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PAPER

Fabrication, characterisation and performance of hydrophilic and super-hydrophilic silica as cell culture surfaces

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We demonstrate a straightforward procedure for the controlled formation of silica films on tissue culture polystyrene (PS) surfaces. The films were formed by sequentially treating PS with polyaniline, glutaric dialdehyde and protein prior to silica formation. The films could be tailored to exhibit super-hydrophilicity (contact angle < 5°) which was retained for more than two months under ambient conditions. Both hydrophilic and super-hydrophilic silica coated surfaces were suitable for the culture of an adherent human melanoma cell line. Proliferation, toxicity and adhesion assays were used to compare cell behaviour. Cells on the silica surfaces showed enhanced adhesion and comparable rates of cell proliferation as compared to cells grown on conventional tissue culture plastic. The results obtained can be understood by considering the surface properties of the different materials and the ability of the silica coated surfaces to adsorb significantly higher levels of serum proteins from the growth medium. One of the outcomes of this study is a re-evaluation of the hydrophobicity/hydrophilicity characteristics required for good cell growth and the possibility of designing new tissue culture materials capable of greater control over cell populations.

Introduction

There has been considerable interest in the use of sol–gel materials for biological and biomedical applications.[1] Sol–gel materials have already been used as host matrices for the entrapment of viable yeast,[2] prokaryotes,[3] and eukaryotes.[4] The sol–gel method has also been used to produce bioactive porous glasses for bone regeneration and to fabricate silica films on glass surfaces for cell-growth.[5,6] It has been observed that biological entities such as proteins and liposomes,[7,8] in addition to mammalian and prokaryotic cells, remain active on sol–gel derived surfaces after immobilisation or adhesion and can demonstrate increased stability and growth.[3,9]

An advantage of using silica as a culture substrate is that silica may be readily modified during and after fabrication to produce a range of materials with varying functionality,[10] wettability,[11] topology and porosity,[12,13] all properties which have been identified as important in cell adhesion and thus important in the development of new cell culture materials.[13] Silica particles of different sizes assembled on stainless steel and titanium foils have, for example, been shown to effect the growth and differentiation of human-bone-marrow derived Mesenchymal Progenitor Cells (MPC's).[14]

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Various research groups have shown that patterned surfaces functionalised with various chemical groups may control the growth of osteoblasts,[15] hepatocytes and endothelial cells,[16,17] while others have shown that silicon nano-pillars with diameters of 100–200 nm may be used to capture tumour cells.[18] Self-assembled monolayers,[19] polymers,[20] short chain peptides and hydrogels with different functional groups and roughness have all been used to culture mammalian cell lines with differing responses observed.[21,22]

Many reports have shown that the wettability and charge density of the substrate's surface may influence the function and fate of attached cells,[23] and as such both are important parameters in the production of new culture materials. The effect of wettability, and topology of silica and organically modified silica films on the growth of Buffalo Green Monkey Kidney (BGM) cells and the differentiation of neuronal cells has been shown.[6] In the case of BGM cells, silica films with an intermediate hydrophilicity (contact angle ~ 70°) were determined to be superior to polystyrene and glass in cell culture performance in serum present conditions. This trend for optimal adhesion with a contact angle of 60–80° has been demonstrated for a range of cell types in the presence of serum.[23] However, the ability of hybrid silica films to promote cell growth under reduced serum conditions has also been shown.[6]

The potential for many of the surface modification approaches noted above is limited, due partly to the time-consuming processes required for their fabrication, an inability to simultaneously control multiple physical and chemical properties of the surface as well as the necessity of using restrictive and/or expensive surfaces such as glass, silicon and gold during fabrication. Little attention has been

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paid to the most widely used and economical tissue culture material, polystyrene, as a basis for forming functional films including silica films. Currently, sulfuric acid or nitrogen plasma treatment to generate charged groups on the plastic surface and coatings of proteins (collagen) and polyamines (poly-lysine) improve the attachment, growth and proliferation of many cell lines.[24–26] Several drawbacks are associated with these techniques in that the modification is often temporary in nature and can require specific conditions such as refrigeration to maintain the materials properties.

However, while several efforts have been made to use inorganic materials as substrates for cell growth, including the use of hybrid silica films to grow (BGM) cells and neuronal model cells (C12 rat pheochomocytoma cells),[6] a comprehensive understanding of cell response to the wide range of inorganic materials available is currently lacking. This is particularly true for materials exhibiting extreme properties such as super-hydrophilicity, in part due to an inability to manufacture the materials in a manner suitable for cell culture, with surface modifications on current materials ineffective over extended periods, requiring costly base materials such as gold or time consuming methods as noted above.[27]

In this contribution, we demonstrate a straightforward procedure for the controlled formation of silica films, including those with super-hydrophilic (contact angle $< 5°$) properties on tissue culture polystyrene (PS) surfaces, Fig. 1.

