



Nanotopography – potential relevance in the stem cell niche

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Nanotopography – potential relevance in the stem cell niche

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Abstract

In this review general properties of the stem cell niche are initially described, using intestinal and bone marrow niches as examples. Understanding signals in the niche that regulate stem cell behaviours is important for applications such as tissue engineering, and limitations in *ex vivo/in vitro* recapitulation of some stem cell niches, particularly the bone marrow, have led researchers to attempt to delineate signalling mechanisms present *in vivo* using a reductionist approach. This is especially important as 'stemness' is not solely an intrinsic property of stem cells but a result of the reciprocal interactions between stem cells and their niches¹. Physical stimuli such as mechanical stiffness and topography are known to significantly impact stem cell behaviours; being translated through adhesions, intracellular tension and mechanotransduction, which can alter gene expression and thus cell fate. This review focuses on the lesser-described physical stimuli of nanotopography and the mechanisms by which stem cells respond and interact with it. Specific examples of nanotopographical influence over stem cell differentiation are highlighted and parallels drawn between the stem cell niche and these 'synthetic' *in vitro* observations. Ultimately if the complex stem cell niche is to be mimicked *in vitro* or stem cells exploited for medical applications the physical microenvironment, including nanotopography, must be optimised.

Biographies



Lesley-Anne Turner

Lesley-Anne is currently a post-doctoral researcher in The Centre for Cell Engineering at The University of Glasgow. As an undergraduate student she studied Biomedical Sciences and in 2012 she completed a PhD in Biomedical Materials Science at The University of Manchester. During her post-graduate training she developed an interest in collaborative research and as such completed her cross-disciplinary thesis (covering materials science, electronic engineering and biology) titled 'Large area patterning techniques for the characterization of nerve and retinal cell responses to nano/micro scale topographies'. Her current interests include investigating the effects of nano topography on mesenchymal stem cell differentiation, with a focus on the metabolome.



Matthew J Dalby

Matt started life in Glasgow as a PDRA on the EU grant Nanomed after completing a PhD in biomedical materials at Queen Mary, University of London in 2000. In 2003 he became a BBSRC David Phillips Fellow, a lecturer in 2008, a Reader in 2010 and Chair in Cell Engineering in 2014. His research focuses on cell interactions at the nanoscale with particular interest in influencing mesenchymal stem cell growth and differentiation. He is academic secretary of the Glasgow Orthopaedic Research Initiative (GLORI).

Main article

Introduction

The stem cell niche² can be considered as a basic unit of tissue physiology³, which both defines the specific anatomical location in which a stem cell resides and confers functionality (regulating stem cell survival, self renewal and differentiation)⁴. Conversely, the attributes that enable stem cells to be so fundamental for tissue generation and repair also prime them for undesirable effects such as unregulated proliferation resulting in tumour formation. Thus, in the body fine control of stem cells through the niche is critical for tissue homeostasis; the niche provides a structured environment that protects the stem cell from the body (protection from depletion or undesired signalling) and the body from stem cells (providing an environment that precisely controls stem cell self renewal in order to avoid tumourigenesis). In the niche stem cells are maintained in a metabolically quiescent state, which protects them from DNA damage, until they are required at which point they produce progenitor/transit amplifying cells that go forward to regenerate damaged tissue⁵. This niche control over progenitor production is in response to the integration of complex, dynamic signals from surrounding tissue, enabling the niche to respond to the needs of the organism in a balanced manner. This specialised niche function, of precisely controlling stem cell self-renewal whilst enabling differentiation when required, is mediated by a specific microenvironment. The niche microenvironment is composed of cell and non-cellular elements that present chemical and physical cues in order to facilitate stem cells central roles in tissue generation and repair (Figure 1). Understanding these cues will enable us to characterise the stem cell niche, which is essential if we are to exploit stem cells for therapeutic and research use, e.g. in regenerative medicine.

1. The stem cell niche

In humans somatic niches, which are required for tissue repair and are thought to be necessary to ensure stem cell longevity and multipotency, are found in many tissues including: muscle (satellite stem cells located beneath muscle fibre basal lamina), brain (hippocampus), skin (bulge of hair follicle), intestines (epithelium) and bone marrow (putatively the sinusoidal surface). The later two are the most well described and investigated in humans, and thus will be used as the main examples in this review. They can be considered as examples of 2D and 3D niches - intestinal stem cells are exposed to a basal lamina on one side and lumen on the other (2D), and bone marrow stem cells encompassed by extracellular matrix (ECM)/cells on all sides (3D). Although there is heterogeneity both between niches in different tissues and also within the same type of niche depending on functional state (e.g. homeostasis, regeneration, development), similarities between different niches in humans as well as other organisms are thought to exist^{1,6,7}, including:

1. Niches contain specialised cells (be that niche cells of different lineages and/or stem cell progeny) and are found in specific anatomical locations (both of which differ depending on stem cell type).
2. The niche functions as a physical anchor for stem cells, which may be through cell-cell or cell-ECM adhesions.
3. The niche appears to function to regulate stem cells and orchestrate their behaviour, be that quiescence, self-renewal or differentiation, in response to signals from the body.
4. The niche is a dynamic structure that integrates cues from potentially many sources, e.g. ECM, chemical factors, metabolic cues, mechanical stimuli, architectural constraints, cell-cell contacts, and the stem cells themselves.
5. Blood vessels are often found near niches, which potentially enable the transport of long range signals and enable recruitment/egress of cells.

The intestinal niche

One of the most well defined stem-cell niches is the intestinal niche, where adult intestinal stem cells enable the epithelial lining of the intestine to renew and repair daily throughout life in response to the aggressive luminal environment, where in humans up to 10^{11} epithelial cells are lost every day⁸. This extensive cell renewal arises from relatively small populations of leucine-rich repeat-containing heterotrimeric guanine nucleotide-binding protein-coupled receptor 5 (LGR5+) stem cells. These LGR5+ stem cells reside in protected niches formed by crypts at villi bases (Figure 2). During tissue renewal transit amplifying cells proliferate upwards towards the top of crypts, where they differentiate into epithelial cells. Thus, the intestinal crypt niche enables LGR5+ stem cells to indefinitely self-renew whilst at the same time regenerating functional epithelia. Although a complex mechanism that is incompletely understood, a number of components in the niche that regulate this bi-directional behaviour have been described. Paneth cells that neighbour LGR5+ stem cells secrete niche specific factors (e.g. epidermal growth factor, WNT3A and notch ligand) and are thought to be essential for the translation of tissue/body factors into signals for stem cells (e.g. calorie restriction induces Paneth cells to reduce mTOR complex1 signalling, which in turn reduces the size of the stem cell pool). The ECM component laminin is thought to be important in the intestinal niche; along the crypt-villus axis variations in laminin composition in the basement membrane are thought to play a role in establishing and maintaining stem cell distribution⁹.

The bone marrow niche

In contrast to the intestinal stem cell niche, the bone marrow supports two stem cell types – mesenchymal stem cells (MSCs) and haematopoietic stem cells (HSC), which have a co-dependant relationship. This niche is less well characterised and far less linear than the simpler intestinal niche and as such a number of competing models have developed to describe it¹⁰. MSCs supply the local tissues with skeletal cells (fat, bone, cartilage, reticular and HSC supporting)¹¹. It should be noted that MSCs can be derived from other tissues (e.g. fat, umbilical cord and dental tissue) and thus have other niches, and furthermore the term MSC is a contested one. A perivascular location for MSCs is hypothesised (suggesting crossover with pericytes¹²), however as MSCs are also extractable from

non-vascularised niche tissue (e.g. cartilage) this may indicate different populations of MSCs. HSCs replace more than 500 billion blood cells everyday (including platelets and red, myeloid and lymphoid cells). In the bone marrow, HSC numbers are kept fairly constant except in times of haematopoietic stress when HSCs are able to mobilise and move to extramedullary sites. During development, the HSC niche is found in diverse tissues including the liver where the number of HSCs expands daily. The MSC/HSCs niche is perivascular and often, although not always, located near trabecular bone^{10, 13} (Figure 2). Endothelial cells (which share a common lineage with haematopoietic cells) are thought to make up the cellular element of the niche and synthesise multiple factors that promote HSC maintenance and localisation (including stem cell factor and C-X-C motif chemokine). There is a great deal of evidence that other cell types are important indirect regulators of the marrow niche, including: sympathetic neurons that confer a circadian rhythm of HSC retention and mobilisation¹⁴, non-myelinating Schwann cells, osteoclasts, monocytes/macrophages and haematopoietic cells^{15, 16}.

In addition to the sinusoidal niche, the endosteum has previously been proposed as a bone marrow MSC/HSC niche. However, research now suggests that rather than comprising an integral part of the niche, i.e. being niche constituent cells, osteoblastic and osteolineage cells of the endosteum are thought to contribute to the HSC niche as indirect regulatory components¹⁷⁻¹⁹. That said, it should be noted that HSCs are predominantly found in the trabecular region of bone marrow and the endosteal region is highly vascularised, suggesting a degree of overlap.

