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Surface Engineering of Synthetic Polymer Materials for Tissue Engineering and Regenerative Medicine Applications

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Summary

When using polymer materials as scaffolds for tissue engineering or regenerative medicine applications the initial, and often lasting, interaction between cells and the material are via surfaces. Surface engineering is an important strategy in materials fabrication to control and tailor cell interactions whilst preserving desirable bulk materials properties. Surface engineering methods have been described that can strongly influence cell adhesion, migration, proliferation, differentiation and functionality. This review aims to categorise the current strategies for modifying surface chemistry and/or topography in terms of the resultant change in cell behaviour.

1. The Requirement for Surface Engineering

A tissue-engineering scaffold serves as a template for tissue regeneration and plays an important role in cell adhesion, migration proliferation and differentiation. Many synthetic biodegradable polymers have been developed to provide a 3D environment for cell growth and tissue formation. These include poly(α-hydroxy acids) [1], poly(propylene fumarate) [2], poly(orthoester) [3], polycarbonate [4], polyurethanes [5, 6], poly3-hydroxybutyrate [7] and polyphosphazenes [8]. Some of the synthetic biodegradable polymers such as poly (α-hydroxyacids) including poly(lactic acid), poly(glycolic acid) and their copolymer poly(lactic acid-co-glycolic acid) have gained FDA approval for certain biomedical applications.

When these synthetic materials are used *in vitro* or *in vivo* they will encounter a protein rich medium that conditions the material surface [9-12]. The protein rich medium may be the cell culture medium added with cells for *in vitro* applications or the local interstitial fluid at the site of implantation for *in vivo* applications. The surface conditioning is driven by the thermodynamics of protein adsorption. The chemical properties of the material will strongly influence the final composition of the adsorbed protein layers and, in turn, this will influence the response of local cells and tissues. The choice of synthetic material to be used in a scaffold is driven by issues such as the processability of the polymer, the chemistry of bulk biodegradation, mechanical properties and logistical issues of cost, compatibility with sterilisation techniques and shelf life. These properties do not necessarily ensure that the surface properties of the final scaffold are optimal for protein and cell interaction.

Hence, there is often a requirement to change the surface properties of scaffold materials without changing bulk properties. Surface engineering strategies to enhance protein and cell interactions fall into 2 distinct categories; chemical and topographical modifications. These strategies are often inspired by the features of extracellular matrices (ECM). The contribution of nano-scale features of ECM to the modulation of cell-matrix signalling and cell behaviour is a well-known phenomenon. ECM consists of multiple proteins and polysaccharides, which act as a natural tissue scaffold. It has a crucial role in cell survival, proliferation and differentiation by providing spatial, biochemical and mechanical cues [13, 14]. ECM intrinsic factors are involved in the activation of intracellular signalling pathways through adhesion receptors, such as integrins [15-17]. Moreover, it has been well documented that physical cues and topography of the ECM orchestrate cell behaviour through a phenomenon known as cellular guidance [18-20].

Techniques and approaches to engineer the chemistry and topography of synthetic scaffolds have been reviewed by others previously [12, 21-26]. Therefore, in the next section we provide a concise summary of approaches and refer the reader to more detailed reviews and original papers for further information.

2. Summary of Surface Engineering Techniques

2.1 Surface Chemistry

Broadly the methodologies for changing surface chemistry can be divided into methods that (i) introduce new functional groups onto the scaffold polymer surfaces or (ii) coat the polymer with a thin layer of another polymer or other chemical species. Table 1 summarises example of these approaches.

The choice of method is driven by the type of polymer used to form the scaffold, the scaffold structure and final surface chemistry required. Direct chemical modification of the surface requires the chemistry of the synthetic polymer to permit the modification (e.g. to undergo hydrolysis). Coating methods are applicable to different scaffolds depending on the surface charge, solvent interaction and surface energy. A final factor to consider is the scaffold structure. Small pore networks within the scaffold can restrict access to coating materials especially in plasma polymerisation.

2.2 Surface Topography

The importance of surface topography on cell behaviour has been realised for nearly a century. According to Moore et al. [27] topographical cues were used for the first time to culture embryonic frog cells on coverslips covered by spider web [28]. Two decades later, Weiss et. al demonstrated the *in vitro* arrangement of aligned nerve fibres on rod-like fibrin [29]. Recently, the utilisation of new nanofabrication technologies have enabled further advancement of the above mentioned pioneering studies in order to investigate the effect of micro- and nanotopography on cell behaviour and function [30-32]. More recently, mathematical algorithms was used to fabricate chips of poly(lactic acid) with 2,176 different random and nonbiased topographic features to study how parameters of the mathematical algorithm correlate with cellular responses [33]. Such an approach can be applied to unravel complex and still incompletely understood interplay between cells and topographic features.

Advances in micro- and nano-scale fabrication techniques have enabled incorporation of micro- to nano-scale topographic features onto various substrate surfaces. Recent developments have come from adaptation of techniques routinely used in the production of semiconductors by the electronics industry. Photolithography was the first among these technologies which was utilised to create topographic patterns of 5-100 micrometers for stem cell research [34]. Increasing demand to create smaller length scale patterns led to the development of alternative methodologies such as electron beam lithography to create topographic features as small as 3-5 nm [35]. Lithographically established patterns can be transferred into supporting substrate through an etch process. In order to fabricate samples directly to substrates for biological experiments, Nickel shims can be prepared from master sample to replicate pattern either by hot embossing or injection moulding [36].