The films exhibited super-hydrophilicity for more than two months under ambient conditions and were suitable for the growth of an adherent human melanoma cell line; FM3.[28] A combination of cell adhesion, proliferation and toxicity assays were used to compare cell behaviour on tissue culture polystyrene, hydrophilic and super-hydrophilic silica surfaces. The results obtained are explained by consideration of the surface properties of the materials and their ability to adsorb serum proteins from the growth medium. One of the outcomes of this study is a re-evaluation of the hydrophobic/hydrophilic characteristics required to ensure good cell growth. A potential application in the development of such tissue culture materials would be selective enrichment of a culture with cells of clinical or scientific interest from the mixed culture.

Experimental methods

Fabrication of silica surfaces on polystyrene

Untreated tissue culture polystyrene (PS) Petri dishes (Sarstedt) were coated with a polyaniline (PANI) film; 0.25 M aniline

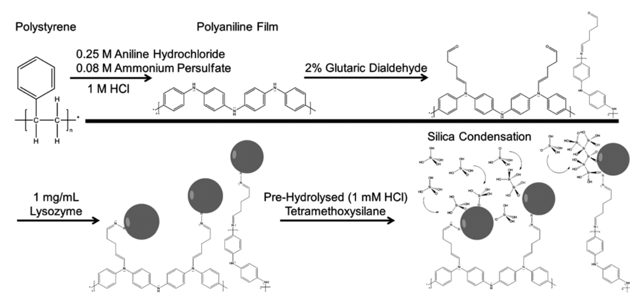

Fig. 1 Scheme showing modifications make to a polystyrene surface permitting the controlled formation of silica films.

hydrochloride (Sigma) in 1 M HCl in the presence of 0.08 M ammonium persulfate (Sigma-Aldrich) in ddH$_2$O in a 1 : 1 molar ratio using a method adapted from Karir *et al.*[29] The PANI coated PS surfaces were treated with 2% v/v glutaric dialdehyde (Sigma-Aldrich) in ddH$_2$O at 57 °C for 2 h, before further treatment with a 1 mg mL^{-1} lysozyme (Fluka) in 0.1 M phosphate buffer (pH 7.2) solution for 24 h before aspiration of the excess reagents and stored at 4 °C. The surfaces (hydrophilic or super-hydrophilic) were prepared as follows. For the hydrophilic surface, the protein coated surfaces were treated with 1.0 M tetramethoxysilane (TMOS) (Aldrich) in ddH$_2$O (pre-hydrolysed with 1.0 mM HCl for 15 min) for 1 hour with 5% glycerol as a drying control agent and then left to dry under ambient conditions. Alternatively, for the super-hydrophilic surface, the protein bound surface was treated with 0.5 M TMOS in ddH$_2$O (pre-hydrolysed with 1.0 mM HCl for 15 min) for 2 hours and then dried in a covered vessel over a 48 h period under ambient conditions.

Characterisation of materials

Materials were characterised by UV/VIS spectroscopy to follow PANI film formation using a Unicam UV2 UV-vis spectrophotometer, scanning between 390 and 1100 nm with a resolution of 0.5 nm. Scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDXa) were used to assess morphology and elemental composition respectively using a JEOL JSM-840A SEM operating in the secondary electron mode at an accelerating voltage of 20–25 kV and a working distance between 15 and 35 mm with EDXa analysis using an Oxford Instruments INCA X-sight system (count rate set to three kcounts s^{-1} for all samples). Samples were coated with gold for imaging, and carbon for EDXa analysis using an Edwards Sputter Coater S150B. Contact angle measurements of a 5 μL drop of ddH$_2$O on the surfaces were made using a Krüss DSA 10 Contact Angle Meter and analysis using Drop Shape Analysis software, with nine replicates for each sample. Atomic force microscopy (AFM) was used to assess surface roughness of the samples using a Pacific Nanotechnology Nano-R2 AFM in close contact mode with Pacific Nanotechnology Close Contact Mounted Cantilevers (P-MAN-SICC-0). Nine replicate scans were treated (levelled) before root mean square roughness (RMS) measurements were made using the software Nanorule. Line analysis of raw image data was used to determine film thickness at each stage of fabrication after a scratch was introduced on the surface in addition to silica particle size and distribution. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to determine the silicon content of the media using a Perkin Elmer Optima 2100 DV Optical Emission Spectrometer with WinLab32 software. Silicon content was determined by measuring signal intensity at 251.611 nm against a standard curve of between 0.01 and 0.75 mg L^{-1} orthosilicic acid (obtained from an industry standard solution (BDH) in RPMI-1450 media (Lonza BioWhittaker) matrix). Silica content of the films was determined after treatment with 2 M NaOH for 1 h at 80 °C with subsequent silica concentration measured from 100 μL aliquots following the method described by Belton *et al.*[30]

