The influence of nanotexturing of poly(lactic-co-glycolic acid) films upon human ovarian cancer cell attachment

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Abstract
In this study, we have produced nanotextured poly(lactic-co-glycolic acid) (PLGA) films by using polystyrene (PS) particles as a template to make a polydimethylsiloxane mould against which PLGA is solvent cast. Biocompatible, biodegradable and nanotextured PLGA films were prepared with PS particles of diameter of 57, 99, 210, and 280 nm that produced domes of the same dimension in the PLGA surface. The effect of the particulate monolayer templating method was investigated to enable preparation of the films with uniformly ordered surface nanodomains. Cell attachment of a human ovarian cancer cell line (OVCAR3) alone and co-cultured with mesenchymal stem cells (MSCs) was evaluated on flat and topographically nano-patterned surfaces. Cell numbers were observed to increase on the nanotextured surfaces compared to non-textured surfaces both with OVCAR3 cultures and OVCAR3-MSC co-cultures at 24 and 48 h time points.

Keywords: nanotexturing, films, poly(lactic-co-glycolic acid), human ovarian cancer cell

(Some figures may appear in colour only in the online journal)

Introduction
Nanotexturing of biomaterials has been widely used to mimic the extracellular matrix and is currently an approach used to modulate, isolate and optimize the response of the cells for multiple applications in regenerative medicine and cancer therapeutics (Zhang and Webster 2012).

Previous studies of the topographical influence of cell-material attachment have been carried out with geometries including grooves, wells, pits, and protrusions on a diverse range of materials. The results for mature cells including cancer cells show that they respond to nano- and micro-textured biomaterial surfaces, where changes in cell adhesion, proliferation, orientation, alignment, migration and morphology were all observed. (Curtis and Wilkinson 1997, Fleming et al 1999, Andersson et al 2003, Matsuzaka et al 2003, Recknor et al 2004, Hsu et al 2005, Falconnet et al 2006, Miller et al 2007, Martinez et al 2009, Sarna et al 2009, Lamers et al 2010, Zhang and Webster 2012). These textured biomaterials may mimic in vivo microenvironments, and thus enable modeling of cell–cell and cell–extracellular matrix interactions and modulation of cell–surface interactions.
Although there are numerous studies on textured materials, the complexity of their role in controlling cell interactions and cell responses is still yet not fully understood and hence is impossible to predict without carrying out the individual cell studies. Also the response mechanisms of different cell lines to textured surfaces are not well defined (Andersson et al., 2003, Hsu et al., 2005, Miller et al., 2007, Martinez et al., 2009, Lamers et al., 2010, Zhang and Webster, 2012). Hence in this study, we have focused on fabrication, characterization and cell culture studies of hemispherical protrusions to provide nanotextured biodegradable biomaterials to characterize the ovarian cancer cell response to this surface topography.

Hemispherical protrusion shaped nanotextured materials were manufactured using a colloidal particle lithography technique employing polystyrene (PS) particles with the diameters from 57 to 280 nm. The PS templates were prepared by using two methods, the first as described in Zhang and Webster’s study (Zhang and Webster, 2012) which enabled formation of a multi-layered templates, and a second approach (Ogaki et al., 2010) by using self-assembly of PS particles at an air–water interface which produced mono-layered particles reducing the occurrence of step edges in the nano imprinted polymer surfaces. The latter method has not been used before to prepare biomaterial surfaces. The PS templates were used to form a negative relief in polydimethylsiloxane (PDMS) from which replication of the particulate topography was obtained in the biodegradable poly(lactic-co-glycolic acid) (PLGA) films (figure 1).

Atomic force microscopy (AFM) was used to characterize the topography, x-ray photoelectron spectroscopy (XPS) to analyze surface chemistry, and contact angle to measure the surface wettability.

Cell attachment on PLGA films was evaluated with an ovarian cancer cell line, OVCAR3, and OVCAR3 co-cultured with mesenchymal stem cells (MSCs). Specifically, the cell responses to non-textured PLGA films and nanotextured PLGA films were compared. With ovarian and other cancer types, the presence of a viable stem cell niche i.e. MSCs is critical to tumor growth and invasion, conferring a metastatic phenotype and chemo- and radio-resistance. Indeed, co-culturing the OVCAR3s with MSCs provides more disease relevance and appropriate cell:cell interactions. Specifically MCSs will differentiate into fibroblasts, thereby providing increased opportunity for cancer cell anchorage (Haller et al., 2000, Lehmann et al., 2011, Touboul et al., 2013).