Nanotopographic geometries can be categorised into anisotropic topography like nanogrooved and aligned fibres or isotropic topography such as nanopillar/post and nanopit. In addition, nanofeatures can be created evenly through substrate or distributed unevenly (i.e. varying groove width) to create topographic gradients (Figure 1 & 2).

2.3 Combined Modification of Topography and Chemistry

Besides the conventional approach of varying an individual surface property (chemical or physical), combinatorial approaches have also been used to investigate the combined effect of both chemical and topographical properties of substrates [37-42]. Furthermore, the effect of orthogonal-gradients of chemical and physical modifications was studied to elucidate the combinatorial effects of both attributes on cell behaviour [43]. Although in most cases a sequential strategy have been taken to impose both modifications onto surface, more recently new methods has been developed to imposed both modifications in one step such as reactive imprint lithography (RIL) [44]. In RIL, deprotection reaction of *tert*-butyl ester groups during imprinting at elevated temperature is exploited to obtain activated and structured surface over large area which can be functionalised with established methods of surface chemistry. RIL has advantages over previously introduced methodology using a strong organic acids for wet-chemical deprotection of the *tert*-butyl ester groups, introduced by Embrechts and colleagues [45]. In addition, lower level of intramolecular anhydride formed during manufacturing of modified surfaced using RIL compare to thermolysis of free surface [44].

3. Functional Outcomes of Surface Engineering

Ultimately the wide range of surface engineering strategies highlighted in section 2 has been developed to influence cell behaviour on contact with the scaffold. In this section we have defined 4 desirable cell behaviours and review the diverse range of surface engineering approaches that have targeted each behaviour type.

3.1 Cell polarity, adhesion and migration

One of the most pronounced effects of topography is on cell polarity. The importance of cell polarity and geometry on cell function is well documented [30, 46]. Cell polarity is critical in organ development and loss of cell polarity has been associated with pathological conditions, such as cancer metastasis [47, 48]. A wide range of cell types respond to nanotopographical cues by elongating and aligning parallel to nanogrooves. Morphological responses of cells to nanopit and nanopost features are subtle and vary from reduction of spreading to constant or increased filopodia formation. Polarity of cultured cells is significantly influenced by the variety of topographical features. On denser and narrower grooves the polarity of cultured cells increased, whereas in wider grooves cells were not as polarized [49, 50].

The alteration of surface topography also influences adherence and migration of cells. In general, nanopits and nanoposts reduce cell adhesion, while enhanced adhesion can be achieved on nanograting substrates. In contrast to random trajectories of migration on flat surfaces, increased overall migration velocities are observed in the direction of the grating axis on nanogratings, while little information is available regarding cell migration on nanoposts and nanopillars (Table 2). Despite extensive knowledge regarding fundamental mechanisms of cell migration, little is known about molecular mechanism by which directional migration promoted by local 3D architectures [51]. Although recent advances suggest decreased mechanical forces through down-regulation and high turnover of a focal adhesion protein known as zyxin as the underlie mechanism of focal adhesion remodelling in human mesenchymal stem cells (hMSCs) cultured on 350nm

gratings [52], further studies must be aimed to fully elucidate mechanisms by which local 3D topography encourages directional cell migration. In addition, further studies are required to further examine the influence of feature geometry and size on adhesion.

Similarly, cell adhesion and spreading vary following the introduction of different chemical groups or combinations of physical and chemical modifications onto substrates (Table 3 & 4). Using model surfaces of self-assembled monolayers of silanes, attachment of human fibroblasts was found to be highest on amine and carboxylic acid terminated surfaces. Cell spreading was also highest on these two surfaces. In comparison, -CH3, -PEG and -OH terminated surfaces showed much lower cell attachment and spreading. The amount of protein adsorbed to the -CH3, amine and carboxylic acid presenting surfaces were similar despite the significantly lower amount of cell attachment and spreading on -CH3 surfaces [53]. In another study, the attachment of rabbit ear chondrocytes on poly(L-lactide) acid (PLLA) scaffold was significantly improved after aminolysis in 1,6-hexanediamine solution, and was furthered improved by coating with a three bilayer of chondroitin sulphate/collagen I. [54]. The aminolysis requires the immersion of PLLA scaffolds in 1,6-hexanediamine at elevated temperature, which can damage the bulk property of the scaffold. Adhesion of smooth muscle cells was also found to be increased on hydrolysed PGA mesh compared to un-treated mesh [55]. However, the degradation of PGA in sodium hydroxide solution during the hydrolysis process was significant, with a fibre diameter loss of 50% in approximately 6 minutes. Physical adsorption of fibronectin also increased cell adhesion of osteoblasts on PLGA porous scaffolds. A concentration of 200nM of fibronectin with an incubation time of 2 hours was found to be effective to promote cell adhesion [56]. This method is a simple one-step procedure; however, the stability of the adsorbed protein layer is a concern. The coverage and conformation of the adsorbed protein layer may also depend on the chemistry, surface energy and surface charge of the substrate. The attachment of MC3T3-E1 osteoblasts was found to be increased on the gelatin entrapped surface [57]. Gelatin particles were used as sacrificial materials to generate porous PLLA scaffolds. During the dissolution and leaching out stage of the gelatin particles within the PLLA scaffolds, some of the gelatin molecules are trapped within the PLLA material, as verified by ATR-FTIR and X-ray Photoelectron Spectroscopy. The entrapment requires the selection of a right solvent that can dissolve the molecules that promote cell adhesion and swells the substrate material. This requirement can hinder the application of this method to different adhesion-promoting molecules and scaffolding materials. Surface chemistry has also been reported to affect the polarity of epithelial cells. Laminin-111 coated PLGA nanofibre scaffolds have been found to promote mature tight junctions [58].