Lysozyme adsorption was measured using the Bradford assay with 20 μL aliquots diluted in 1 mL working reagent (4× stock

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containing 100 mg Coomassie G-250, in 50 mL methanol, 100 mL 85% phosphoric acid and made to a final volume of 200 mL with ddH_2O). Samples were incubated for 5 min at room temperature before absorbance was read at 595 nm using a Unicam UV2 UV-vis spectrophotometer, concentration being determined from a calibration curve of different lysozyme concentrations. Fibrinogen (Fluka) and bovine serum albumin (BSA) (Fluka) adhesion to the surfaces after a 24 h period was monitored using the amido black assay as described by Roach *et al.*[31]

Cell culture, proliferation, toxicity, vitality and adhesion assays

FM3 cells were cultured in RPMI-1640 (Lonza BioWhittaker) growth media supplemented with 1% L-glutamate (Lonza) and 10% bovine Foetal Calf Serum (FCS) extracts (HyClone). Temperature was maintained at 37 °C with a CO_2 concentration of 5%. Upon confluence, cultures were passaged or introduced onto the culture surfaces by removal of growth media, washed twice with Dulbecco's Phosphate Buffered Saline (DPBS) (Lonza BioWhittaker), and washed with 1× trypsin-versene solution (Lonza BioWhittaker). After a five min incubation 250 000 cells were introduced onto the (ultra-violet (UV) sterilised for 15 min) culture surfaces. Samples were studied in the presence and absence of foetal calf serum and the media for all assays was not changed after initiation of the assay.

Cell imaging was carried out with a Nikon Eclipse TS100 light microscope. Images were digitised with a Nikon DN100 Digital Net Camera with 0.7× magnification. Apoptotic cells were stained with 0.4% trypan blue solution (Sigma) 1 : 4 (v/v) dye to media. Cells were counted manually from microscope images (20 × 0.25 (WD 6.2) objective) segregated into a 9 × 7 grid. Counts were taken from three separate grid sections with total cells determined by multiplication. Three replicate images were used per condition and time point.

The Toxilight® Plus ATP assay (Lonza) was conducted by reconstituting the lyophilised 'adenylate kinase detection reagent' and allowing all reagents and samples to equilibrate to room temperature. From each sample 20 μL of media was added to 100 μL of reconstituted 'adenylate kinase detection reagent' and after a 15 min incubation at room temperature the Relative Light Units (RLU) intensity was read with a Berthold Detection Systems Microplate Luminometer, integration time 1 sec with three replicates taken per sample.

The Vialight® Plus ATP assay (Lonza) was conducted by reconstituting the lyophilised 'ATP monitoring reagent plus' and allowing the reagent and all samples to equilibrate to room temperature. Cells on each sample plate were lysed by incubation at room temperature for 10 min with 2.5 mL of the supplied cell lysis reagent. From each sample plate 25 μL of media was added to 100 μL of reconstituted 'ATP monitoring reagent plus', after a 2 min incubation at room temperature the RLU intensity was read in the same manner as the Toxilight® Plus ATP assay.

Cell adhesion was examined using a centrifugal assay adapted from the method described by Reyes and Garcia,[32] using an Eppendorf 5804R centrifuge with A-2-DWP rotor. Numbers of adherent cells after exposure to 0-200 RCF was assessed after a 24 h period from seeding cells to the surface by a manual count based method in the manner of the proliferation assay described above. Three replicate images were used per surface at each applied RCF in addition to three replicate surfaces.

Statistical analysis

Statistical analysis was conducted using the Genstat 11th Edition software (VSN International). Significance was assessed using an unpaired *T*-test with a confidence interval of 95%, with error represented as standard error. For proliferation, toxicity, vialight and adhesion analysis, general linear regression was applied with the assumptions of normality and constant variance assessed and a P value of <0.05 considered significant.

Results and discussion

Functionalisation and characterisation of tissue culture polystyrene

Hydrophobic tissue culture polystyrene (PS) surfaces were functionalised with polyaniline (PANI) by the polymerisation of aniline using ammonium peroxodisulfate as an oxidising agent. A green PANI film adhered due to hydrophobic interaction on the PS surface (Fig. 2A) with a characteristic absorption band at ~826 nm due to the polaron band transition of the emeraldine salt.

The position of the polaron band is sensitive to pH and with increasing pH, the band shifts towards a higher wavelength of the electromagnetic spectrum (Fig. 2A).[33,35] The rate of polymerization was pH dependent and affected the uniformity of the film (Fig. 2B and C). PANI films prepared in the presence of 1 M HCl showed uniform coatings over the exposed PS surface (Fig. 2C) compared to films formed with 0.4 M HCl which did not uniformly coat the surface (Fig. 2B).