Materials and methods

Preparation of PS templates

Multi-layer PS templates. Multi-layer PS templates were prepared as described previously (Carpenter et al., 2008, Zhang and Webster, 2012); borosilicate glass coverslips (18 mm in diameter, Fisher) were cleaned and degreased by acetone, ethanol (70%) and dH₂O. Then PS suspensions (300 μl, 10 wt%, Bangs Labs) with the diameters of 57, 99,
210 or 280 nm were pipetted onto the coverslips, and vacuum desiccated to remove the solvent (~2 days).

**Mono-layer PS templates.** Mono-layer PS templates were prepared as described previously (Rybczynski et al. 2003, Ogaki et al. 2010). Silicon wafers (Sigma-Aldrich) were cut to small (~1 × 1 cm²) and large (~1 × 3 cm²) sizes and sonicated with ethanol. Afterwards they were washed several times with Milli-Q water (MilliPore, resistivity of 18.2 MΩ cm⁻¹ at 25°C), and blown dry with nitrogen at room temperature. After drying, the silicon wafers were exposed to UV to increase wafer hydrophilicity for 20 min (UV/Ozone ProCleaner, BioForce Nanosciences, Inc.). The large silicon wafer was placed in a petri dish at an inclined plane, and the Petri dish was filled with Milli-Q water. PS particle suspension (100 μl, 10 wt%) was mixed with an equal amount of ethanol. This mixture was applied slowly over the large silicon wafer to water surface using an Eppendorf pipette, the silicon wafer was then slowly submerged in the water. To obtain hexagonal close packed particles, a dodecylsulfosucinate (SDS) solution (2%, ~10 μl) was added to Petri dish. The PS mono-layers on the water–air interface were then lifted off from the water surface by using the small-size silicon wafer, and were dried at room temperature. The surfaces were prepared by using PS particles with the diameters of 99, 210 or 280 nm.

**Preparation of PDMS templates**

PDMS (Sylgard 184 silicone elastomer, Dow Corning) was mixed thoroughly (curing agent: base, 3.5 ml: 26.5 ml), and then centrifuged for 15 min at 2000 rpm. It was poured over the PS coated coverslips (or silicon wafers) placed in a borosilicate glass Petri dish. PS templates were removed by vacuum extraction and the wafer was heated to 40°C for 2 h to accelerate the curing process. Once cooled, the PDMS template was separated from PS template. The PDMS template was washed with acetone to remove the remaining PS particles on the surface.

**Preparation of PLGA films**

PLGA (1g, 50:50 PLA/PGA, 7000-17000; Sigma Aldrich) was dissolved in chloroform (10 ml), and poured over the PDMS moulds, then placed into a vacuum desiccator to avoid bubble formation. After allowing evaporation of the solvent for 48 h, PLGA films were peeled off from the moulds and placed on glass coverslips. To remove the residual PDMS, PLGA films were washed with hexane (Sigma-Aldrich). For control studies, non-textured PDMS and PLGA films were prepared without PS beads following the procedure described above.

**AFM studies**

Topography images and analysis (section and bearing) of PS, PDMS and PLGA films were obtained in air using a D3000 AFM instrument with a NanoScope IIIa controller (Bruker) operating in Tapping™ mode. RTESPA AFM probes (nominal resonant mechanical frequency: 300 kHz, spring constant: 40 N m⁻¹, Bruker) were used, and images were acquired using an E-scanner, at scan rates between 0.6 and 1 Hz, with a resolution of 512 × 512 pixels. Image data was analyzed, section and bearing analysis were carried by NanoScope Analysis software-Version 1.20 (Bruker).

**Optical microscopy studies**

The samples were viewed using an optical microscope (Prior Scientific Instruments Ltd) equipped with a digital camera (QICAM Fast 1394, QImaging).