Plasma polymers with carboxylic acid and nitrogen containing functionalities are found to increase the adhesion of keratinocytes [59, 60]. These plasma polymerised polymers are formed on the top of substrates under mild temperatures which don't destroy the functionalities of the monomers. The thickness of the plasma polymer layers can be controlled within nanometer range by varying parameters such as deposition time. The diffusion of plasma polymer into pores is dependent on the pore size [61], which limits the application of this technique to scaffolds with small pores and relatively large construct sizes.

Covalently grafting of proteins and peptides offers advantage over physical adsorption in terms of improving the stability of the biomolecules. Chondrocytes from cartilage tissue of rabbit ears showed higher adhesion after 24 hours culture on PLLA with covalently tethered collagen compared to bare PLLA [62]. To graft collagen to PLLA, hydroperoxide groups were first introduced onto the PLLA surface by treating the material with UV and hydrogen peroxide. Carboxyl groups were then introduced onto the PLLA surface by grafting methacrylic acids to form PLLA-g-PMAA which was later activated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide for subsequent conjugation to collagen. This method requires the treatment of scaffolding materials in harsh chemicals and processing conditions which can damage the bulk properties and requires a multiple-step procedure including extensive washing to remove chemical residues. A similar procedure was applied to covalently graft collagen to PCL. [63].

Surface chemistry has also been employed to pattern surfaces with two distinct chemistries for the co-culture of hepatocytes and NIH 3T3-J2 cells. In this study, borosilicate wafers were first coated with a layer of photoresist, patterns were then applied to the substrate using UV through a mask, and the photoresist on these patterned areas was removed for subsequent covalent tether of collagen. The remaining photoresist was then removed in acetone by sonication. Hepatocytes selectively adhered to the collagen coated areas and NIH 3T3-J2 cells attached to the rest of borosilicate surfaces [64]. A simpler method of patterning surface has been achieved by inkjet printing collagen solutions onto (2-[methoxy(polyethyleneoxy)propyl] trichlorosilane treated glass slides. The collagen-free parts of the surfaces were then coated with poly(L-lysine) (PLL). Hepatocytes were first seeded, and adhered only to the collagen coated surfaces. Fibroblasts were then allowed to adhere to the PLL coated regions [65]. More recently, a novel micropatterning technique introduced using combination of aerosol deposition (airbrushing) of ECM proteins through microstencil and plasma polymerisation in order to create complex patterns of hydrophilic regions on poly(dimethylsiloxane) (PDMS) surface [66].

It is important to note that some surface modifications can change the bulk properties, e.g. biodegradation, of the biodegradable polymers. For example, surface modification using wet-chemical processes can lead to a faster degradation rate and reduction of mechanical performance [67]. Ozone oxidation, UV- and γ-radiation also lead to degradation of biodegradable polymers [68-70]. In contrast, plasma-assisted surface modification offers a method to incorporate functional groups on biodegradable polymers without altering the bulk properties [67].

3.2 Proliferation

Nanotopography has been shown to affect other cellular functions including self-renewal and proliferation (Table 5). Several studies have been conducted to evaluate the effect of nanotopography on proliferation of various cell types including mouse embryonic stem cells (mESC) [71], human embryonic stem cells (hESC) [72] and endothelial cells [73]. Enhancement of self-renewal and proliferation was observed in mouse embryonic stem cells cultured on a nanofibrilar scaffold in comparison with a tissue culture plastic surface in the presence of leukemia inhibitory factor. Enhanced expression of Nanog and activation of small GTPase RAC and

phosphoinositide 3-kinase were suggested as molecular mechanisms for higher self-renewal of mESC cultured on nanofibrilar scaffold in comparison to flat surface [71]. In another study, a significant enhancement of proliferation was observed in surfaces with optimal groove width and wettability following combinatorial modifications of surfaces [43].

In a study by Schernthaner and colleagues, nuclear accumulation of β-catenin and activation of specific β-catenin target genes was suggested as the mechanism responsible for higher rates of proliferation in endothelial cells (ECs) cultured on a polymer culture substrate with laser-generated nanopatterns [73].