Uniform PS–PANI films prepared using 1 M HCl were further treated with glutaric dialdehyde (GDA) and used for protein immobilisation and silica film fabrication. Lysozyme was the protein of choice as it has been previously used to generate uniform silica films.[34]

The further functionalisation of the films with GDA prior to protein adsorption was performed to keep the protein further from the surface as increased protein adsorption, with retention of

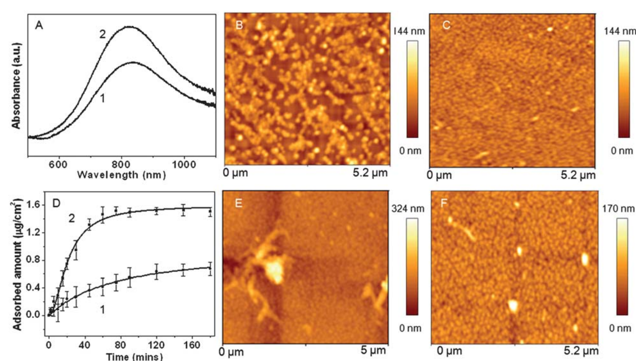

Fig. 2 (A) UV-vis spectra of the PS–PANI films prepared in the presence of 0.4 M (curve one) and 1 M (curve two) HCl. AFM images of the PS–PANI films prepared in the presence of 0.4 M (B) and 1 M (C) HCl. Lysozyme adsorption (D) on PS–PANI (curve one) and PS–PANI–GDA (curve two) surfaces with increasing time. AFM scans of PS–PANI (E) and PS–PANI–GDA (F) surfaces after lysozyme treatment.

activity had been observed for sponge silicatein, another protein able to generate silica films.[36] GDA reacts with the primary and secondary amine groups of PANI, reducing the absorbance maxima of polyaniline due to disruption of the conjugate system while introducing free aldehyde groups to react with the amine groups of lysozyme *via* covalent interaction.[37] The adsorption behaviour of lysozyme on PS–PANI is shown in Fig. 2D. Curve one shows adsorption on the PS–PANI film alone and curve two shows adsorption on the PS–PANI–GDA treated films. Adsorption on the GDA treated PANI films was ~2.5 times higher at ~1.57 ± 0.06 μg cm^{-2} and saturation was reached within one h of treatment. Lysozyme (LYZ) adsorbed uniformly on the PS–PANI–GDA surface as with the plain PS–PANI surface (Fig. 2E and F). The few structures found on the surfaces could be aggregated protein settled on the surface during adsorption.

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Growth and characterisation of silica on modified polystyrene surfaces

Silica films were formed on the PS–PANI–GDA–LYZ films either by treatment with pre-hydrolysed tetramethoxysilane (TMOS) (1 M) for 1 h using a drying control agent (5% glycerol) to eliminate cracking (–SiG, silica-glycerol film) or by reducing the initial concentration of TMOS to 0.5 M and treating for 2 h with greater control of the drying process through the use of a covered chamber, controlling humidity and airflow (–SiH, silica film). The presence of silica on both film types was confirmed by EDXa analysis with spectra showing the characteristic signature for silicon at 1.74 KeV (Fig. 3 spectra 1 and 2).

Surfaces treated with the silica precursor (1 M pre-hydrolysed TMOS for 1 h) on PS–PANI–GDA surfaces without lysozyme did not show this characteristic signal (Fig. 3 spectrum 3), indicating the importance of lysozyme in silica formation.

The most probable mechanism by which lysozyme aids in precipitating silica on the functionalised PS is by the electrostatic interaction of the positively charged protein molecules and the negatively charged silica particles.[34,38] This interaction promotes condensation of silica around the protein, which in turn leads to film formation. Analysis of the PS–PANI–GDA–LYZ–SiG surface by AFM showed a coating of interconnected silica particles (1.054 ± 0.057 μm and ~1.2 particles per nm^2) on the lysozyme treated surface with a Root Mean Square (RMS) roughness of 61.1 ± 3.3 nm (Fig. 4B), interconnected structures of silica particles (1.442 ± 0.109 μm and ~1.5 particles per nm^2) were also observed but found to be significantly larger on the PS–PANI–GDA–LYZ–SiH surface ($p < 0.05$, $n = 140$) with an RMS 75.17 ± 1.6 nm, both observations being supported by SEM analysis (Fig. 3D and E). Crack free and uniform coatings of silica, along with a few larger silica particles, likely formed in the reaction solution and settled on the silica surface were observed (Fig. 3D and 4A).

The molybdenum blue assay was performed to estimate the concentration of silicic acid consumed in the formation of the silica films. Measurements of the levels of silicic acid estimated this concentration to be 2.45 ± 0.70 mM or 0.04 mg cm^{-2} on the PS–PANI–GDA–LYZ surface. No silica was detected on the PS–PANI–GDA and untreated PS surfaces.