2. Signalling in the stem cell niche

The impact and control that stem cell niche signalling has over stem cell behaviour is exemplified by experiments carried out on *Drosophila*^{20, 21} and in mice²² whereby committed daughter cells can resort to stem cells (dedifferentiate) if returned to the niche – suggesting self-renewal and multipotency control by the niche rather than intrinsic cell control. It therefore follows that stemness is considered as not solely an intrinsic property of stem cells but a result of the reciprocal interactions between stem cells and their niches¹. The complex stem cell niche microenvironment is composed of

cell (be that niche cells of different lineages and/or stem cell progeny) and non-cellular elements that present chemical and physical cues on macro, micro and nano scales. Using a 'reductionist' approach we can delineate the complex signalling of the stem cell niche and gain deeper understanding of the factors controlling stem cell renewal and differentiation. Much of this analysis of the impact of specific signals on stem cell behaviours has been carried out *in vitro*, e.g. with the aim of recapitulating the stem cell niche.

Mimicking the niche *in vitro* - intestinal organoids

With the great potential of stem cells in regenerative medicine, recapitulating the stem cell niche *in vitro/ex vivo* is a point of interest. The simpler intestinal crypt is a focus of bioengineering and a number of methods have been used for the growth of intestinal organoids *in vitro/ex vivo*; the most successful of which⁸ used lamin-rich MatrigelTM (an animal derived ECM product) and a cocktail of growth factors found in the endogenous stem cell niche²³. The crypt-villus organoid units that formed (Figure 3) were organised into discrete crypts with both stem and Paneth cells at their base and villus-like structures at the apex. As these organoids were grown from single mouse LGR5+ stem cells embedded in a laminin matrigel the importance of both ECM molecules and niche relevant chemicals for intestinal niche formation are highlighted. In addition the observation that laminin is important in this system correlates with its enrichment at the crypt base *in vivo*²⁴.

Mimicking the niche *in vitro* – bone marrow

Although *in vitro* recapitulation of the bone marrow HSC niche has been extensively investigated, researchers have had less success than that observed with the intestinal niche and as of yet HSCs are unable to be expanded *in vitro*. It has been known since the 70s that co-culture with stromal cells, such as MSCs, promotes HSC survival²⁵. *Ex vivo* expansion protocols often contain varying concentrations of growth factors (although many other factors are involved *in vivo*)¹⁶. A number of ECM molecules have been identified with structural roles in the HSC niche and some are thought to

have specialised, niche-specific functions, including fibronectin, laminin, hyaluronic acid and osteopontin¹⁶. Although clearly beneficial to HSC survival and expansion, neither growth factors, cytokines, chemokines, individual cells or individual ECM molecules have proven sufficient to produce environments conducive to significant HSC maintenance and expansion *in vitro*. Rather it is likely that a complex combination of factors will be required and research is moving in this combinatorial direction.

Together with a cocktail of cytokines, nanofibre scaffolds have been shown to increase the expansion efficiency of HSCs, with enhancement of stemness thought to be related to increased adhesion²⁶ (Figure 4). In another study, when cultured with cytokines on fibronectin coated microwells that were 10 μm in height and 15 – 80 μm in diameter, HSCs remained quiescent in smaller diameter wells where the cavity housed individual cells²⁷ (Figure 4). These observations suggest that cell-substrate contact area is important for HSC renewal and retention of the immature state, which in turn may be related to the number of engaged adhesion structures. The importance of dimensionality/adhesion for maintenance of HSC stemness has been further described by Raic *et al.*, whereby HSC stemness was retained to a greater degree in HSCs co-cultured with MSCs, using a cytokine cocktail, in a three dimensional system compared to a standard two dimensional culture system²⁸ (Figure 4).

Effect of physical stimuli on stem cell behaviours

The effects of chemical stimuli (be that solid or free states) on stem cell behaviours are relatively well characterised²⁹⁻³⁶ and defined growth factors are routinely used *in vitro* to control stem cell renewal and differentiation³⁷⁻³⁹. Accordingly, the remainder of this review will focus on the effects of physical stimuli on stem cells, with emphasis on how these signals may be interpreted by cells and the relevance of nanotopography in the stem cell niche, which are less well defined⁴⁰.

Physical signals in the stem cell niche, and tissue in general, come from both cells and the ECM. The ECM is recognised as an important signalling factor in tissues⁴¹, especially in the form of basement membranes in the niche³. For example in mice hyaluronic acid⁴² appears to have an important role in maintaining HSCs⁴³ and neuronal stem cell populations⁴⁴ and in humans different integrin expression on skin stem cells constrains them to what is presumed to be ECM glycoprotein ligands^{45, 46}. Aside from its important role in chemical signalling (both through intrinsic chemistry and binding of soluble factors), the physical influence that ECM has over cells can be broken down into architectural, mechanical and topographical. ECM architecture can confine cells, expose them to 2D or 3D environments and regulate their geometry, e.g. cell shape is known to influence stem cell differentiation with well spread flattened cells and rounded cells differentiating down osteogenic and adipogenic lineages respectively⁴⁷. Mechanical stimuli, e.g. shear flow, compression or substrate stiffness, influence stem cell differentiation. Finally topography, which is the least studied of the three and in particular nanotopography, will be reviewed in the following section with emphasis on niche specific behaviours such as differentiation and proliferation.

3. Mechanisms by which cells may interpret nanotopographical signals

Cells interact with topographic stimuli via cell adhesion molecules at their periphery, which allow the cell to physically connect with the adjacent structure and functionally provide a route for bi-directional communication by which the topographical environment can be translated into intracellular messages (mechanotransduction). Mechanotransduction can propagate through the cell as biochemical (indirect) or physical (direct) mechanotransduction (discussed below) and effect behaviours as diverse as growth, apoptosis, morphology, proliferation and differentiation. It is the type and arrangement of adhesions that determines cell responses to topographical stimuli.

Adhesions

Different cells have differing types and concentrations of adhesion molecules mediating cell-extracellular and cell-cell attachments, which include: cadherins, integrins, CD44, syndecans and discoidin domain receptors (DDR) (although syndecans and DDRs 'true' status as adhesion receptors remains to be clarified)⁴⁸. Integrin proteins and CD44 proteoglycans are thought to provide high affinity cell-ECM adhesions in anchorage dependent cells. Integrins are well-characterised; they bind a number of ECM molecules, including laminins and fibrillar collagens⁴⁹. The cell surface proteoglycans CD44 and syndecan bind, respectively: glycoproteins, glycosaminoglycans and hyaluronic acid^{50,51} and a wide range of molecules including adhesive ECM molecules and growth factors⁵². Whilst the structure of individual ECM components vary widely, many share common motifs; for example, the arginine-glycine-aspartic acid (RGD) motif found on the hydrophilic loops of a number of ECM molecules including fibronectin, vitronectin and tenascin. It is these motifs, or ligands, which cell-substrate adhesion molecules bind to with specificity, for example integrins bind RGD. Adhesions can be enhanced by synergistic cell binding to additional ECM structures/motifs, for example syndecans and DDRs in particular are thought to synergistically cooperate with integrins^{53,54}. Adhesions (be that cell-cell or cell-substrate) have been identified as important in stem cell functions such as maintenance, proliferation and differentiation^{42,55,56} as well as niche functions such as cell anchoring, recruitment/egress and potentially control of division (with adhesion formation potentially regulating cell polarisation and thus switching between symmetrical and asymmetrical stem cell division)⁵⁷. In particular, integrin mediated adhesions are thought to be important in both HSC and skin niches⁵⁸.

In non anchorage-dependent cells adhesion is less pronounced, which when viewed *in vitro* manifests as spherical cells rather than flattened as with anchorage dependent cells (Figure 5). Although less pronounced, cell-substrate adhesions are specific. Research carried out by Franke *et al.* demonstrated that when HSCs (non anchorage dependent cells) were cultured upon a range of ECM molecules stronger adhesion was observed between fibronectin and integrins, weaker adhesion

between heparin and selectins and in some instances, for example on tropocollagen, no adhesion was observed at all⁵⁹.