It has been shown that chemically modified polyacrylates and polystyrene surfaces can support stem cell self-renewal. Self-renewal of embryonic stem cells in defined medium was found to be similar on carboxylic acid containing acrylate surfaces tethered with peptides from vitronectin and bone sialoprotein and on Matrigel [74]. Both oxygen plasma and UV/ozone treatment were found to be able to modify a polystyrene surface to support self-renewal of human embryonic stem cells [75, 76].

High throughput methods have been developed to screen large numbers of different materials and their surface chemistries [77, 78]. Some polyacrylates after coating with serum have been identified to support self-renewal of human embryonic stem cells [78]. Certain integrins binding proteins, such as vitronectin, have been found to play an important role in controlling stem cell fate.

3.3. Differentiation

Nanotopography can be utilized to promote the differentiation of cells into various lineages (summarised in Table 6) as demonstrated in case of ESCs [79] and hMSCs [32, 80].

Nanotopographical features have been used to direct hMSCs differentiation particularly into an osteogenic lineage [32, 81-83]. Induction of osteogenic differentiation has been achieved by long-term culture of hMSCs on varying degree of disordered nanopits made from poly(methylmethylacrylate) (PMMA) in the absence of inducing signals, suggesting physical nanostructures might be sufficient to induce differentiation [32]; However, enhanced osteogenic differentiation of hMSCs was achieved following induction of cultured hMSCs on surfaces with nanofeatures in combination with osteogenic medium suggesting optimal condition can be achieved by using combination of both physical cues and soluble factors [83]. Work by Zouani et al. suggests that not only the width but also the depth of nanopattern is important to elicit differentiation [84]. These studies demonstrated that surface topography can be used to directly bias cell fate decision.

Nanotopographic induction of differentiation toward other fates has also been investigated such as myogenic [85] and neurogenic differentiation [80, 86, 87]. Higher differentiation tendency toward neural lineages have been demonstrated in embryonic or adult stem cells cultured on engineered surfaces in various studies. One such example is the differentiation of hMSCs into neuronal-like cells following culture on nanograting of 350 nm width [80]. Similar to You and colleagues' observation [83], further enhancement of differentiation was achieved by the synergistic effect of both nanotopography and biochemical cues such as retinoic acid [80]. In

addition, enhanced neuronal differentiation have been reported in neuronal progenitors when co-cultured with astrocytes on micropatterns larger than 10 µm [88, 89] and electrospun polyamide nanofibers [90] due to synergistic effect of soluble factors released by astrocytes. Although the molecular mechanism driving differentiation by nanotopography is largely unknown, recent studies suggested involvement of integrin-activated focal adhesion kinase [91, 92].

Nanotopographic features have also been investigated to encourage formation of multicellular structures with enhanced functionality. The importance of nanoscale cues in directing organization and function has been shown in human endothelial progenitor cells (hEPCs) cultured on both planar and nanograting substrates where hEPCs cultured on nanograting substrate formed multicellular band structures while cells cultured on planar substrate formed confluent monolayers [93]. In a more recent study, it was demonstrated that endothelial cells (ECs) were aligned on both nanofibrilar and micropatterend channels, down-regulated adhesion proteins and chemokines and reduced adhesiveness to monocytes and platelets [94]. Using a combination of electron beam and photolithography, a 3 layer tubular scaffold with a coaxial arrangement were fabricated which can be used as a vascular tissue engineering scaffold [95].

Since contractile property of cardiac tissue is directly related to cellular elongation and orientation, formation of anisotropic myocardium has also been investigated through various topographic features. In a pioneering study, faster propagation of action potential was observed in neonatal rat ventricular myocytes cultured on abraded microchannels on a poly(vinyl) chloride substrate [96]. In another study, the effect of microtopography on intracellular calcium dynamics was investigated on cardiomyocytes cultured on a PDMS substrate with trapezoidal grooves with a depth of 50 µm and 120 µm spacing between adjacent triangular ridges [97]. Increased diastolic and systolic intracellular calcium following electrical stimulation at higher and all stimulation frequencies, respectively, demonstrated that microstructure can directly influence cardiomyocytes intracellular calcium dynamics. To further investigate the influence of topography on functionality of cardyomyocytes, contractile forces generated by cardiomyocytes cultured on flat and a 10 µm-wide microcantilevers was measured in another study [98]. Cells cultured on microcantilevers showed anisotropic actin organisation and had 65-85% higher contractile forces compared with flat surface. In addition, it was shown that expression of junctional markers such as N-cadherin and connexion-43 upregulated in presence of certain arrangements of micropillars, further suggesting enhancement of cardiomyocytes functionality by surface topography [99].

Neural tissue engineering is another area that topographical guidance have been utilise to further enhance functionality. Several approaches have been taken to fabricate conduits containing microchannels using various materials including PLGA and PCL [100, 101]. Moore et al. fabricated PLGA conduits with distinct channels running parallel along the length of the scaffold using injection moulding with rapid solvent evaporation [102]. Krych et al. demonstrated that greater regeneration can be achieved following implantation of Schwann cell-seeded PLGA conduits with 450 µm over 600 µm diameter microchannels [103]. Rutkowski and colleagues also demonstrated that improved functionality can be achieved following introduction of

grooves within lumens with a width of 10 µm, depth of 4.3 µm and spacing of 10 µm in rats with 1 cm sciatic nerve transections [104].