The roughness of the films as measured by AFM after each stage of the deposition process showed a general increase except when protein was added, where roughness decreased (Fig. 5B). This effect has been noted in previous studies with a tentative explanation of this effect being that proteins (either lysozyme or serum proteins) act as a 'filler' across the topological features of the surface.[39] In addition to roughness, the thickness of the films generally increased with the addition of each layer indicating the deposition of an increasingly irregular layer of material on the PS surface (Table 1). Thickness measurements, considering the size of lysozyme to be ~45 × 35 × 35 nm also indicate a multilayer on the surface for the PS–PANI–GDA–LYZ surface.[40]

The surface wettability of the films was assessed by measuring the water contact angle (Fig. 5A). The contact angle decreased as the surface was progressively functionalised with the lowest contact angle being measured for the –SiH films that exhibited a contact angle < 5° (super-hydrophilic) which was maintained over a period of two months. This result is in contrast to others who have shown the fabrication of super-hydrophilic surfaces using lithography and electrochemical methods, with maintenance of this property for only a few days.[27]

Wetting of a surface depends on chemical composition and the micro/nano-texture for a given chemical composition, increasing the surface roughness can render a film more hydrophilic or hydrophobic depending upon the initial wetting property of the material.[18,41,42] The high surface roughness of the –SiH film coupled with the intrinsically hydrophilic network of silanol

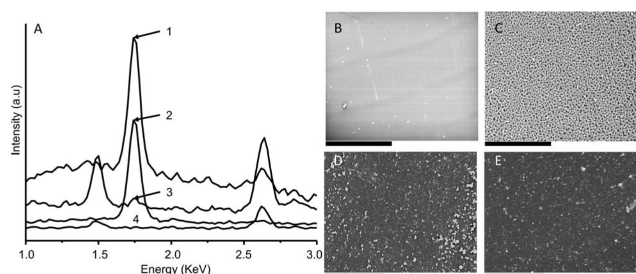

Fig. 3 (A) EDXa of –SiH (1), –SiG (2), PS–PANI (3) and PS–PANI–GDA–Silica (no lysozyme addition) surfaces (4). SEM images of PS (B), PS–PANI (C), –SiH (D) and –SiG films (E). Scale bar represents 50 μm (B), 5 μm (C) and 30 μm (D and E) respectively.

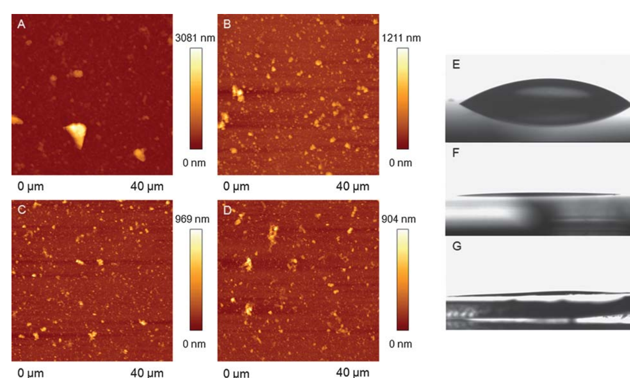

Fig. 4 AFM scans of silica films deposited on PS-PANI–GDA–LYZ (A), with glycerol (B) and the same surfaces after exposure to FCS (C and D). Images of water droplets on –SiG (E), –SiH surface after preparation (F) and after two months (G).

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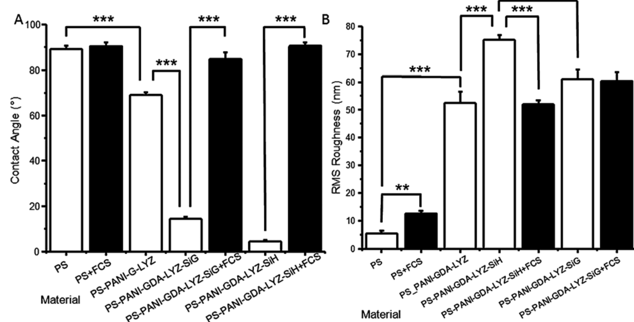

Fig. 5 Surface contact angle (A) and RMS roughness (B) of selected films before and after exposure to FCS.

Table 1 Film thickness measured at each stage of fabrication

Material	Thickness (nm)
PS–PANI	119.5 ± 4.4
PS–PANI–GDA	114.4 ± 3.8
PS–PANI–GDA–LYZ	138.3 ± 11.0
PS–PANI–GDA–LYZ–SiH	213.7 ± 20.4
PS–PANI–GDA–LYZ–SiG	192.9 ± 37.9

groups on silica particles establishes ideal conditions for super-hydrophilic behaviour.[43]

Other studies have shown that silica films fabricated on silicatein bound gold surfaces using a fast drying (N_2 gas stream) process lead to the formation of hydrophilic (wetting contact angle ~ 15°) surfaces.[34] The findings of this previous study and the results presented in this contribution point to the importance of the drying process in giving rise to the super-hydrophilic properties of the slow-dried silica surfaces. While the precise mechanism for this behaviour is not currently understood, rapid dehydration has long been known to collapse sol–gel derived structures,[44] this would decrease roughness and increase the contact angle of the material. It is also possible that the slow drying of silica films under controlled conditions leads to the packing of the silica particles on the surface in such a way that renders roughened silica films with a high level of porosity, also leading to a large number of silanol groups presented on the surface.