**Water contact angle measurements**

Water contact angle measurements of the films were measured by a Krüss DSA 100 instrument by dispersing an ultrapure Milli-Q water droplet (Millipore, resistibility of 18.2 MΩ cm⁻¹ at 25°C) with a volume of ~400 pl.

**XPS measurements**

The samples were analyzed using XPS in order to determine surface elemental composition using a Kratos AXIS Ultra Spectrometer (Kratos Analytical, Manchester, UK) with a mono-chromatic Al Kα x-ray source (1486.6 eV) operated at 10 mA emission current and 12 kV anode potential. The ULTRA was used in fixed analyzed transmission mode, a pass energy of 80 eV was used for the wide scans, and 20 eV for the high resolution scans. Data analysis was carried out using CASAXPS software with empirically derived sensitivity factors to determine the composition (atomic percent) from the peak areas. The photoelectrons were collected normal to the sample surface. The measurements were carried only with a mono-layer textured 280 nm films and non-textured films which had been cast against a flat PDMS mold.

The residual PDMS layer thickness over the mono-layered 280 nm textured PDMS films and non-textured films were calculated by using the silicon signal in the XPS data. Topofactors for hemispheres were used to calculate the equivalent conformal PDMS thickness of the textured surfaces (Shard et al. 2009, Shard 2012).

**Human ovarian carcinoma cell and MSC adhesion assay**

Human ovarian carcinoma cell line, OVCAR3 (US National Cancer Institute) was cultured only/or co-cultured with MSC line, hm-MSC-bm (Sciencell Research Laboratories, CA, US) in Dulbecco’s modified eagles medium (DMEM, Sigma, UK) with 10% fetal bovine serum (FBS, Sigma, UK).

For sterilization of the films, PLGA films were removed from the coverslips by soaking in 70% ethanol for approximately 4 h, and later soaking in clean 70% ethanol for approximately 20 min. The surfaces were sterilized by UV light for 5 min in ethanol, and later 5 min, without ethanol. After rinsing with media the films were placed in 3 ml of DMEM + 10% FBS.
OVCAR3 cells were labeled with Cell tracker™ CM-DiI red fluorescent protein reporter (Life Technologies, UK) and cultured on three different surfaces including a control well of a six well plate (flat plastic), non-textured PLGA films, and textured PLGA films prepared from mono-layered 280 nm PS templates for 48 h. For each condition the cells were plated (OVCAR3 only, OVCAR3 and MSC’s combined at a 1:1 ratio) on duplicate wafers (one per well, six well plates) with a final density of $6 \times 10^5$ and monitored over 48 h for cell attachment. Three plates, one for each time point were set up (4, 24 and 48 h) and in each case the wafers were removed for counting.

At 4, 24 and 48 h, the cells in all conditions were counted manually over three regions of interest (ROI) for each duplicate well where possible. Cells emitting red light were photographed at 10X objective magnification in three areas of each well (duplicate wells). Each image was divided into four equal (dimensions) ROI’s and the cells counted. In this study, number of cells attached to the films at the 4th hour, 24th hour, and 48th hour were studied.

**Statistical analysis**

The validation and analysis of cell attachment data was carried out in SPSS 21, (IBM) using Wilcoxon signed ranks test. Other statistical analysis was performed by a two sample t-test assuming both equal and unequal variances. Statistical significance was accepted at $p < 0.05$.

**Results**

In these studies we have focused on fabricating regular nanotextured and defect-free materials over large length scales compatible with the cell culture experiments (1 cm × 1 cm).

**Nanotextured surfaces prepared from multi-layer PS templates**

Utilizing the Zhang and Webster method (Zhang and Webster 2012), we successfully produced PDMS templates from multi-layers of PS spheres. Using AFM imaging, we determined that the form of the PS templates were transferred to PDMS templates and then to the PLGA films (figure 2). This data also indicated that the diameters of the features in PS templates and PLGA films remained unchanged during the fabrication process of the films. (See also figure S3, ESL.)

From the AFM images of the PS particles, the PDMS mould and the PLGA surfaces presented in figure 2, hexagonal close packing of the particles is evident for the 210

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**Figure 2.** AFM topography images of PS surfaces (A), PDMS surfaces (B), and PLGA films (C) prepared from multi-layer PS templates. The surfaces were prepared by use of 280, 210, 99, and 57 nm PS beads. All images were 2 μm × 2 μm.
and 280 nm assemblies, but this was accompanied by retention of PS particles in the structure imprinted in to the silicone mould for the 99 nm surface and the order was not present in the 57 nm beads.