It has been demonstrated that differentiation potential can be altered following chemical modification of surfaces (Table 7). The differentiation of bone marrow derived mesenchymal stem cells was studied on a range of silane modified surfaces. -CH3 surfaces maintained the MSC phenotype. -NH_2 and -SH modified surfaces promoted and maintained osteogenesis both in the presence and absence of biological stimuli [105]. Human mesenchymal stem cells were cultured on polystyrene surfaces modified with photoreactive azidophenyl derivatives of three different chargeable polymers: poly(acrylic acid), polyallylamine and poly(ethylene glycol). The polyallylamine surface supported cell adhesion and proliferation and also promoted chondrogenic differentiation [106]. The synthesis of sulphated glycosaminoglycan from bovine chondrocytes have been found to be highest on cationic PLGA microcarriers coated with PLGA-g-poly(L-lysine) graft copolymers compared with on hydrophobic and negatively charged PLGA, respectively [107]. In addition, functional groups have been tethered to induce differentiation of human mesenchymal stem cells. Primary amine, t-butyl, phosphate, tetrafluorobutyl and methacrylic acid functional groups containing methacrylates were incorporated into poly(ethylene glycol) dimethacrylate at sufficiently low concentration to study the effect of these small molecules on the differentiation of mesenchymal stem cells. Protein and gene expression altered by these molecules were analysed for stem cells cultured in the gels [108].

Table 8 summarises reports in which topography and chemistry were both controlled with the aim of controlling cell differentation. For example Lie et al demonstrated that not only adhesion of MC3T3-E1 osteoblasts was enhanced on nano-fibrous gelatin scaffolds with *in situ* formed apatite, enhanced proliferation and differentiation were also observed following incorporation of apatite compared with nano-fibrous gelatine alone [109].

Prospective

This review has attempted to consider the role of surface engineering from the perspective of the functional outcomes in terms of changes in cell response. It is apparent that cell polarity, adhesion, proliferation and differentiation can be influenced by a wide range of surface properties. The breadth of available surface engineering techniques should be beneficial for clinical translation because these cell responses can be achieved on virtually any bulk material and for many tissue types.

In summarising the current literature it is apparent that there is a shortage of studies on the combined effects of chemistry and topography. In the body, most cells interact with surfaces arranged within a 3D architecture. The relationship between multiple surface-to-cell interactions and 3D space are essential in determining tissue patterning, repair or regeneration. The combination of topographical and chemical changes opens up a huge design space for new surfaces. This, in turn, requires high throughput and perhaps combinatorial mechanisms of creating surfaces and analysing their interactions with cells.

More mechanistic studies are required to shed light on the response of cells to surface properties. In most studies we measure single (or a small selection) of desirable functional outcomes. These outcomes can be achieved via multiple cellular pathways and understanding, and possibly controlling, these pathways through combined chemistry and topography will more closely mimic the role of the ECM.

Surface engineering methods should be attractive for clinical translation of synthetic scaffolds. The technologies reviewed here are generally inexpensive to use and from a regulatory perspective are scalable and easy to quantity control. Separating bulk and surface properties should allow enhancements in cell and tissue interaction without the need to redesign bulk properties.

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Figure 1: Schematic illustration of commonly used topographic features as cell culture substrate. Geometries can be divided into two categories of isotropic and anisotropic. Isotropic geometries are uniform in all directions including nanoposts/pillars (A) and nanopits (B). Anisotropic geometries like nanogrooves (C) are directionally dependant and provide cues along a single axis. Cues can be provided through topographic gradient along a particular axis (D). Schematics are not drawn to scale.

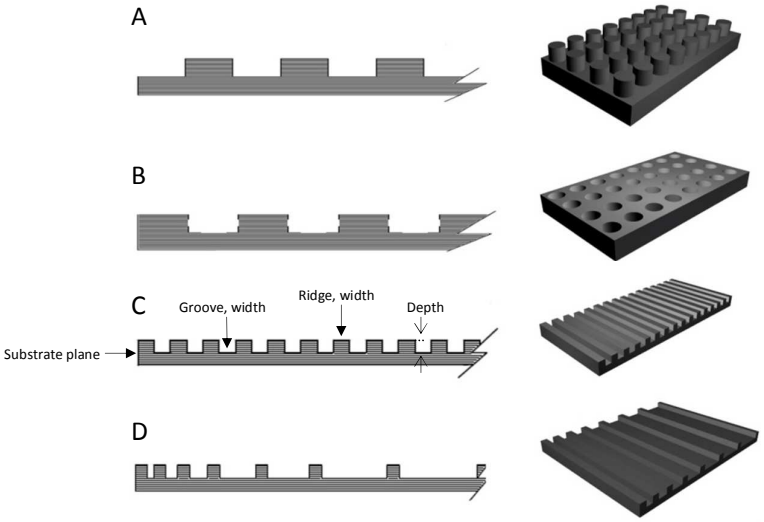

Figure 2: Scanning electron microscopy of nanopites with hexagonal, square, near square order with a random displacement of ±50 nm and random arrangements, respectively. Reprinted by permission from Macmillan Publishers Ltd: Nature Materials [32].