Proliferation and toxicity assays of adherent melanoma on hydrophilic and super-hydrophilic silica surfaces

To investigate the efficacy of our hydrophilic (–SiG) and super-hydrophilic (–SiH) silica functionalised PS we explored the behaviour of the human adherent melanoma cell line FM3. Experiments were performed in the presence and absence of foetal calf serum (FCS). From previous studies and the known properties of these surfaces we expected a negative influence on cells in comparison to the PS control.[6,23] As determined by haemocytometer 250 000 cells were plated onto the surfaces on day zero. A day after plating, cells on all surfaces exhibited changes characteristic of spreading seen after cell adhesion (Fig. 6).[45]

Over the next three to four days the cells were monitored as they progressed to confluence (Fig. 7) with proliferation monitored using optical microscopy, cellular ATP, cell death through adenylate kinase release. Contrary to expectation, the hydrophilic and super-hydrophilic nature of the silica surfaces did not appear to prevent cell growth or promote increased cell death.

Over the seven days of the experiment, cells grown with FCS progressed to confluence by day four after which the rate of growth slowed due to increasing confluence and media depletion, with a significant change in cell numbers over time ($F_{(1,819)}$ = 85.98, P < 0.001, R_2 = 0.05) (Fig. 7A and D). No significant difference was determined between PS or either of the silica surfaces. Cells grown without FCS over this period also showed no significant difference in cell proliferation between surfaces, with population growth static over the seven days and no significant increase in cell numbers. This result could be expected,

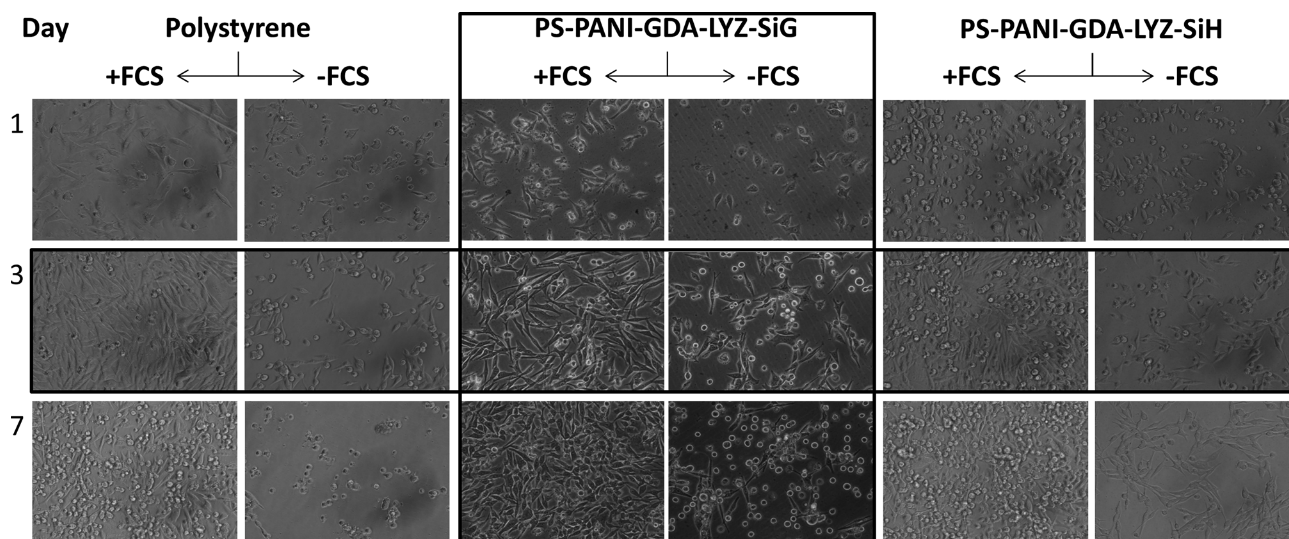

Fig. 6 Micrographs of FM3 cells taken on polystyrene, PS–PANI–GDA–LYZ–SiG (silica-glycerol) and PS–PANI–GDA–LYZ–SiH (silica) after one, three and seven days of culture for cells grown in the presence or absence of foetal calf serum.