AFM studies with larger scan areas (8 μm × 8 μm) indicated the presence of flat layers of particles, separated by step edges between areas of disorder in the PLGA films (figure 3). These step edges were attributed to the formation of multi-layers during PS template preparation (figure S4, ESI). By analysis of the AFM images, the percentage of the protrusions by boundary structures in the PDMS surfaces was found to be up to ~7% of the overall scan area.

Nanotextured surfaces prepared from mono-layer PS templates

In an alternative method, adapted from Ogaki et al (Ogaki et al 2010), PS templates were prepared by self-assembly of the PS beads on the water–air interface; a single layer of PS particles were spread on the wafer during the preparation of the templates. Mono-layered PS templates were prepared from beads with the diameters of 280, 210 and 99 nm, but 57 nm particles did not prove effective from beads with the diameters of 280, 210 and 99 nm, but 57 nm particles did not prove effective (figure S5, ESI). For the templates prepared from 280 to 210 nm, AFM results indicated that PS mono-layer templates were prepared successfully, without the retained particles as found in the multilayer moulding, and the nanotexturing was again transferred to PDMS and PLGA films. For 99 nm templates prepared by this new method, multi-layers were observed and surface regularity was disrupted compared to larger particle diameters (figure 4). For 210 and 280 nm PS particles, step edges were not observed on the surfaces (figure S6, ESI). Optical microscopy images have also confirmed the elimination of the macroscopic defects (figure S7).

When AFM data obtained from both methods with different PS particle diameters were considered, the films prepared from mono-layer 280 nm particles formed defect free highly ordered hexagonal packing both over small and large scales. The presence of step edges and nano-grooves were fully eliminated, as well as macroscopic non-ordered cracks. For these reasons, PLGA surfaces prepared from monolayered 280 nm templates were selected for further characterization.

XPS and water contact angle measurements

Since chemistry, as well as topography, effect cell response to materials, XPS studies were carried out with the PLGA samples to determine whether the samples have similar chemistries after a hexane wash to reduce PDMS residuals. The studies were undertaken with textured and non-textured PLGA surfaces as well as a PLGA powder reference. XPS data indicated the presence of silicon at a similar thickness in textured (1.2 ± 0.3 nm) and non-textured (1.0 ± 0.1 nm) surfaces (table 1). This was assigned to PDMS oligomers carried over from the silicone mould. All other spectral features were found to be equivalent in the textured and non-textured surfaces indicating chemical equivalence.

Overlayer PDMS thickness on PLGA particles were calculated from XPS data with the method described previously (Shard et al 2009). By using the topofactors given for the hemispheres, the equivalent conformal thickness was calculated via the thickness of PDMS as for a flat sample. According to the calculations, both flat samples and topofactor corrected textured surfaces have ~1 nm overlayer PDMS remaining. A two sample t-test was carried on the film PDMS film thicknesses and the P value was found to be 0.09, indicating that there was no statistical significance between the textured and non-textured films.

Contact angle measurements indicated that the wettability values of the surfaces were similar. In both surfaces, contact angle values were found higher than 90°, which indicates that the wetting of the surfaces were poor, and the surfaces were hydrophobic (table 1).

Cell culture studies

OVCAR3 cells were studied in monoculture and in co-culture with MSC’s in order to assess their response to 280 nm hemispherical topographies. Cell culture studies were carried out with PLGA surfaces prepared from mono-layered
280 nm templates and non-textured flat PLGA surfaces. Flat plate wells (tissue culture plastic only with no other added matrix/wafer/gel) were used for control studies. OVCAR3, and OVCAR3 seeded with MSCs at a 1:1 ratio were cultured onto the surfaces. In the study, attachment of the cells to the films at 4th hour, 24th hour and 48th hour were studied.

**Figure 4.** AFM topography images of PS surfaces (A), PDMS surfaces (B), and PLGA films (C) prepared from mono-layered PS templates. The surfaces were prepared by use of 280, 210, and 99 nm PS beads. All images were 2 μm × 2 μm.