In addition to the type of cell adhesion molecule and cell/ECM ligand, cell adhesion is dependent on ligand density and arrangement., Integrin clustering (multimerisation or gathering), which enables more complex adhesive structure formation (please see next section 'adhesion structures' for more details), only happens in anchorage dependent cell adhesion to RGD sequences if a maximum inter-ligand distance of 58-72 nm between attachment sites is presented^{60,61}. In addition, the minimum unit of clustered integrins that forms a functional cell adhesion has been described as a tetrameric configuration⁶². In contrast, non-anchorage dependent cells such as HSCs have different threshold dimensions, for example HSCs adhering to RGD required ligand density thresholds in the range of 32 – 45 nm^{63,64} (Figure 5). Interestingly, threshold distances have been found to vary depending on the type of ligand that HSCs are adhering to, with osteopontin requiring less than approx. 75 nm and FNRRGD approximately 100 nm⁶⁴. Differences in threshold distances between anchorage dependent cells and non-anchorage dependent HSCs are hypothesised to be a result of differences in the actin cytoskeleton and adhesion related proteins in the two cell types. In integrin mediated focal adhesions of anchorage-dependent cells, the principle adhesion kinase is focal adhesion kinase (FAK), whereas in integrin-linked multiprotein complexes of HSCs the primary kinase is Pyk2⁶⁵⁻⁶⁷. The actin cytoskeletons of the two cell types differ, being fibrous in anchorage dependent cells but ring like in HSCs, which enable them to maintain their round shape even when adhered⁶⁴. We note that the different ligand specificity of different RGD formats (linear, place in sequence etc.) may lead to alterations in some of these values and this needs to be checked between reports⁶⁸.

Adhesion structures

In the integrin adhesome ('the network of protein interactions that potentially link integrins to the actin cytoskeleton') with integrin clustering comes structural and signalling protein recruitment and

connection with the cell cytoskeleton, which leads to the formation of larger adhesion structures (adhesion maturation). Many adhesion structures have been described, including: nascent adhesions, focal complexes, focal adhesions, podosomes, fibrillar adhesions and three dimensional matrix adhesions⁶⁹(Figure 6). All are dense, complicated structures (for a detailed review see⁷⁰) and although heterogeneous, they share common features. One of the best-defined adhesions is the focal adhesion, comprised of integrins, structural proteins, adaptor proteins, signalling molecules, and cytoskeletal components.

There are differing theories as to how early adhesions nucleate and elongate (there is likely to be overlap), including: initiation by integrin binding and clustering followed by assembly of the cytoplasmic adhesome complex (including actin binding) and alternatively, assembly of cytoplasmic adhesome components, co-localisation and then integrin binding⁷¹. Integrin adhesion structures are dynamic and can change with time, potentially maturing from small and transient nascent adhesions to progressively larger and more stable focal complexes then focal adhesions and finally fibrillar adhesions that are able to rearrange ECM. As focal contacts mature, in addition to increasing in size they are thought to change in composition, structure and function; going from contacts that transmit strong tractional forces to adhesions that are thought to be more passive anchoring structures that maintain a spread cell morphology⁷². Adhesion size appears to be an important factor for cell mechanics; increasing adhesion size has been found to correlate with increased cellular tension [17]. Recruitment of molecules and cross linking expands and strengthens the adhesion as well as decreasing the force generated/experienced per molecule [18], thus feeding back into force generated signalling controlling adhesion maturation^{73,74}. Adhesive structures vary between cell types, for example: migrating cells don't have fibrillar adhesions, neutrophils/macrophages show nascent adhesions and focal complexes and fibroblasts and smooth muscle cells display focal complexes and focal adhesions⁷¹. Cells also appear to be able to bridge adhesions between integrin clusters – i.e. if integrin clusters are large enough cell structural proteins can bridge between the integrin clusters to form larger adhesions. This appears to be protein dependant with cells on fibronectin able to bridge between 500 nm diameter clusters and cells on vitronectin only requiring 200 nm diameter clusters^{75,76}. It should be noted that there is some evidence that the adhesions

formed *in vitro* may differ in some ways to those formed *in vivo*, especially when considering cells that exist naturally in a three dimensional environment. Differences are thought to arise in adhesion composition, localization and function; for example three dimensional adhesions generally show enhanced biological activities, less integrin usage, fewer stress fibres and less stringent cytoskeletal organisation compared to their two dimensional counterparts⁷⁷.

Biochemical (indirect) mechanotransduction

Upon ligand binding intracellular integrin domains undergo conformational change, which, through signalling protein activation (such as FAK) and/or physical transmission through cytoskeletal proteins (such as talin and alpha actinin), results in signal propagation through the cell. Both direct signalling protein activation and cytoskeletal protein interactions can result in activation of downstream biochemical signalling cascades, hence the term biochemical/indirect mechanotransduction. Mechanotransduction via direct signalling protein activation follows mechanisms similar to signalling cascades generated by soluble chemicals such as growth factors – once a mechanotransducer is activated, multiple pathways can cause inhibition or enhancement of any one particular signalling cascade (for example FAK activation of the ERK/MAPK (extracellular signal related kinase/mitogen activated protein kinase) pathway) and is known to effect various cellular responses such as proliferation and differentiation⁷⁸⁻⁸⁰

Many molecules and structures are immobilised on the insoluble cytoskeleton anchored to adhesion sites; the cytoskeleton is thought to act as both a scaffold for solid-state biochemical reactions and as a reservoir for sequestered soluble factors^{81, 82}. It therefore follows that if integrin signalling is sufficient to influence cytoskeletal molecular conformation thus modifying cytoskeleton shape, tensions, structure or kinetics, any of the structures associated with the cytoskeleton may also be influenced⁸³. Where these structures are signalling molecules, if sequestered molecules are released or binding sites exposed or hidden, downstream biochemical signalling cascades may be enhanced or

inhibited following mechanisms similar to biochemical signalling cascades initiated by soluble chemicals e.g. cytokines and growth factors⁸⁴. In this way, mechanical force can be transduced through the cytoskeleton and using solid-state chemistry can be converted into biochemical reactions (Figure 7).

Physical (direct) mechanotransduction

As well as considering cells as biochemical units, they can also be considered as mechanical units with perhaps both features contributing to cell responses to materials. Perhaps the best theory presented to date of how cells could respond as mechanical units can be found in tensegrity theory. The cell cytoskeleton can be described as a tensegrity structure, whereby opposing forces act in unison to maintain the shape of the structure, whilst providing strength and resilience; this means that cells are maintained in a prestressed state and are in equilibrium under a balance of forces⁸⁵. That is, the cytoskeletal network is under isometric tension, which removes any slack in the system; 'lack of slack' means that any mechanical stresses applied to the system (cytoskeleton) are immediately sensed. The tensegrity architecture model therefore provides a mechanism whereby mechanical stimulation of the cell cytoskeleton via attachment proteins, at a point on a cell's membrane, causes the cell to react as a whole and results in integrated, global changes in cytoskeletal structure⁸⁶. Cytoskeletal stresses tend to dissipate less in stiffer structures, thus the stiff prestressed cytoskeleton is able to concentrate and focus stresses and facilitates longer distance force propagation compared to less stressed structures.

As an extension to physical mechanotransduction through the cytoskeleton, mechanical pull on integrins can be further propagated through the cytoskeleton to cell nuclei, causing nuclear envelope⁸⁶ and whole nuclei⁸⁷ distortion. The cytoskeleton is contiguous with the nucleoskeleton (also called the karyoskeleton and nuclear matrix⁸⁸) and is connected via LINC (linker of nucleoskeleton and cytoskeleton) complexes (Figure 7). LINC complexes span the nuclear envelope; on the cytosol side

they bind cytoskeletal proteins including F-actin, intermediate filaments and microtubules, and intranuclearly LINC complexes transmit forces to the nucleoskeleton and chromatin (for example by directly binding lamins, which in turn can bind DNA either directly or indirectly through matrix attachment regions (MARS) on telomeres). Cytoskeletal forces are transduced through LINC complexes into the nucleus, analogous to force transmission through focal adhesions to the cytoskeleton. Also similarly to the adhesion complexes described previously, LINC complexes can be transient or stable and are thought to form a range of 'adhesion' types. Stiffness differentials between the cytoskeleton and nucleus (the nucleus is the stiffest organelle and is approximately nine times stiffer than the surrounding cytoskeleton⁸⁶) are thought to facilitate force propagation over the relatively large distances between the cell membrane and nuclear envelope⁸⁹. Within the cytoskeleton intermediate filaments are thought to play a more significant role in force transfer to the nucleus compared to actin microfilaments due to mediation of force transfer at both high and low strains, whereas actin mediation is only conferred at low strains⁸⁶.

The nucleoskeleton is a permanent network of core filaments underlying thicker fibres and is composed of proteins including lamins, titin, actin, nuclear myosins and kinesins. Not only does the nucleoskeleton confer specific shape, mechanical properties and functionality to the nucleus and genome, similarly to the cytoskeleton, many molecules and structures are immobilised on the insoluble structure. This immobilisation is proposed to act as a platform for functional complexes required for nuclear activities, such as transcription and DNA repair^{88,90,91}. The nucleoscaffold appears to exhibit precise spatial order to the nucleus in terms of chromosome organisation, DNA replication, transcription and processing of RNA⁸³. Distortion of the cell nucleus or nuclear envelope may alter the molecular conformation of the nucleoskeleton similarly to the manner in which the cytoskeleton is altered in response to adhesion dynamics. Nucleoskeletal proteins have been shown to influence a number of processes, including: transcription, replication and DNA repair, thus alterations in their shape, tensions, structure or kinetics may also influence these associated structures⁹². Interestingly, mutations in the genes encoding A-type lamins of the nuclear envelope have been linked to ageing of adult stem cells and their niches^{93,94}.

