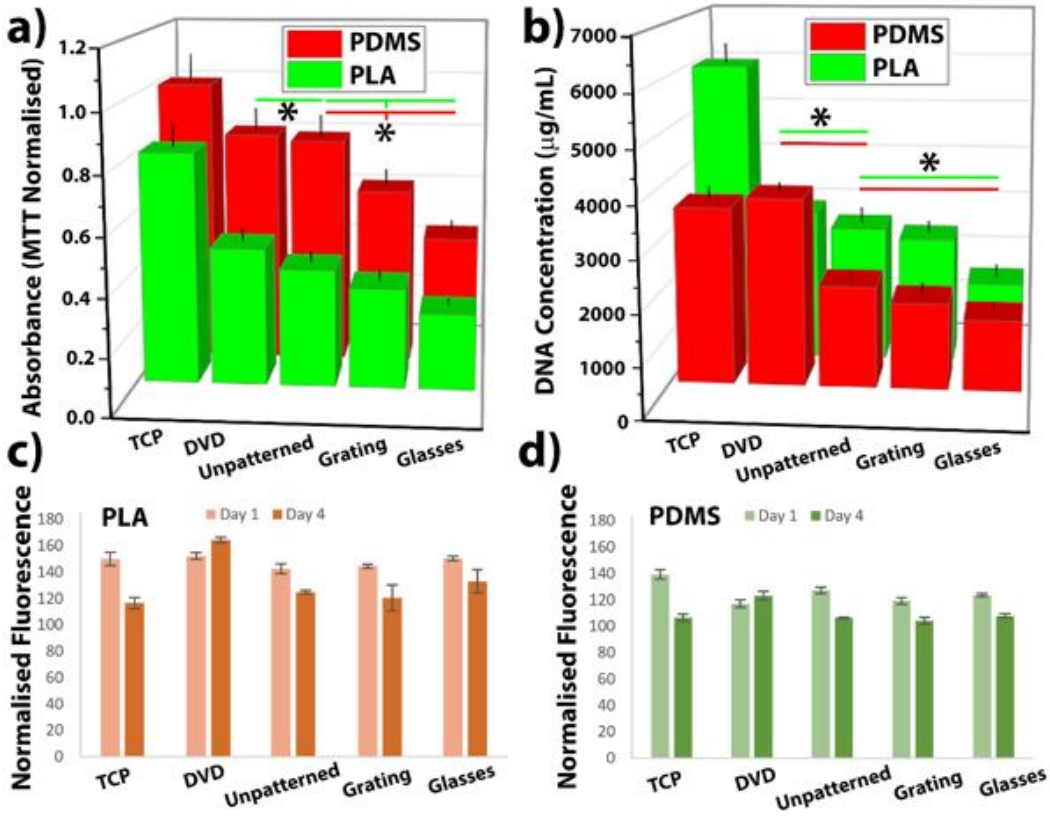

Figure 3: Cultivation of HAoSMC on patterned substrates with different micro- and nanofeatures a) MTT metabolic activity analysis of HAoSMC on patterned PDMS (red) and PLA (green) substrates, b) DNA concentration quantification (µg/mL) on PDMS (red) and PLA (green) substrates, AB cell viability assay on c) PLA and d) PDMS substrates after 1 and 4 days of the incubation of HAoSMC (n=3). * $p > 0.05$

In addition to the MTT and t-DNA assays, Alamar Blue cell metabolic activity assay was also performed. Consistent with the earlier results, DVD nano-patterned substrates presented good cell viability and activity across the culture period, Figure 3c-d. All surfaces, including TCP, showed a decreased trend in metabolic activity by culture day 4, whereas only the DVD patterned substrate showed a higher metabolic activity on both PLA and PDMS surfaces, and overall highest activity on PLA substrates.

Cell Adhesion and Cytoskeletal Assembly

Cellular response is a crucial consideration when designing biomaterials and primarily entails the following steps: initial attachment, cytoplasmic meshing and cell spreading. Smooth muscle cells have a fusiform shape, which is circular in the middle and tapered at both ends. Most smooth muscle cells have a differentiated, contractile phenotype with few cell organelles and a myofibril-rich cytoplasm in adult arterial media. However, when they are cultured *in vitro*, they often change to a non-contractile, myofibril-poor phenotype with a cytoplasm that is enriched in organelles and rough endoplasmic reticulum [34].