**Table 1.** XPS results, PDMS thickness calculations with and without topofactor correction, and contact angle measurements of the samples. The studies carried out with nanotextured PLGA films prepared from mono-layered 280 nm templates, and non-textured PLGA films after a hexane wash of the samples. For XPS studies, PLGA powder was also analyzed as a reference. The conformal coating thickness of PDMS was calculated using the stoichiometry of PDMS and a topofactor correction for the particle topography developed by Shard. (Shard et al 2009, Shard 2012).

| Samples           | XPS results | PDMS thickness (nm) |
|-------------------|-------------|---------------------|
|                   | C 1s %      | O 1s % | Si 2p % | Non-corrected | Topofactor corrected | Contact angle (θ) |
| PLGA powder       | 62.9 ± 0.28 | 37.1 ± 0.28 | —       | —             | —                 | —                 |
| Textured films    | 58.7 ± 2.44 | 33.1 ± 0.78 | 8.2 ± 1.9 | 1.6 ± 0.5     | 1.2 ± 0.3          | 104.4 ± 0.5       |
| Non-textured films| 63.2 ± 0.52 | 31.2 ± 0.51 | 5.6 ± 0.37 | 1.0 ± 0.1     | —                 | 107.9 ± 0.8       |

**OVCAR3 alone**

It was observed that OVCAR3 cells were adhered to the surfaces by 4 h and the mean number of cells attached per wafer were similar for both textured and non-textured surfaces but less than the flat plate well, cell culture plastic control (figure 5, figure S8, ESI).
The mean number of cells per wafer increased for both textured and non-textured surfaces over time, however in the flat plate well cell culture control, a decrease was observed after 24 h with OVCAR3s probably due to having reached confluency and then overgrowth of the cells on the well plate. The mean number of cells per wafer were higher on textured surfaces as compared to non-textured surfaces at the 24 and 48 h time points and were significantly greater (*p < 0.05) at the 24 h time point. Also the cells at these time points were clustered in islets on the tissue culture plate control and wafers indicative of proliferation (figure S8).

**Discussion**

There has been recent interest in the use of biomaterials to modulate cancer cell adhesion (Hartman et al 2010, Wan et al 2012, Kim et al 2013, Sharma et al 2014, Islam et al 2015) motivated by the few studies in this area and to our knowledge none with ovarian cancer cells, we investigated the modulation of ovarian cancer cell adhesion fabricating novel regularly nanotextured and defect-free materials over large lengths scales. Indeed, neither of these factors has been explored to date. Our first method of nanotexturing was to use a colloidal lithography approach similar to that reported by Zhang and Webster (Zhang and Webster 2012) where we observed with AFM, step edges in PLGA films as a result of formation of multi-layers during PS template preparation (figures 2 and 3). These step-edges appear to be points of weakness in the interparticulate bonding, and resulted in PS particulate transfer to PDMS along these edges and subsequent PLGA films. Since it has been reported that cells can be aligned with surface features (known as ‘contact guidance’) (Curtis and Wilkinson 1997, Rajnicek et al 1997, Flemming et al 1999, Wilkinson et al 2002), we then sort to find another colloidal lithography that could remove these features in the topography. Eliminating this response of the cells to non-ordered topography, allows study of cell response to nanotextured substrates only and to achieve this monolayer PS templates were prepared. Monolayers of PS particles...
have been reported previously (Ogaki et al 2010), however here for the first time we used these to template nanotexture into PLGA surfaces for a biomaterial application. We found that we could create defect free nanotextured surfaces with nanotextured domes either 210 or 280 nm in diameter. However when smaller PS particle diameters were used in template preparation (99 and 57 nm), the order of small scale nanotexturing was disrupted. With this method, hexagonal packing is achieved by addition of SDS and indeed the particle diameters and SDS solution concentrations are crucial. Hence only the nanotextured surfaces with 280 nm nanotextured domes were progressed further where these films and a flat surface control were rigorously characterized using XPS and contact angle measurements. These measurements proved chemical equivalence in surface chemistry and hence we can be certain that any difference in cell response is due to topography i.e. nanotexturing.