Cytoskeleton mediated physical mechanotransduction may present a number of advantages over soluble biochemical mechanotransduction conferred via diffusion. The quick response system means that mechanical forces can cause rapid effects, more so than biochemical signalling that takes diffusion time. In addition to quick response times, cytoskeletal mediated biochemical signalling channels forces along discrete fibres of the cytoskeleton providing a mechanism by which to concentrate stresses on specific molecules at particular locations, whilst protecting other cellular components from these mechanical forces, meaning that only a subset of molecules may experience force levels strong enough to alter their activities⁹¹.

Alterations in gene expression as a result of mechanotransduction and intracellular tension

Alterations in intracellular tension are thought to have important consequences for stem cells, with high, low and intermediate levels of tension promoting osteogenic differentiation, adipogenic differentiation and stem cell renewal respectively^{47, 95, 96}. The mechanisms that translate mechanotransduction into alterations in gene expressions are less well understood compared to cytosolic mechanotransduction, although a number of mechanisms have been proposed. One mechanism proposes that when nuclei are deformed, chromosome positions within the nuclei are distorted⁹⁶⁻¹⁰⁰. Chromosomes occupy discrete territories within the interphase nucleus and because transcription factors and machinery are thought to be in varying concentrations in different regions of the nucleus, altering chromosome positions may affect their accessibility for transcription and therefore alter gene expression¹⁰¹. Nuclear envelope distortion alone may be a factor in the mechanotransduction of forces^{102,103}; Itano *et al.* proposed that calcium ion (Ca^{2+}) release from the perinuclear space after envelope distortion acts on calcium ion regulated transcription factors, which in turn alter gene expression levels¹⁰³. Other mechanisms include the translocation or activation of transcription factors. Focal adhesion signalling may result in translocation of focal adhesion proteins to the nucleus where they can act as transcription factors; for example once released, research has

shown that zyxin may translocate and alter transcription of genes such as endothelin-1⁷⁸ and that FAK can shuttle from focal adhesions to operate within the nucleus, where it targets ubiquitination of the cell-cycle mediator p53 (tumour protein 53) and can act as a transcription co-regulator with the GATA4 zinc-finger transcription factor linked to embryogenesis¹⁰⁴⁻¹⁰⁶. Two transcriptional co-activators involved in altering gene expression regulating cell growth are YAP and TAZ. Mechanical properties have been shown to alter the locations and activity of YAP and TAZ: in stiff cells they are active and located in the nucleus whereas in compliant cells they are inactive and located in the cytosol¹⁰⁷⁻¹⁰⁹. YAP and TAZ are also proposed to mediate a mechanical memory in cells, acting as rheostats that store information about a cells physical environment and influencing cell fate¹⁰⁷.

Signal integration

Signals mediated through adhesions can be integrated with other local signals, such as those initiated by soluble chemical factors. For example, integrins co-localise with other signalling structures, including growth factor receptors and stress-sensitive ion channels¹¹⁰. Many molecules involved in signalling cascades other than those initiated by integrin signalling (such as growth factors and cytokines) are immobilised on the focal adhesion cytoskeleton, bringing these downstream molecules and mechanotransduced pathways into close proximity^{81, 111-113}. In addition to co-localisation and focal adhesion orientation, different signalling molecules and biochemical pathway components can be brought into close association by scaffolding proteins, which act as 'hubs' facilitating the recruitment and organisation of target proteins, resulting in tethering of multiple components of signalling pathways to one location. This effect is further extended by scaffold proteins interacting directly with one another or through bridging proteins to form higher order macromolecular complexes¹¹⁴.

In focal adhesions, scaffolding proteins include FAK, paxillin and RACK1. Scaffolding proteins are an integral part of adhesome signal organisation and their interactions follow specific trends, which can be described using network motifs (defined as unique patterns of interactions between proteins that appear significantly more often in the real network compared with randomised networks¹¹⁵). The

adhesome has been found to be composed of network motifs that consist of binding interactions regulated by on/off switches, the most common of which were three-node motifs consisting of a scaffolding protein, signalling molecule and its down-stream target⁷⁰. That these were the most common of the adhesome motifs highlights the importance of the adhesome in integrating mechanical and chemical signalling.

Complimentary to spatial control over signal integration, tensegrity architecture offers a model by which mechanical signals can be globally integrated. Tensegrity architecture describes a way in which forces transmitted through localised adhesion sites induce rearrangements throughout the cell within a tensionally integrated cytoskeleton¹¹³. The sensitivity of the system is dependent on the concentration of prestresses in the cytoskeleton⁹¹ and connection to adhesion molecules⁸⁶. Taken together these two mechanisms illustrate how signal integration can be achieved on both local and global scales, being mediated by for example focal adhesions (integration 'hubs') and the cytoskeleton respectively.

In order for stem cells to effectively function in the stem cell niche, the different signals that they are exposed to must be integrated to give a 'whole-cell' response. In addition the complex signals relayed to the niche itself must be integrated, e.g. by niche support cells, to ensure precise temporal and spatial control over stem cell proliferation and differentiation.

Synthetic materials

Using techniques traditionally associated with electronic engineering and materials science, such as photolithography and reactive ion etching¹¹⁶⁻¹¹⁸ defined topographic patterning can be achieved in biomaterials enabling researchers to home-in on specific physical cues influencing cell behaviours. This is particularly useful for characterising the effects of topography and mechanotransduction on the stem cell niche.

Where biomaterials are incorporated into culture systems, new functionality will also be conferred. Cells do not tend to interact directly with biomaterials, rather, when cell solutions are brought into contact with biomaterials (be they naturally derived or synthetic) a layer of proteins adheres to the surface within milliseconds¹¹⁹. Protein adsorption is affected by the properties of the material¹²⁰ (Figure 8), including surface charge, wettability (hydrophobicity) and topography. It is this adsorbed layer of protein that is thought to influence cell interactions with the underlying material¹²¹.

4. Stem cell responses to nanotopography

The importance of scale

Many researchers have shown that cells are able to respond to differences in topography with behaviours as diverse as adhesion, morphology, proliferation and differentiation being influenced (e.g.^{100, 122-126}). Over 100 years ago, early work described cells as being responsive to topographic features¹²⁷ and the term 'contact guidance' was first published by Weiss in 1945¹²⁸ (Figure 9).

More recently the importance of topography scale has been identified. Adhesion formation and dynamics occurs on the nanoscale; for example integrins span the 10 nm thick plasma membrane and the attached actin cytoskeleton is separated by a 40-nm-high focal adhesion core-region consisting of strata with specific roles. These strata include a signalling layer consisting of integrin cytoplasmic tails, FAK and paxillin (signalling proteins), an intermediate force transducing stratum containing talin and vinculin; and an actin-regulatory stratum containing VASP (vasodilator-stimulated phosphoprotein), zyxin and α -actinin¹²⁹. Furthermore, to connect the nanoscale actin filaments to the microscale actin contractile stress fibre bundles cells appear to use adhesion related particles which are small enough to fit between gathered integrins¹³⁰. Thus the effects of scale are important; due to their sizes being in the same order of magnitude, micron scale features can be considered as 'housing' cells whereby cells are confined by substrate features. In contrast, because nanoscale features are an order of

magnitude smaller than cells and on the same size scale as their molecular components, nanotopography can be considered as interacting with cells, whereby nanoscale structures act as multiple signalling points (Figure 9).

Nanotopography is known to influence the way in which adhesions form on substrates and is thought to be the signal initiation point of cell-nanotopography interactions^{69, 131, 132}. By controlling adhesion size and type, adhesion composition, function and strength (tractional force as a result of the actin-myosin cytoskeleton) can be influenced. Cytoskeletal tension has been shown as an important transducer of physical stimuli through cells, with e.g. *in vitro* studies show that MSCs differentiate down differing lineages when cultured on substrates of varying stiffness, and in the developing embryo early embryogenesis is halted if myosins are knocked out in mice^{47, 73, 133}. Thus tractional force is thought to play a large part in cell responses to nanotopographical stimuli due to its influence on cytoskeletal tension (which as described previously can effect biochemical and physical mechanotransduction and ultimately niche relevant cell behaviours such as proliferation and gene expression). Tractional force is known to vary between adhesion structure types, for example, focal complexes are under great tractional force (their size is not thought to correlate with the force experienced^{72, 134}) whereas focal adhesions are under less tractional force (their size shows linear correlation with tractional force for a given substrate stiffness¹²²). Thus a route between nanotopography influenced adhesions and niche-relevant stem cell signalling, via cytoskeletal tension, is described.