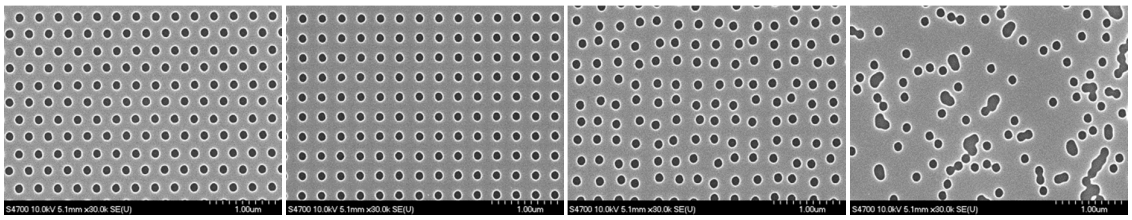

Table 1: Summary of Methods of Modifying Surface Chemistry

Technique	Summary of approach	Surface Chemistry Modification	References
Introduce new functional groups onto scaffold polymer surface			
Surface hydrolysis	Soaking of polymer scaffold in an acidic or alkaline solution.	Hydrolysed ester groups to form carboxylic acid and hydroxyl groups.	[55]
Oxygen plasma etching	Etch the substrate material with oxygen plasma to introduce functional groups.	Presentation of oxygen containing functional groups.	[110]
UV/Ozone treatment	Oxidise the substrate material with UV/Ozone at ambient conditions.	Presentation of oxygen and nitrogen containing functional groups.	[75]
Covalent grafting of peptides	Graft peptides onto scaffolding materials using coupling reactions such as reactions involving carbodiimide. If function groups for coupling reaction are not present on the polymer, then it will need to be modified to introduce functional groups for subsequent grafting.	Presentation of grafted peptides.	[74, 111]
Coating with thin layer of another polymer or chemical species			
Plasma polymerisation	Polymerisation of monomer vapours under mild temperatures on the substrate scaffolding materials for retaining the functional groups of the monomers.	Presentation of the functional groups of the plasma polymers.	[60, 112, 113]
Physical adsorption	Immersing scaffolding materials in protein or peptide grafted polymer solutions. The adsorbed layer physically attaches to the scaffolding material.	Presentation of the physically adsorbed layer.	[56, 114]
Surface entrapment	A region of the material close to and including the surface is swollen by a partial solvent. The surface modifying agent s dissolved in the partial solvent. When the solvent is removed the modifying agent is trapped at the surface	Presentation of biomolecules or polymers containing cell adhesion peptides.	[57, 115]
Layer by layer assembly	The substrate is first modified with a charged layer; another layer with opposite charge is then applied onto the first layer. Thicker coatings can be achieved by repeating the process.	Presentation of self-assembled bilayers.	[54, 116]
In situ apatite formation	Apatite is formed on the scaffolding materials by soaking the scaffold in simulated body fluids.	Surface formed apatite.	[109, 117]

Table 2: Alteration of cell adhesion following physical modification.

Cell type	Substrate Material	Physical Modification	Functional outcome	References
bPASMCs	PDMS	Microposts 2-10 μm in diameter 3-50 μm in height	cells adhesion, spread across and deflected multiple posts	[31]
hCECs	Si	Nanogrooves 70 nm width 600 nm depth	Elongation and alignment along micrometer- and nanometer-sized grooves and ridges	[118, 119]
hECs	PDMS	Nanogrooves 1200 nm width 600 nm depth	Elongation along ridges, formation of well-defined capillary tubes following induction by Matrigel	[93]
NIH 3T3 fibroblast	PUA	Gradient Microgrooves 1-9.1 μm	Enhanced adherence to denser features; alignment, elongation and bias migration along the direction of ridges	[50]

(PDMS, poly(dimethylsiloxane); Si, Silicon; PUA, poly(urethane acrylate);)

Table 3: Alteration of cell adhesion following chemical modification.

Cell type	Substrate Material	Chemical Modification	Functional outcome	References
bVSMCs	PGA	Surface hydrolysis, Hydrolyse ester groups and form carboxylic acid and hydroxyl groups	Increased cell adhesion and seeding density	[55]
Rat osteosarcoma cell line	TCPS	Plasma copolymerisation of acrylic acid and 1,7 octadiene	Improved cell adhesion to plasma copolymer surface	[112]
Human keratonocytes	TCPS	Plasma copolymerisation of acrylic acid/1,7 octadiene and allyl amine/1,7 octadiene	Improved adhesion of keratinocytes on acrylic acid/1,7 octadiene with low concentration of carboxylic acid groups in similar level to collagen-I	[60]
Human osteoprogenitor	PDLLA & PLGA	Physical adsorption of RGD-PLL or fibronectin to PDLLA substrate	Enhanced adhesion and spreading following both modifications, successful osteogenic differentiation into mature osteogenic phenotype	[56]
bovine aortic endothelial cells	PDLLA	Adsorption of PLL-GRGDS	Increased in spreading, inhibition of spreading at high concentration of PLL-GRGDS	[114]
Human Fibroblast	Glass or silicon	Self-assembled monolayers, Silanisation of glass or slicon surfaces with silanes terminated with CH_3, Br, $CH=CH_2$ or PEG.	Strong adhesion, spreading, fibronectin formation and growth and enhanced activity of integrins on -COOH and $-NH_2$ terminated surface, weak interaction with $-CH_3$, -PEG and -OH	[53]
MC3T3-E1 osteoblasts	PLA	Surface entrapment of gelatin	Increased hydrophilicity following gelatine entrapment, Significant enhancement of cell adhesion and proliferation	[57]
3T3 fibroblast	Glass	Plasma polymerisation of allyl amine and hexane	Increased cell even distribution throughout the core and the sheath of millimetre-scale size scaffolds	[113]
NIH 3T3 fibroblasts, human megakaryocytic M07e	Glass, gold, titanium oxide, various polymers such as PTFE and PS	Covalent bond to inorganic oxides and amine containing organic surfaces.	Water contact angle change to similar values after coating various substrates with dopamine, Significant attachment of fibroblasts after coating various substrate with dopamine and PEG-SH	[120]
SaOS-2 osteosarcoma cell line	Gelatin/bioglass composite	*In situ* apatite formation	Enhanced attachment and secretion of ECM	[117]