Using these 280 nm nanotextured defect free films and a flat surface control of chemical equivalence, we then progressed to evaluating OVCAR3 cell adhesion on these films, in monoculture and in co-culture with MSCs. Our data shows that OVCAR3 cell adhesion, whether in monoculture or co-cultured with MSCs, to all surfaces and similarities between textured and non-textured surfaces at the four hour time point as also reported by Zhang and Webster (Zhang and Webster 2012) for breast cancer cells. It should also be noted that these cell attachment studies are also the first to our knowledge using a co-culture with MSCs.

However at 24 and 48 h time points, our results demonstrated that textured surfaces have more cells attached compared to non-textured surfaces when alone or co-cultured with MSCs. Our data is in agreement with previous studies with cell lines where reported changes in cell attachment were observed compared to non-textured surfaces. In some papers, a decrease in cell adhesion/proliferation was reported due to texturing (Curtis et al 2001, Wilkinson et al 2002), and oppositely some reported an increase in cell adhesion/proliferation (Miller et al 2007, Carpenter et al 2008) as was observed in our studies.

Conclusions

In this study, we aimed to investigate the effect of hemispherical protrusion topographies on ovarian cancer cell adhesion. For this purpose, flat and nanotextured PLGA films obtained from (1) mono-layered and (2) multi-layered PS templates were characterized by AFM and optical microscope.

According to our results:

- Films obtained from multi-layered templates had non-ordered, uncontrolled surface topographies (macroscopic structures and step edges).
- Films obtained from monolayered templates were defect-free at large length scales, and demonstrated nanotexturing in small scale. However it was found that when the PS particle diameters for template preparation decreased to 99 nm and 57 nm, the order of small scale nanotexturing was disrupted.
- Whilst in the cancer cell adhesion studies, OVCAR3 seeded alone, and seeded with MSCs, cancer cells adhered to all surfaces, and increased cell numbers on textured surfaces compared with the non-textured surfaces was observed at 24 and 48 h time points.

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References

Andersson S A, Backhed F, von Euler A, Richter-Dahlfors A, Sutherland D and Kasemo B 2003 Nanoscale features influence epithelial cell morphology and cytokine production Biomaterials 24 3427–36
Carpenter J, Khang D and Webster T J 2008 Nanometer polymer surface features: the influence on surface energy, protein adsorption and endothelial cell adhesion Nanotechnology 19 505103
Craighead H G et al 1998 Chemical and topographical surface modification for control of central nervous system cell adhesion Biom. Microdevices 1 49–64
Curtis A and Wilkinson C 1997 Topographical control of cells Biomaterials 18 1573–83
Curtis A S G, Casey B, Gallagher J O, Pasqui D, Wood M A and Wilkinson C D W 2001 Substratum nanotopography and the adhesion of biological cells. Are symmetry or regularity of nanotopography important? Biophys. Chem. 94 275–83
Dalby M J, Giannaras D, Riehle M O, Gadegaard N, Alfissson S and Curtis A S G 2004 Rapid fibroblast adhesion to 27 nm high polymer demixed nano-topography Biomaterials 25 77–83
Falconnet D, Csucs G, Grandin H M and Textor M 2006 Surface engineering approaches to micropattern surfaces for cell-based assays Biomaterials 27 3044–63
Flemming R G, Murphy C J, Abrams G A, Goodman S L and Nealey P F 1999 Effects of synthetic micro- and nano-structured surfaces on cell behavior Biomaterials 20 573–88
Haller D, Bode C, Hampes W P, Pfeifer A M A, Schiffrin E J and Blum S 2000 Non-pathogenic bacteria elicit a differential cytokine response by intestinal epithelial cell/leucocyte co-cultures Gut 47 79–87
Hartman O, Zhang C, Adams E L, Farach-Carson M C, Petrelli N J, Chase B D and Rabott J E 2010 Biofunctionalization of electrospun PCL-based scaffolds with perlecian domain IV peptide to create a 3D pharmacokinetic cancer model Biomaterials 31 5700–18
Hsu S H, Chen C Y, Lu P S, Lai C S and Chen C J 2005 Oriented Schwann cell growth on microgrooved surfaces Biotechnol. Bioeng. 92 579–88
Islam M, Sajid A, Mahmood M A I, Bellah M M, Allen P B, Kim Y T and Iqbal S M 2015 Nanotextured polymer substrates show enhanced cancer cell isolation and cell culture Nanotechnology 26 225101