***In vitro* Observations**

In terms of stem cell responses to nanotopography, MSCs are the most well-described and will be summarised in this section. When cultured on nanopits (310 nm deep and 30 μm diameter) increased MSC spreading has been observed compared to cells cultured on both planar and groove patterned (327 nm deep, 50 μm wide) substrates¹³⁵. When cultured on nanopillars (6 nm high, 200 nm wide, centre to centre spacings of 290 nm), MSCs showed increased adhesion compared to those grown on planar substrates¹³⁶. This trend was also observed when MSCs were cultured on titania nanopillars (28 nm diameter, 15 nm high and 40 nm centre to centre spacing)¹³⁷. Height was found to be an

important factor in MSC adhesion to these nanopillars; for nanopillars with heights ranging from 15 to 100 nm, cell adhesion was increased on smaller pillars and decreased on larger ones¹³⁷. One of the most commonly characterised cell responses to topographies are those of cell alignment and orientation in the direction of, or perpendicular to, grooves. This observation holds true for MSCs; for example cell cytoskeletal and nuclear elongation and alignment has been reported in response to nanogrooves (350 nm deep, 350 nm wide and 700 nm pitch) compared to planar substrates where no elongation or alignment was observed¹³⁸. It is noted that grooves wide enough to be step-cues rather than guidance cues can be more osteogenic than planar or narrow grooves⁶⁹.

Yim *et al.* demonstrated that MSCs respond to nanogrooves (dimensions of 350 nm depth, 350 nm width and 700 nm pitch) by up-regulating expression of neuronal, vascular and muscle cell markers¹³⁸. Neuronal marker expression was most significant, suggesting that nanogrooves can induce neuronal lineage differentiation in MSCs¹³⁸. It should be noted that this behaviour represents a transdifferentiation and although neural proteins were expressed (MAP2 and beta tubulin), a functional role has yet to be demonstrated. In this work nanogrooves induced significantly greater MSC differentiation compared to micro grooves, highlighting the importance of scale and demonstrating the potential power of nanotopography over micro topography in terms of cell differentiation. This influence of nanotopography was linked to focal adhesion formation, with smaller and more elongated cell focal adhesions on the nanogrooves compared to micro grooves and planar substrates, in addition topography induced gene expression was shown to be dependent on actomyosin contractility and FAK activity¹³². The importance of topography scale was also observed in MSCs cultured upon nanotube titanium oxide with diameters ranging from 30 – 100 nm. Specifically, on tubes with 100 nm diameters cells differentiated down an osteogenic lineage, whereas on 30 nm tubes no noticeable differentiation was observed¹³⁹.

The degree of disorder in nanopit arrangements have been shown to effect MSC differentiation; ordered nanopits (120 nm diameter, 100 nm depth, 300 nm centre to centre spacing) stimulated MSCs to proliferate within a multipotent state¹⁴⁰, whereas when a degree of disorder was introduced

to the pits (a randomly orientated deviation of 50 nm from the centre) MSCs differentiated down an osteogenic lineage¹⁴¹ (Figure 10). This osteogenic differentiation was comparable to differentiation stimulated using traditional chemically defined media. The responses of MSCs to pit disorder were hypothesised to be related to differences in adhesion formation (with pits being non-adhesive areas) resulting in differences in intracellular tension. MSCs on the highly ordered pattern had smaller adhesions than those on the disordered topographies, which developed super mature, or fibrillar, adhesions that were hypothesised to result in increased intracellular tension and thus differentiation down an osteogenic lineage^{131, 142}.

Biomimicry of nanotopography has been investigated in the culture of MSCs on helical synthetic nanoribbons, where helices with a 63 nm periodicity (thus mimicking type 1 collagen, the most common collagen found in bone, which displays a repetitive topographic banding pattern of 67 nm along the length of its fibrils¹⁴³) induced osteogenic differentiation whereas a periodicity of 100 nm did not¹⁴⁴. Importantly, this differentiation effect was found to be dependent on the mechanotransduction of physical stimuli. Biomimicry has also been shown to influence adipose derived MSC behaviour when cultured on cell-imprinted substrates (the original cells were removed from the substrate before MSC culture, leaving behind a topographic pattern of their surface and residual cellular fragments)¹⁴⁵. MSCs adopted the shape and certain gene expression profiles of the original cells, which was hypothesised to be at least partially a result of the micro and nanotopographies of the imprinted substrates.

Embryonic stem cells

The importance of the stem cell niche is exemplified with embryonic stem cells (ESCs); in order to culture ESCs *in vitro*, ESCs must be grown on specialised substrates, such as feeder layers (for example mouse embryonic fibroblasts) or MatrigelTM. The necessity of such substrates makes it difficult to investigate the effects of nanotopography on ESC behavior, although a few studies have been carried out. When cultured on nanopit topographies (120 nm diameter, 100 nm depth, 300 nm centre to centre spacing and a randomly orientated deviation of 50 nm from the centre) ESCs differentiated

down a mesodermal lineage towards the osteoblastic phenotype to a greater degree than cells cultured on planar substrates¹⁴⁶. On nanogrooves (350 nm wide, 500 nm high, 700 nm pitch) coated with gelatin ESCs differentiated down a neuronal lineage¹⁴⁷. It should however be noted that this observation is perhaps more complex as there is some evidence that neural differentiation is a default response in ESCs cultured without specific lineage differentiation stimuli¹⁴⁸.

This overview of stem cell responses to nanotopographical stimuli is summarised in Table 1.

	Topography features	Topography dimensions	Cell response (compared to planar substrates unless stated)	References
Mesenchymal stem cells	Nanopits	310 nm deep, 30 µm diameter	Increased cell spreading.	135
		120 nm diameter, 100 nm deep, 300 nm centre to centre spacing, ordered geometry	Retention of multipotency, linked to focal adhesion formation.	131, 140, 142
		120 nm diameter, 100 nm deep, 300 nm centre to centre spacing, disordered geometry	Osteogenic differentiation, linked to focal adhesion formation.	97, 131, 149
	Nanopillars	6 nm high, 200 nm wide, centre-centre spacings of 290 nm	Increased adhesion.	136
		28 nm diameter, 15 – 100 nm high, 40 nm centre to centre spacings	Increased cell adhesion on shorter pillars.	137
	Nanogrooves	350 nm deep, 350 nm wide, 700 nm pitch	Cytoskeletal and nuclear elongation and alignment.	138
		350 nm deep,	Increased neuronal marker	132, 138

		350 nm wide, 700 nm pitch	expression compared to both planar and micro grooved substrates. Differentiation response linked to focal adhesion formation.	
	Nanotubes	30 – 100 nm diameter	Larger tubes stimulated osteogenic differentiation compared to smaller diameter tubes.	¹⁵⁰
	Biomimicry - banding	Helical nanoribbons with 63 nm helical periodicity	Osteogenic differentiation compared to nanoribbons with 100 nm periodicity. Differentiation response dependent on mechanotransduction of mechanical stimuli.	¹⁴⁴
Embryonic stem cells	Nanopits	120 nm diameter, 100 nm deep, 300 nm centre to centre spacing, ordered geometry	Differentiation down a mesodermal lineage towards the osteoblastic lineage.	¹⁴⁶
	Nanogrooves	350 nm wide, 500 nm high, 700 nm pitch (coated with gelatin)	Neuronal differentiation.	¹⁴⁷

Table 1: Summary of stem cell responses to nanotopographical stimuli.

Signal Gradients

Signal gradients are known to be important in morphogenesis during development and may also play a role in the stem cell niche, for example soluble/ECM-tethered chemical gradients may enable fate decisions (e.g. self renewal or differentiation) to be controlled by the distance of the cell from the niche ⁷. Signal gradients may also be conferred by topographical signalling; for example, studies have shown that in some cell types cultured on grooved substrates topographic cues are able to propagate (in terms of cell migration) up to nine cells away from the original signal ^{151, 152}.

5. Correlations between nanotopographical control of stem cell behaviour *in vitro* and observations of niche components *in vivo*

We have described how nanotopography can influence and direct stem cell behaviours including gene expression and illustrated how nanotopographical signals acting via adhesion formation and cytoskeletal tension can be integrated with other chemical signals both locally and globally to give a whole-cell response. *In vitro* observations have enabled us to delineate and understand how specific nanotopographical cues influence stem cell behaviours and in some cases parallels between *in vitro* and *in vivo* observations can be drawn. Nanotopography is present in the bone marrow MSC niche on both ECM and cells. Endothelial cells form fenestrated sinusoidal capillaries in bone marrow with nanopores between 50 and 300 nm in diameter¹⁵³ (Figure 11). Collagen type X, which is found at sites of large fractures and endochondral ossification, display a nanopattern where nanofeatures are separated by approximately 100 nm in a disordered lattice arrangement¹⁵⁴ (Figure 11). These two topographic structures bear resemblance and dimensions similar to the ordered and disordered 120 nm diameter pit structures described previously that *in vitro* were found to stimulate MSC phenotype retention and osteogenic differentiation respectively^{140,141}, highlighting a potential role for nanopits in the bone marrow MSC niche. The cell behaviours observed on these topographies *in vitro* (retention of multipotency and osteogenic differentiation on ordered and disordered pits respectively) also bear similarities to the cells found in these locations *in vivo* (perivascular MSCs and osteoblasts on sinusoidal fenestrations and type X collagen respectively).