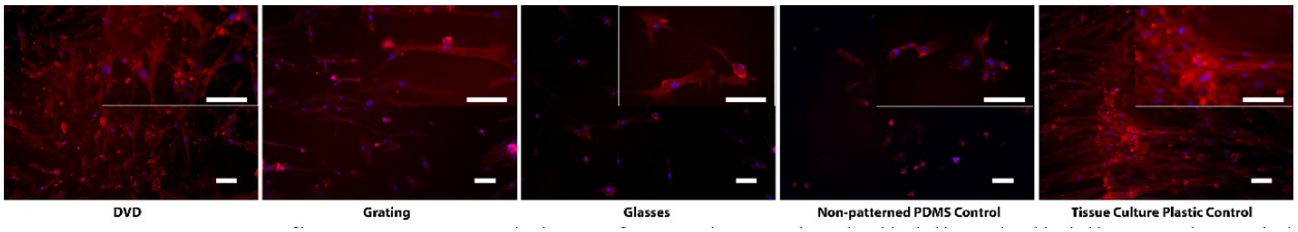

Figure 4: Immunofluorescence staining of F-actin (red, phalloidin-Phalloidin) and nuclei (blue, DaPI) of HAoSMC on DVD, Grating, Glasses, Non-patterned PDMS control substrates, and Tissue Culture Plastic control, respectively. Scale bars represent 100 μm.

Internal cytoskeletal structures of HAoSMC cultured and attached to patterned substrates is shown in Figure 4 to compare with morphological assessment overall in brightfield imaging shown in Figures 2.A and 2.B, demonstrating that all substrates enabled cell attachment to differing degrees, with cell morphologies, proliferative capacity and clustering demonstrating both material chemistry and topography differences. Not all materials could be assessed in this way due to autofluorescence of the PLA, however, on PDMS samples it can clearly be seen that the DVD patterned surface has the highest cell number and actin filament spread which also seen on the tissue culture plastic surface attachment.

Discussion

Well defined nanostructures were transferred to PLA and PDMS substrates with some feature dimensions as low as 400 nm, highlighting the efficiency and high degree of fidelity of such methods, whilst overcoming numerous limitations associated with conventional techniques. This simple and powerful technique has the potential to use the same substrate master through multiple replications. It is apparent that cells can sense the different patterned features on the substrates; the behaviour of the cultured HAoSMC on PDMS and PLA was found to be governed to some extent by the underlying substrate. Topographical surfaces with ordered grooves/ridges are known to impact on cell characteristics [35], and here this is demonstrated clearly in terms of cell attachment, morphology and metabolic activity. In the current study patterned substrates presenting features from diffraction glasses and gratings were shown to exhibit an inhibitory effect on the attachment of the cells, with more rounded cell morphology being presented. This is complementary to other research presenting limited cell spreading on silicon nanopillar arrays, possibly as a result in actin arrangement within the cytoskeleton and its localisation around surface structural features disallowing extended morphologies [36].

Materials are often compared for culture of cells *in vitro*, with the ambition to better understand material properties and their surface structural characteristics [37]. In this study cells were found to favour the PLA, which is a natural occurring polymer that possesses higher degree of hydrophilicity compared to PDMS. Image analysis revealed that both PLA and PDMS substrates with grooves having width of ~400 nm has the most impact on the behaviour of the HAoSMC cells regarding cell attachment/surface coverage and metabolic activity compared to the other topographies. Further, we found that there were drastic differences in cell coverage on some of the patterned surfaces depending on the materials used, e.g., cell coverage on PDMS DVD patterned surfaces was ~30% compared to ~60% on PLA surfaces; conversely ~8 % coverage was observed on PDMS diffraction glass patterning compared to only ~3.5% on PLA materials presenting the same pattern. These results could be due to changes in the protein layer com-