(AHDCS: adult human-derived corneal stromal cells; bVSMCs, bovine vascular smooth muscle cells; HBC, hydroxylbutyl chitosan; hCECs, human corneal ephithelial cells; hECs, human endothelial cells; PAA: poly(acrylamide); poly(methyl mathacrylate): PMMA; ppAAm: plasma polymerised allylamine; TCPS, tissue culture poly(styrene).

Table 4: Combinatorial effect of chemical and physical modifications on cell adhesion.

Cell type	Substrate Material	Chemical Modification	Physical Modification	Functional outcome	References
Rat hippocampal neurons	Glass	Adsorption of PLL	Microgrooves 20-40 & 50-100 µm width 5 µm depth	Effective guidance of neuritis outgrowth and number	[37]
MC3T3-E1 S14 Osteoblasts	PHBV	Adsorption of fibronection or immobilisation of alkaline phosphatase	Microgrooves 1-10 µm width 10-30 µm depth & micropites 4 µm width 5 µm depth	Improved cell adhesion and alignment	[38]
Human keratinocyte	PET	Plasma deposition of acrylic acid (cell adhesive) and poly(ethylene oxide (cell repulsive)	Conical nanoposts 117±5 nm height	Improved cell adhesion	[39]
Human umbilical vein endothelial cells	PET	Plasma deposition of acetaldehyde	Aligned fibres 100 µm diameter	promotion of cell attachment and spreading, formation of focal adhesion	[41]
rHPN	Glass	Adsorption of PLL	Microgrooved pattern 2 & 15 µm width 1 µm depth	promotion of specifically polarized morphology by guidance cue pattern	[42]

(PLL: poly-L-lysine; PHBV: Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate); PET: Poly(ethyleneterephalate); rHPN, Rat hippocampal pyramid neurons;)

Table 5: The effect of surface modification on self-renewal of ESCs and proliferation of stem cells.

Cell type	Material	Chemical modification	Physical modification	Functional outcome	References
mESCs	Polyamide	-	Randomly oriented nanofibres 280 nm average diameter	promotion of proliferation and self-renewal of mESCs through Rac, PI3K/AKT signalling	[71]
hMSCs	Glass	Self-assembled monolayers, Silanisation of the surface.	-	The –CH3 surfaces maintained the hMSC phenotype. The –NH2 and –SH-modified surfaces promoted and maintained osteogenesis both in the presence and absence of biological stimuli. The –OH and –COOH-modified surfaces promoted and maintained chondrogenesis under both basal and chondrogenic stimulated conditions.	[105]
3T3 dermal fibroblast	PMMA	Plasma deposition (gradient of ppAAm)	Gradient of microgrooves 5-95 µm width 3 µm depth	Significant increase in cell proliferation in area with optimal groove width and wetability	[43]
rNSC	PES	-	Nanofibres 273±45 to 1452±312 nm diameter	lower proliferation compared to cells cultured on laminin-coated 2D surface in the presence of bFGF, lower degree of cell aggregation and higher degree of proliferation and cell spreading as the fibre diameter decreased	[86]
hESCs	PAS	Covalent conjugation	-	Supporting self-renewal in chemically-defined, xeno-free medium comparable to that on Matrigel™, retain of normal karyotype	[74]
hESCs	PS	Oxygen plasma etching	-	Maintenance of self-renewal and stable karyotype comparable to that on Matrigel™, multi-germ layer *in vitro* differentiation	[76]
hESCs & hiPSCs	PS	UV/Ozone treatment	-	Maintenance of self-renewal at optimised UV dose comparable to that on Matrigel™	[75]
hMECs	PET	-	Ripples & Walls 300 nm & 1.5 µm	Induced proliferation as a result of nuclear accumulation of β-catenin	[73]
HUVECs	PCL	Aminolysis and covalent grafting of collagen	-	Significantly improved cell adhesion and proliferation	[63]
hESCs	Polystyrene	-	Nanopillars 50-400 nm height	Maintenance of Oct4 expression in absence of bFGF, downregulation of Oct4 in presence of bFGF in honeycomb configuration	[72]

(bFGF, basic fibroblast growth factor; hiPSCs, human induced pluripotency stem cells; hMECs, human microvascular endothelial cells; HUVEC, human umbilical vein endothelial cells; mESCs, mouse embryonic stem cells; PAS, peptide-acrylate surfaces; PCL, poly(caprolactone); PES, poly(ethersulfone); PET: Poly(ethyleneterephalate); PMMA, poly(methyl mathacrylate; ppAAm: plasma polymerised allylamine; PS, spin-cast thin polystyrene; rNSC, rat neural stem cells; TCPS, tissue culture poly(styrene).