Conclusions and perspectives

Traditional tissue engineering whereby cells are cultured on a scaffold *in vitro* then implanted have shown some success¹⁵⁵. However, future directions for tissue engineering are likely to involve engineering scaffolds with signals that promote *in vivo* population and potentially even attract and direct the behaviour of endogenous stem cells^{4,156}.

Ultimately if the complex stem cell niche is to mimicked *in vitro* or *in vivo* for tissue engineering applications the physical environment, including topography, must be optimised. Scale is important in this context because the natural stem cell niche presents topographic cues on macro, micro and nano scales, which each scale have differing types of interactions with cells. Micron scale features can be considered as 'housing' cells, where cells are confined by substrate features; in contrast nanotopography can be considered as interacting with cells, whereby nanoscale structures act as multiple signalling points. The power of nanotopography to direct stem cell behaviours is observed *in vitro* and parallels between these observations and the stem cell niche confound the importance of understanding the role of nanotopography in the stem cell niche.

For nanotopography, future directions will likely include:

- 1) Development of new tissue culture plastics for stem cell growth. Because topography is a purely physical principle it can be easily injection moulded into the base of traditional cell culture plates, wells and flasks. I.e. the next generation of cell culture materials can appear, at the macro scale, the same as the old generation. This will aid with MSC growth and targeted differentiation using standard culture protocols.
- 2) High throughput screening 1. For testing of drugs for effects on stem cells, having stem cells in the correct phenotype will be very important. Topographically patterned e.g. 96 well plates will help control the stem cell population while drug trials are undertaken. This should reduce variability and artefact.
- 3) High throughput screening 2. For understanding the range of stem cell responses achievable, nanotopographical arrays, as has been performed with microtopographical

topochips¹⁵⁷, will help increase our understanding of the range of control we can gain over MSCs with nanotopography.

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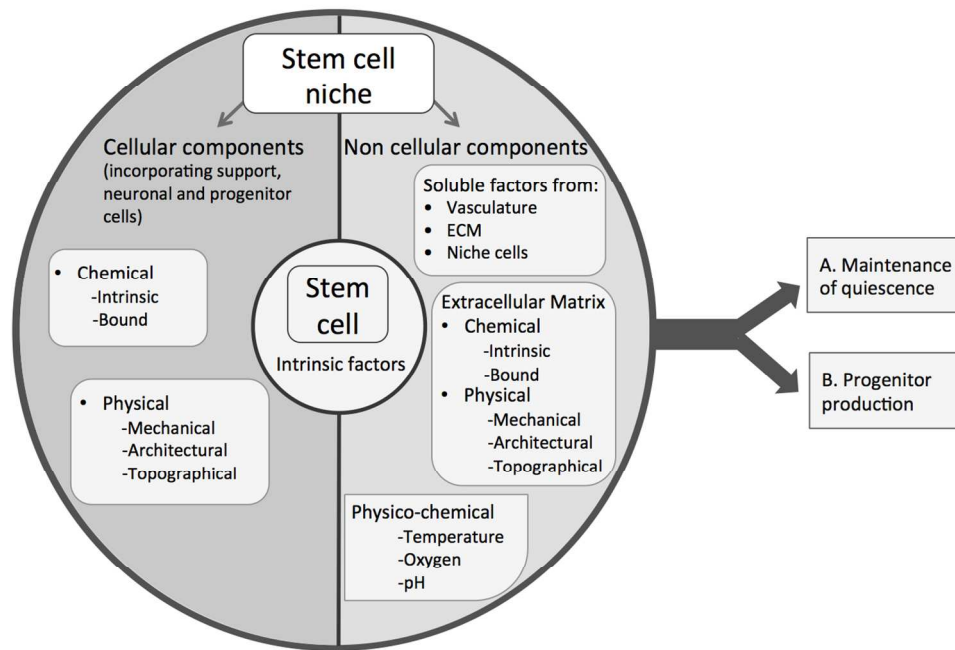


Figure 1: Potentially important signalling factors in the stem cell niche. Multiple factors in the niche are thought to influence stem cells: from stem cell intrinsic factors to physical signals such as mechanical cues from the extracellular matrix and chemical signals from adjacent cells. In this review we will focus on the effects of topography, and specifically nanotopography.

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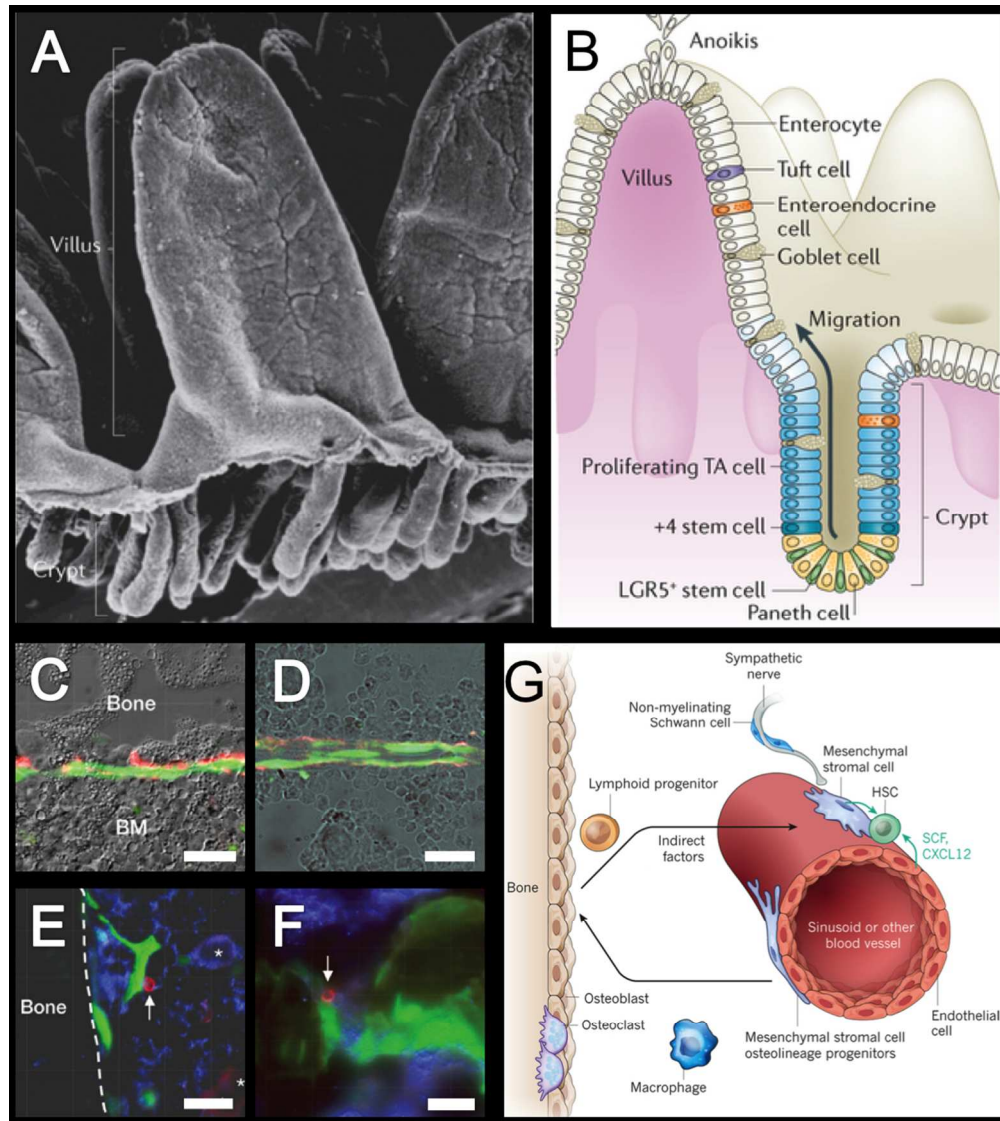


Figure 2: Intestinal and bone marrow haematopoietic stem cell niches
 Intestinal villi and crypts are shown in scanning electron microscope (A) and schematic (B) images, highlighting the location of the intestinal stem cell niche at crypt bases. Optical images of the endosteal region (C) and bone marrow parenchyma (D) in mice with fluorescent images overlaid show mesenchymal stem cells (nestin+ cells) (MSCs) and neuronal cells (catecholaminergic fibres) in green and red respectively. Fluorescent images of the endosteal region (E) and sinusoids (F) of mice, highlighting haematopoietic stem cells (CD150+CD48-Lin-), haematopoietic progenitor cells (CD48+) and MSCs in red with arrows, blue and green respectively. Megakaryotes (CD150+CD48+Lin+) are highlighted with asterisks. Schematic (G) of a proposed bone marrow stem cell niche. Scale bars are 50 μ m (C-F). Images A, B [8], C-F [13] and G [10].