Kim H N, Jiao A, Kim M S, Kang D H, Kim D H and Suh K Y 2013 Nanotopography-guided tissue engineering and regenerative medicine Adv. Drug Deliv. Rev. 65 536–58

Lamers E, Walboomers X F, Domanski M, te Riet J, van Delft F, Luttge R, Winnubst L, Gardeniers H and Jansen J A 2010 The influence of nanoscale grooved substrates on osteoblast behavior and extracellular matrix deposition Biomaterials 31 3307–16

Lehmann A D, Daum N, Bur M, Lehr C-M, Gehr P and Rothen-Rutishauser B M 2011 An in vitro triple cell co-culture model with primary cells mimicking the human alveolar epithelial barrier Eur. J. Pharmaceutics Biopharmaceutics 77 398–406

Martinez E, Engel E, Planell J A and Samitier J 2009 Effects of artificial micro- and nano-structured surfaces on cell behaviour Ann. Anat.—Anatomischer Anz. 191 126–35

Matsuzaka K, Walboomers X F, Yoshinari M, Inoue T and Jansen J A 2003 The attachment and growth behavior of osteoblast-like cells on microtextured surfaces Biomaterials 24 2711–9

Miller D C, Haberstroh K M and Webster T J 2007 PLGA nanometer surface features manipulate fibronectin interactions for improved vascular cell adhesion J. Biomed. Mater. Res. A 81 678–84

Ng R, Zang R, Yang K K, Liu N and Yang S-T 2012 Three-dimensional fibrous scaffolds with microstructures and nanotextures for tissue engineering RSC Adv. 2 10110–24

Ogaki R, Lyckegaard F and Kingshott P 2010 High-resolution surface chemical analysis of a trifunctional pattern made by sequential colloidal shadowing ChemPhysChem 11 3609–16

Rajnicek A M, Britland S and McCaig C D 1997 Contact guidance of CNS neurites on grooved quartz: influence of groove dimensions, neuronal age and cell type J. Cell Sci. 110 2905–13

Recknor J B, Recknor J C, Sakaguchi D S and Mallapragadaa S K 2004 Oriented astroglial cell growth on micropatterned polystyrene substrates Biomaterials 25 2753–67

Rybczynski J, Ebels U and Giessig M 2003 Large-scale, 2D arrays of magnetic nanoparticles Colloids Surf. A 219 1–6

Sarna M, Wybiersalska E, Miekus K, Drukala J and Madeja Z 2009 Topographical control of prostate cancer cell migration Mol. Med. Rep. 2 865–71

Shard A G 2012 A straightforward method for interpreting XPS data from core–shell nanoparticles J. Phys. Chem. C 116 16806–13

Shard A G, Wang J and Spencer S J 2009 XPS tofactors: determining overlayer thickness on particles and fibres Surf. Interface Anal. 41 541–8

Sharma A, Sharma N L, Lavy C B, Kiltie A E, Hamdy F C and Czernuszka J 2014 Three-dimensional scaffolds: an in vitro strategy for the biomimetic modelling of in vivo tumour biology J. Mater. Sci. 49 5809–20

Touboul C, Lis R, Al Farsi H, Raynaud C M, Warfa M, Althawadi H, Mery E, Mirshahi M and Rafii A 2013 Mesenchymal stem cells enhance ovarian cancer cell infiltration through IL6 secretion in an amniochorionic membrane based 3D model J. Transl. Med. 11 28

Wan Y, Mahmood M A I, Li N, Allen P B, Kim Y T, Bachoo R, Ellington A D and Iqbal S M 2012 Nanotextured substrates with immobilized aptamers for cancer cell isolation and cytology Cancer 118 1145–54

Wilkinson C D W, Riehle M, Wood M, Gallagher J and Curtis A S G 2002 The use of materials patterned on a nano- and micro-metric scale in cellular engineering Mater. Sci. Eng. C 19 263–9

Zhang L and Webster T J 2012 Poly-lactic-glycolic-acid surface nanotopographies selectively decrease breast adenocarcinoma cell functions Nanotechnology 23 155101