Table 6: Induction of differentiation following physical modification of surface.

Cell type	Material	Physical modification	Functional outcome	References
hMSCs & osteoprogenitors	PMMA	Nanopits 120 nm diameter 100 nm depth	Stimulation of hMSC osteogenic differentiation on disordered nanoscale features in absence of osteogenic supplements	[32]
hMSCs	HBC & HBC/collagen	Aligned nanofibres 200-900 nm average diameter	Enhanced alignment, upregulation of myogenic gene markers	[85]
hMSCs	PDMS	Nano- and microgratings, 350 nm, 1 or 10 µm width 350 nm depth	Significant up-regulation of neuronal markers compared to micropatterned and unpatterned, further enhancement of differentiation in presence of biochemical cues such as retinoic acid	[80]
mESC	PCL	Aligned nanofibres 250 nm average diameter	Neural differentiation of mESCs seeded directly onto PCL nanofibres, minimal astrocytic differentiation.	[121]
hMSCs	PUA	Nanoposts, 150, 400 & 600 nm diameter Nanogratings, 150, 400 & 600 width	Higher ALP activity and higher expression of osteogenic markers in cells cultured on patterned surface compared to unpatterned PUA in presence of osteogenic medium	[83]
hESCs	PDMS	Nanopillars, 35-400 nm diameter	Enhanced neuronal yield by increasing pillar height from 25-400 nm, ~80% neuronal differentiation on higher pillar height in first 96 h in absence of biochemical factors	[87]
Human primary osteoblast	PCL	Micropits, 300 nm depth 20, 30 & 40 µm diameter	Osteogenic differentiation with most pronounce effect in 30 µm pits	[122]
hMSCs	PDMS	Nanogratings, 250 nm depth 250 nm width	Up-regulation of neurogenic and myogenic differentiation markers in hMSCs cultured on nanograting compared to microgratings and unpatterned	[92]
hESCs	PCL	Nanopits, 100 nm depth 120 nm diameter	Enhanced mesodermal differentiation in comparison with planar surface	[79]

(HBC, hydroxylbutyl chitosan;)

Table 7: Induction of differentiation following chemical modification of surface.

Cell type	Material	Chemical modification	Functional outcome	References
mMyoblasts	Alginate	Covalent coupling	Adherence, proliferation, fusion and expression of heavy-chain myosin (a differentiation marker) following GRGDY modification of alginate surface	[123]
Human osteoprogenitor	PDLLA & PLGA	Physical adsorption of RGD-Poly(L-lysine) or fibronectin to PDLLA substrate	Enhanced adhesion and spreading following both modifications, successful osteogenic differentiation into mature osteogenic phenotype	[56]
hECs	PLLA	Layer by layer assembly	Increased adhesion, proliferation and secretion of von Willebrand factor	[116]
hMSCs	Glass	Surface entrapment	$-NH_2$ and –SH-midified surfaces promoted and maintained osteogenesis, chondrogenic differentiation on $-NH_2$-modified surface in presence of chondrogenic medium but not on –SH-modified surface, control and $–CH_3$-modified surface maintained MSC phenotype but lack differentiation stimuli	[105]
Rabbit ear chondrocytes	PLLA	Layer by layer assembly of chondroitin sulphate and collagen type-I onto PLLA	Improved cell attachment, proliferation, cytoviability and GAG secretion following introduction of chondroitin sulphate and collagen type I onto PLLA	[54]
hMSCs	PAAc, PAAm & PEG	Physical coating	Negatively charged surface supported adhesion and proliferation while positively charged PAAm supported cell adhesion, proliferation and differentiation, enhanced chondrogenic differentiation on PEG and PAAm-modified surface	[106]

(PAAc, poly(acrylic acid); PAAm, poly(allylamine); PEG, poly(ethylene glycol)

Table 8: Induction of differentiation following combinatorial modification of surface.

Cell type	Material	Chemical modification	Physical modification	Functional outcome	References
MC3T3-E1 osteoblasts	Gelatine	*in situ* apatite formation	Nanofibre	Enhanced cell adhesion and proliferation, higher mechanical strength and enhanced osteoblastic differentiation following incorporation of apatite	[109]
PC12	PAA	Adsorption of BSA	Microwells (10 µm in diameter) connected by 1 µm microchannels	Selective attachment, growth and differentiation, control over number of neuritis outgrowth	[40]
hMSCs	PET	Covalent immobilisation of –RGD peptide	Nanopits, 10-100 nm depth	Promotion of adhesion without noticeable differentiation on 10nm, induced differentiation into osteoblast-like cells on 100 nm features	[84]

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