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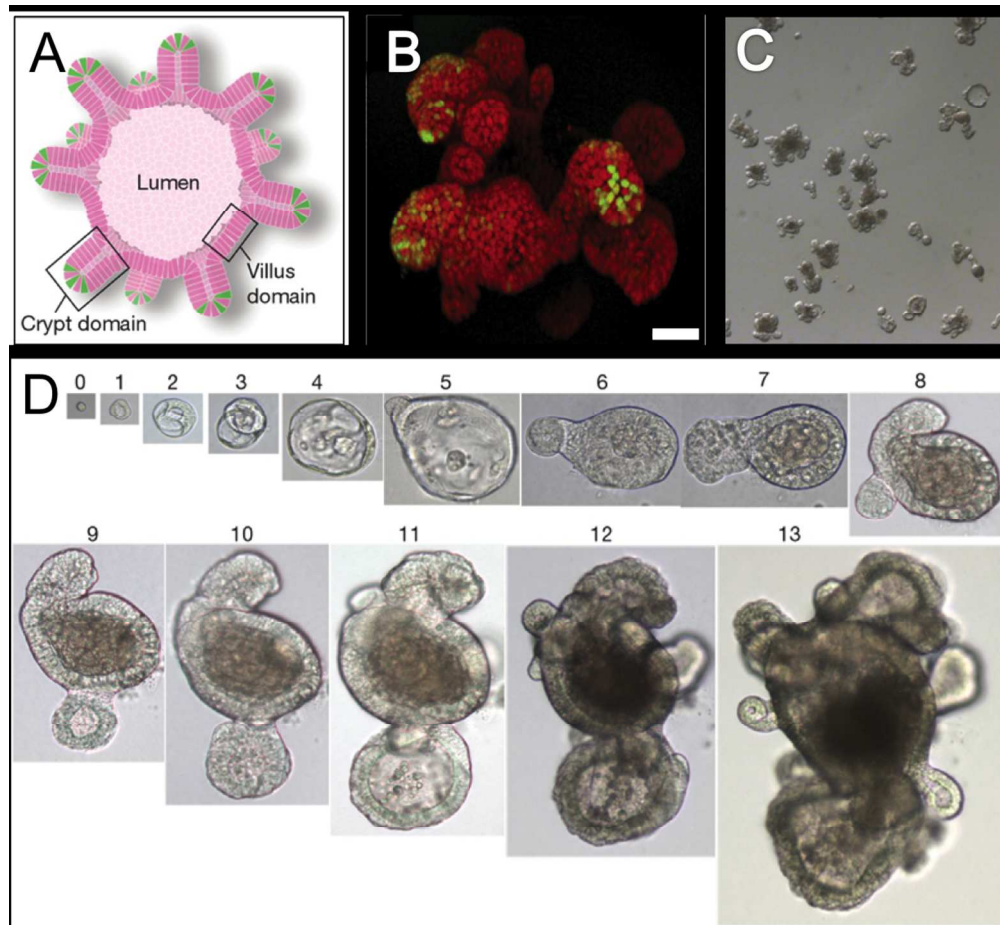


Figure 3: Mimicking the intestinal niche in vitro: organoids

Schematic representation (A) and three-dimensional reconstructed confocal image (B) of an intestinal organoid, highlighting leucine-rich repeat-containing heterotrimeric guanine nucleotide-binding protein-coupled receptor 5 (LGR5+) stem cells (green) at crypt bases and other cells (red). Optical image (C) shows an organoid suspension derived from a single cell organoid. Optical images (D) showing organoid growth with time from a single LGR5+ stem cell exposed to appropriate conditions (see text). Numbers above images = days of growth. Scale bar is 50 μm (B) and magnification (D) is x40, x20, x10 and x4 for days 0-4, 5-7, 8-11 and 12-13 respectively. All images [23].

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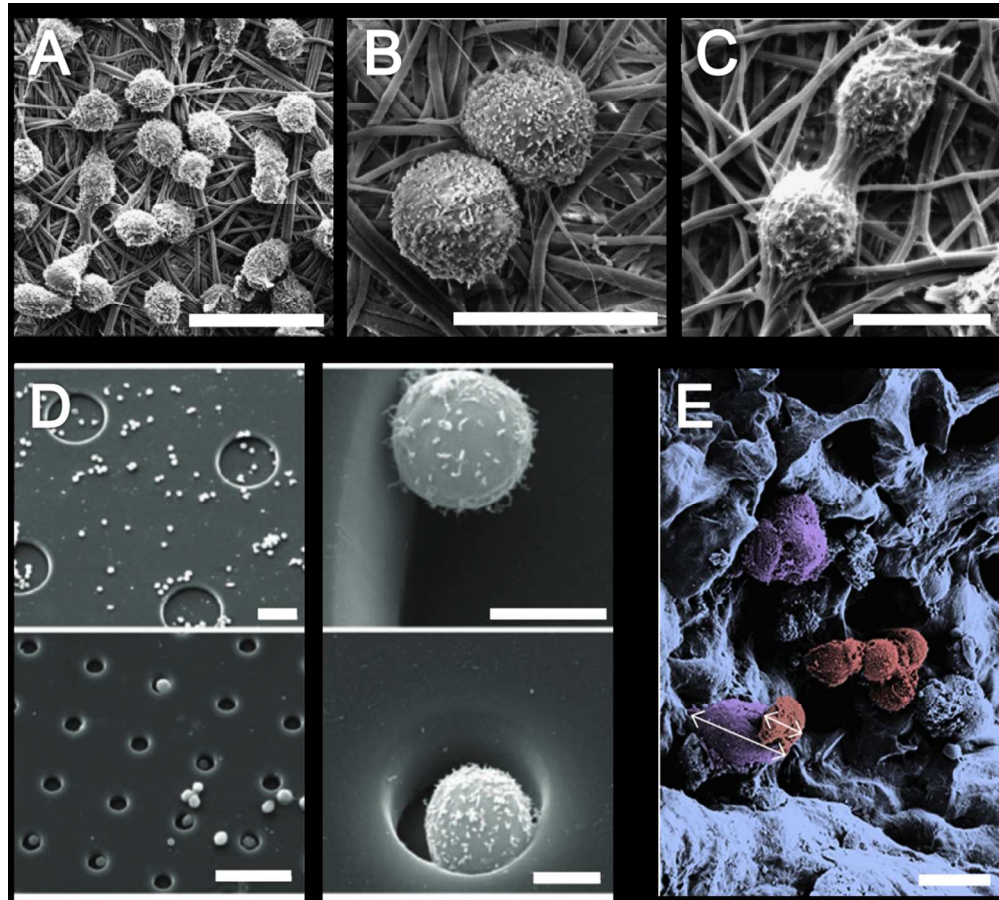


Figure 4: Mimicking the bone marrow niche in vitro: haematopoietic stem cell growth on materials. Scanning electron micrograph images (A-C) of haematopoietic stem cells growing on polyethersulfone 1,4-butanediamine nanofibre meshes, highlighting cell filopodia (B) and division (C). HSC adhesion on fibronectin coated microwells fabricated in poly(ethane-alt-maleic anhydride) on silicone (D). Images on right hand side are magnifications of left hand side images. Pseudo-coloured SEM image of MSCs (purple) and HSCs (red) on a porous hydrogel (E); white arrows highlight different cell dimensions. Scale bars = 20 μm (A), 10 μm (B and C), 50 μm (left images in D) and 5 μm (right images in D) and 20 μm (E). Images A - C [26], D [28] and E [27].