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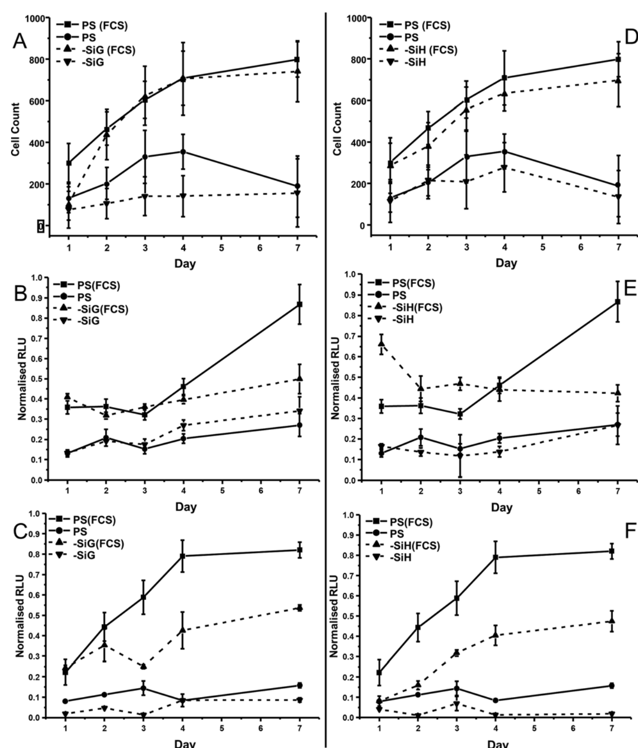

Fig. 7 Cell proliferation (A and D), cellular ATP (B and E) and adenylate kinase (C and F) assays for FM3 cells grown on PS, –SiG (A–C) and –SiH surfaces (D–F) with and without FCS.

as without FCS to supply the necessary growth factors required, cells arrest in the G1 phase.

The cellular ATP assay showed a significant ($F_{(1,388)} = 41.8$, $P < 0.001$, $R_2 = 0.09$) difference over time between cells grown with FCS (Fig. 7C and F) and those without FCS, again suggestive of a static population. Additionally a significant difference between the silica surfaces and PS was detected for both –SiH ($F_{(1,116)} = 119.2$, P 0.044, $R_2 = 0.40$) and –SiG surfaces ($F_{(1,128)} = 52.8$, P 0.012, $R_2 = 0.26$), though no significant difference was determined between the two silica surfaces themselves. This data suggests that though both silica surfaces permit cell growth, a higher rate of growth was observed on PS over this period. The discrepancy between the two assays can perhaps be explained by the higher sensitivity of the biochemical assay technique in assessing large cell populations, as compared to a microscopy based method.

The adenylate kinase assay also showed a significantly higher level of adenylate kinase in cells grown with FCS than those without (Fig. 7B and E), likely due to the higher level of cell turnover in these cultures ($F_{(1,738)} = 326.7$, $P < 0.001$, $R_2 = 0.44$). There was a significant difference in cell death between PS and –SiG ($F_{(1,128)} = 15.3$, $P < 0.001$, $R_2 = 0.08$) and –SiH ($F_{(1,131)} = 41.3$, $P < 0.001$, $R_2 = 0.21$) surfaces with FCS. There was no significant difference in cell death over time between polystyrene or silica cultures without FCS. For the polystyrene surfaces with FCS, a significantly higher rate of cell death was observed over time, indicative of a higher turnover of cells on this surface though the potential difference in cell numbers towards the end of the assay as evidenced by the ATP assay may also explain the trend.

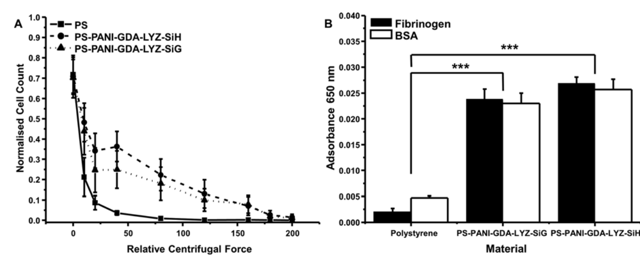

Fig. 8 Cell adhesion assay (A); measuring loss of cells from a surface with increasing RCF. (B) Adsorption of BSA and fibrinogen to PS, –SiH and –SiG surfaces.