position, with surfaces being known to dictate not only the amount of protein binding [38], but also the nanostructure presented impacts on the ability of the protein molecules to unfold [39], and therefore can skew the overall protein layer composition [40]. Further investigations into the mechanisms underpinning the changes in cell response are certainly of broad interest and opening up the ability to nano-pattern in this way to those who do not have access to nanofabrication facilities can only support the growth of knowledge in this area. All the outcomes from cell-surface interaction investigation are pointing out a more flattened, more spread cellular body on DVD surfaces with greater surface area, orientation, aspect ratio for both polymer types which supports the suitability of that surface type for the growth and development of HAoSMC.

Results from MTT, Alamar Blue and t-DNA assays strongly suggest significant regulatory effects on HAoSMC's cell attachment, activity, and proliferation. In the case of PDMS with ordered micro-structured squared holes (diffraction gratings), the cell survival rate was reduced significantly, while in the case of PLA patterned substrates the reduction was also significant. The ability of patterned substrates with grooves and ridges at the micro- and nanoscale to enhance cell adhesion and alignment of different cell types was demonstrated, with the greatest cell adhesion (inferred from morphological assessment), alignment and spreading were found within the cultured HAoSMC cells on patterned substrates compared to flat controls (Figure 3b); this is comparable to other works in this field [41].

Patterned substrates with diffraction gratings exhibit inhibitory effects on the attachment of the HAoSMC cells compared to the unpatterned substrates, which could be attributed to changes in cell adhesion ability on these substrates [42]. Subsequently, cell adhesion, growth, spread and proliferation can be significantly reduced. Poor cell adhesion could significantly promote cell dormancy or even cause cell apoptosis [43]. MTT and t-DNA assays further confirmed these results and showed that the pattern with ordered square holes with length of 3 μm (diffraction gratings) can significantly reduce the cell adhesion, spreading and survival rate of the HAOSMC. Significant changes in the behaviour of the HAoSMC from the surface topography obtained in this study can provide further understanding to pathological differentiation of HAoSMC in atherosclerosis or vascular injury. The adhesion and activity of the smooth muscle cells are shown to be critical in enhancing several mechanical properties in optimizing tissue-engineered vascular grafts [44].

We have shown we are able to alter the response of only a single cell type, whilst micro-and nano-patterning in synergy with changes in surface chemistry, are known to play an important role for many other cell types [45,46]. We hope that this approach will be widely accessible to other researchers for the investigation of other protein and cell-surface interactions. There are limitations in this approach, largely the need for the initial template presenting the pattern required – in this work we present the use of varied household items, but other materials could be utilised to extend the range and type of patterns studied, e.g., plant leaves [47]. In the near future, many different reusable items can be utilized in this method to create defined or random surface features which can induce, elevate, suppress, limit or even prevent cellular interactions with the surface.

Conclusion

Multiscale physical features can be easily induced on PLA and PDMS substrates by using a simple, accessible, inexpensive, efficient yet robust technology, based on nanopatterns of consumer items as master moulds. Dramatic changes on several cellular functions of the HAoSMC can be seen by induced topography on different pattered substrates. Cell adhesion, spreading, morphology and alignment were significantly improved on topographical surfaces with ordered nano-grooves/ridges compared to the ones cultured on unpatterned substrates. Additionally, patterned substrates with diffraction gratings and glasses were shown to cause a significant reduction in cell adhesion, spreading and viability when compared to the cells cultured on unpatterned substrates. Our results suggest that consumer items containing complex and well-defined nanopatterns that otherwise would be discarded, can be used to fabricate effective topographic surfaces for high value technological applications, thus dramatically reducing the cost and environmental impact of controlled surface modification of biomaterials and reducing the lack of access by many laboratories interested in cell topography interactions, to complex topographies, that, otherwise, would require expensive techniques of fabrica-

tion and thus promote the use of more sustainable and accessible technologies.

Conflicts of Interest

The authors declare that they have no known competing financial interests that could have influenced the work reported in this paper.

Associated Content

Supporting Information: The following files are available free of charge. Supplementary information (file type, i.e., PDF)

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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