To determine if the effects observed could have been influenced by silica leaching into the media, Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) was performed on the media after seven days of culture. The amount of the leached silica was 0.096 ± 0.020 mg L^{-1} and 0.026 ± 0.021 mg L^{-1} respectively for the –SiH and –SiG surfaces respectively with the amount from the PS control being less than 0.01 mg L^{-1}. For all of these samples, the level observed was considerably lower than the silicon content of most tap waters at ~10 to 20 ppm suggesting that the surfaces are stable under the culture conditions used and that the concentration of soluble silicon species was unlikely to be a major factor in the cell response observed over the culture period.[46]

Cell and protein adhesion to hydrophilic and super-hydrophilic silica surfaces

A modified centrifugal assay based on a prior study by Reyes and Garcia was used to assess the adhesion of FM3 cells to the –SiH, –SiG and PS surfaces after a 24 h period of culture (Fig. 8A).[32] The data show decreasing numbers of cells after exposure to increasing centrifugal force and that FM3 cells adhered least to PS, as compared to the –SiH and –SiG surfaces. The difference between the two silica surfaces and PS was significant (Chi ≤ 0.001, d.f. = 277) for –SiH, and for –SiG (Chi ≤ 0.001, d.f. = 247), though no significant difference was detected between the adhesion of FM3 to the –SiH and –SiG surfaces. With the enhanced adhesion to the silica surfaces the cells resisted centrifugal forces around two to three times higher than polystyrene, before detachment of 50% of the population and increased the population of cells which could adhere under forces up to 160 RCF. This data shows that despite comparable growth and toxicity, the silica surfaces were able to modify other aspects of cell interaction with the material. We postulate that the enhanced adhesion seen for surfaces containing silica is through the enhanced adsorption of protein to the silica surfaces.

Both nano-scale topology and chemical functionalisation of a surface affect protein adsorption,[31,47] which is itself a prerequisite for cell adhesion.[48] In this study, the adsorption of two individual serum proteins (bovine serum albumin (BSA) and fibrinogen) to the hydrophilic and super-hydrophilic surfaces was explored. Both silica surfaces significantly enhanced the adsorption of serum proteins to the surface (Fig. 8B).

We hypothesise that rather than having a direct influence on cell adhesion, the silica surfaces may enhance the adsorption of extracellular matrix and adhesion proteins from the media, which in turn influences cell adhesion and facilitates proliferation. Contact angle

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measurements made on the silica films after exposure to serum support this with the measured contact angle increasing to a value comparable to PS while the roughness of the surfaces decreased (Fig. 5A and B). This alteration of the contact angle and topology of the culture surface through the physical adsorption of proteins at the surface may be what renders the surfaces comparable as far as the cells are concerned.

The results presented above are in general agreement with other works that state that an intermediate surface wetting angle ($\sim$70°) favours the culture of cells.[6,23] However, our results demonstrate that the boundary of initial surface wettability of a surface for the successful adhesion and proliferation of cells in serum can be adjusted from $\sim$70° to below 5° without compromising the ability of cells to proliferate, at least for the FM3 cell line in the presence of serum.

We propose that the roughened nano-textured silica surfaces provide an appropriate surface topology and surface chemistry to facilitate the adhesion and proliferation of cells in the presence of FCS through the uptake of proteins to the surface.[49,50] Vetrone *et al.* have also shown that different types of nano-textured surfaces may control the extent of osteoblast cell growth.[15] These reports concur with our results in demonstrating how irregular nano-textured silica films can favour cell growth.[6] However, at present we do not have a detailed understanding at the molecular level of how the roughened silica surfaces exert their effects on cell adhesion and growth.

Conclusions

This study demonstrates a simple and reproducible method for the fabrication of hydrophilic and super-hydrophilic silica films on PS surfaces under benign conditions. Lysozyme immobilised on the surface acts as an agent to condense and fabricate uniform silica films *via* a sol–gel mechanism. The resulting silica films when dried under controlled conditions produce a super-hydrophilic surface with a contact angle < 5°. The surfaces developed were robust against cell culture conditions and time. The methods used to create them would be applicable to a wide range of applications and materials.

Adherent melanoma cells were shown to both adhere and proliferate on the silica films. Through examination of proliferation, cellular ATP and adenylate kinase, this response was seen to be comparable to that observed for tissue culture polystyrene surfaces. The performance of the super-hydrophilic and hydrophilic silica surfaces undermines conventional thinking in biomaterials design where the tissue culture surface is generally of a moderately hydrophobic nature.[6,23]

We hypothesise that the nano-textured nature and chemical functionality of the silica films, in concert with culture environment, assists in cell adhesion and proliferation through the recruitment of serum proteins. This represents an important rationale for tissue culture materials design; should surfaces be designed to emphasise indirect cell or general protein adhesion and if serum is used in culture then is a narrow view of what constitutes a viable cell culture surface valid?

The ability to vary material properties at the material/culture interface in a controlled manner may provide economical surfaces suitable for a wide range of cell related applications; for example culture systems with unique hydrodynamic properties for 3D tissue culture.[51] Though a greater level of biological understanding of how cells respond to different surfaces is currently missing, the application of modern 'omics' techniques such as transcriptomics and proteomics to well understood model systems such as those described in this contribution will increase our understanding further and advance the development of materials that can influence growth, differentiation and selection in a controlled manner through the properties they exhibit.[52]

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