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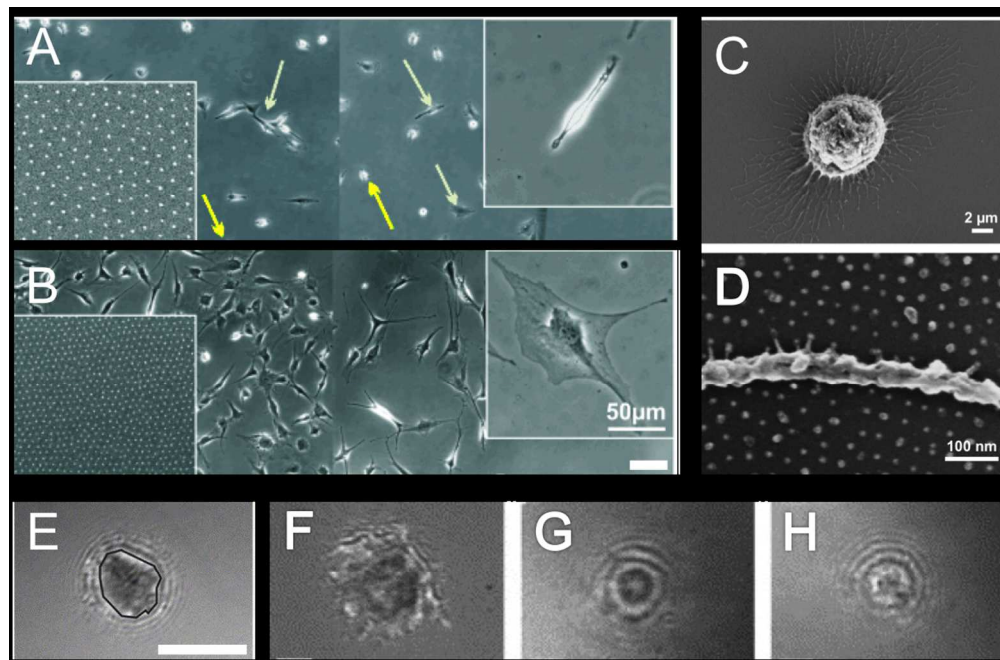


Figure 5: Adhesion in anchorage dependent and independent cells

Optical micrographs of osteoblasts cultured on a non-adhesive surface patterned with adhesive arginine-glycine-aspartic acid (RGD) ligands separated by approximately 85 nm (A) and 28 nm (B) (the right side of the main images are non-adhesive areas). Left insets show nanodot patterns and right insets magnified images of a typical cell on each surface. Green and yellow arrows (A) highlight migrating and quiescent cells respectively. Optical micrograph images (C and D) of human acute myeloid leukemia cell line KG-1a cells (model cell line for immature haematopoietic stem cells) on a non-adhesive surface patterned with adhesive RGD ligands separated by 36 nm show cell protrusions and adhesion points. Reflectance interference contrast microscopy (RICM) images of HPCs grown on: fibronectin (E and F), heparin (G) and tropocollagen (H). An example of a cell-substrate adhesion area is highlighted with a black line (E). Three types of adhesion are illustrated: irregularly shaped areas with dark contrast highlight tight membrane-substrate contact indicating integrin mediated adhesion (F), small circular shaped dark areas show a smaller contact zone and indicate selectin mediated adhesion (G) and small circular shaped bright areas highlight non-adherent cells (H). Scale bars are 100 μm (A and B) and 5 μm (E-H). Images A and B [61], C and D [64] and E-H [59].

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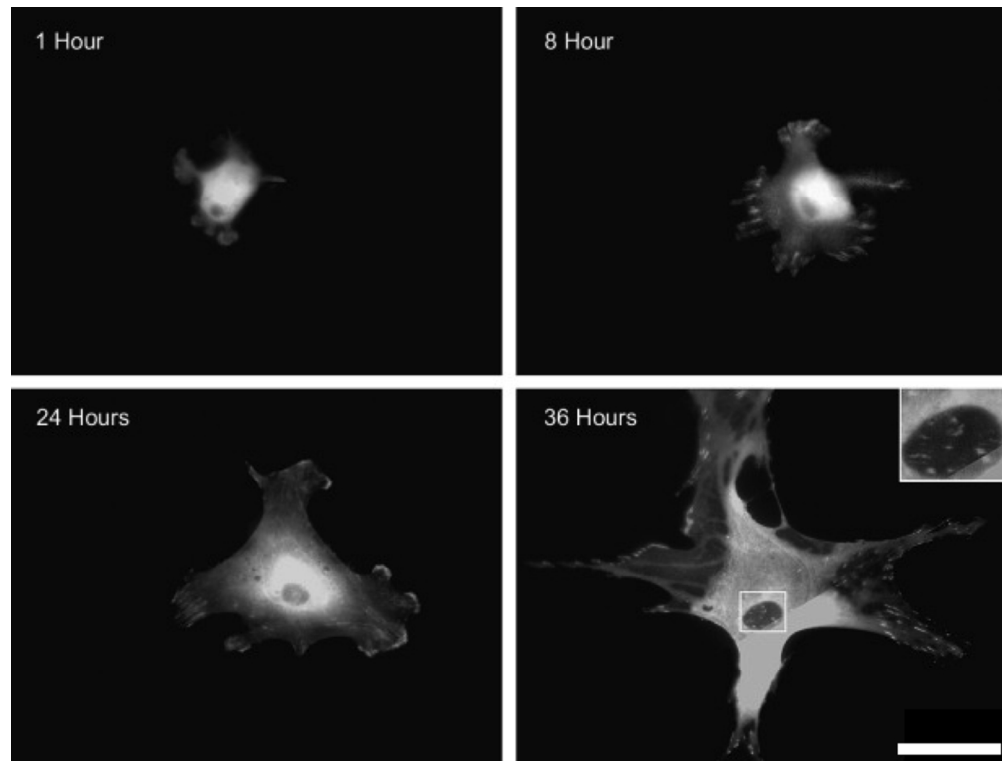


Figure 6: Integrin mediated adhesion structures
Flourescent microscopy images of vinculin stained human osteoblasts cultured on planar poly(methyl methacrylate) substrates. As cells spread, the focal complexes present 8 hours after seeding were slowly replaced by focal adhesions and then super mature adhesions by 36 hours. Boxed area shows sub nuclear adhesion formation on the cellular ventral membrane. Scale bar = 50 μm . Images [69].

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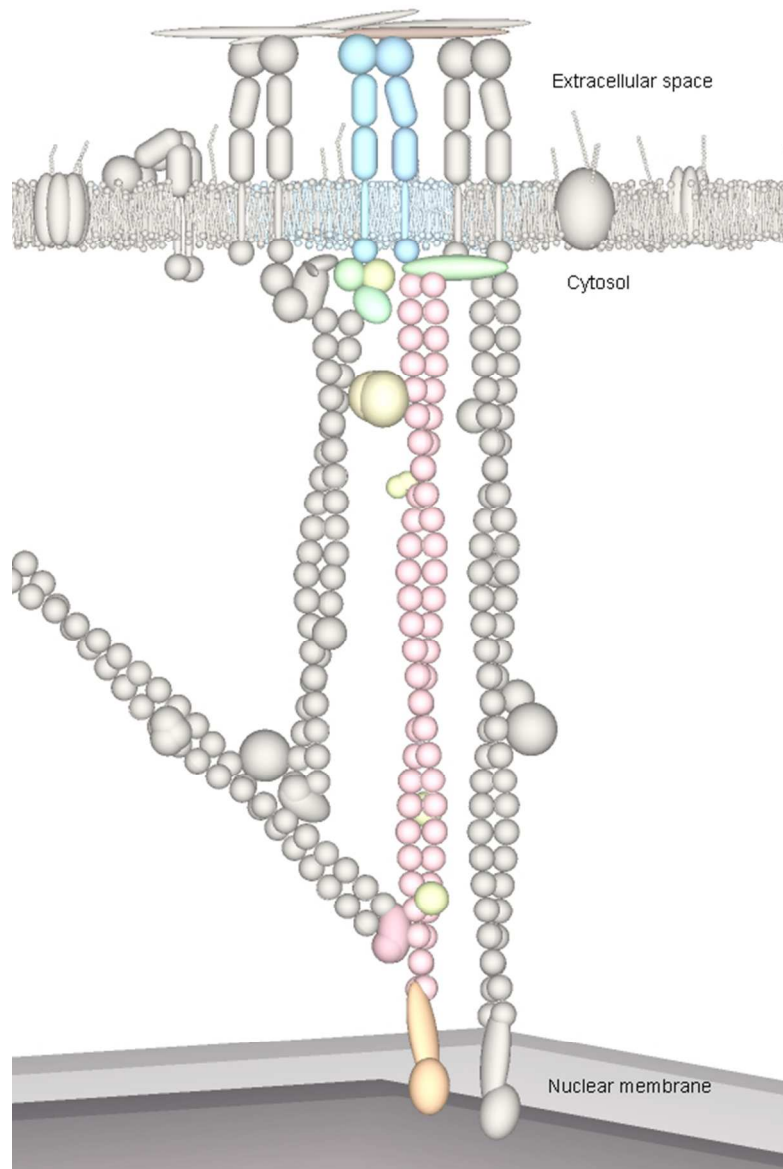


Figure 7: Schematic illustration of the simplified adhesome. Integrins (blue) connect extracellular fibronectin (brown) with an intracellular complex containing, amongst other structures, adaptor proteins (green) and signalling molecules (yellow) and the actin cytoskeleton (pink). Connectivity between these signalling and structural molecules enables mechanical forces to be converted into intracellular signals and relayed to the nucleus. Connections between the actin cytoskeleton and nucleus interior are mediated by LINC structures (orange) that span the nuclear envelope (dark grey).

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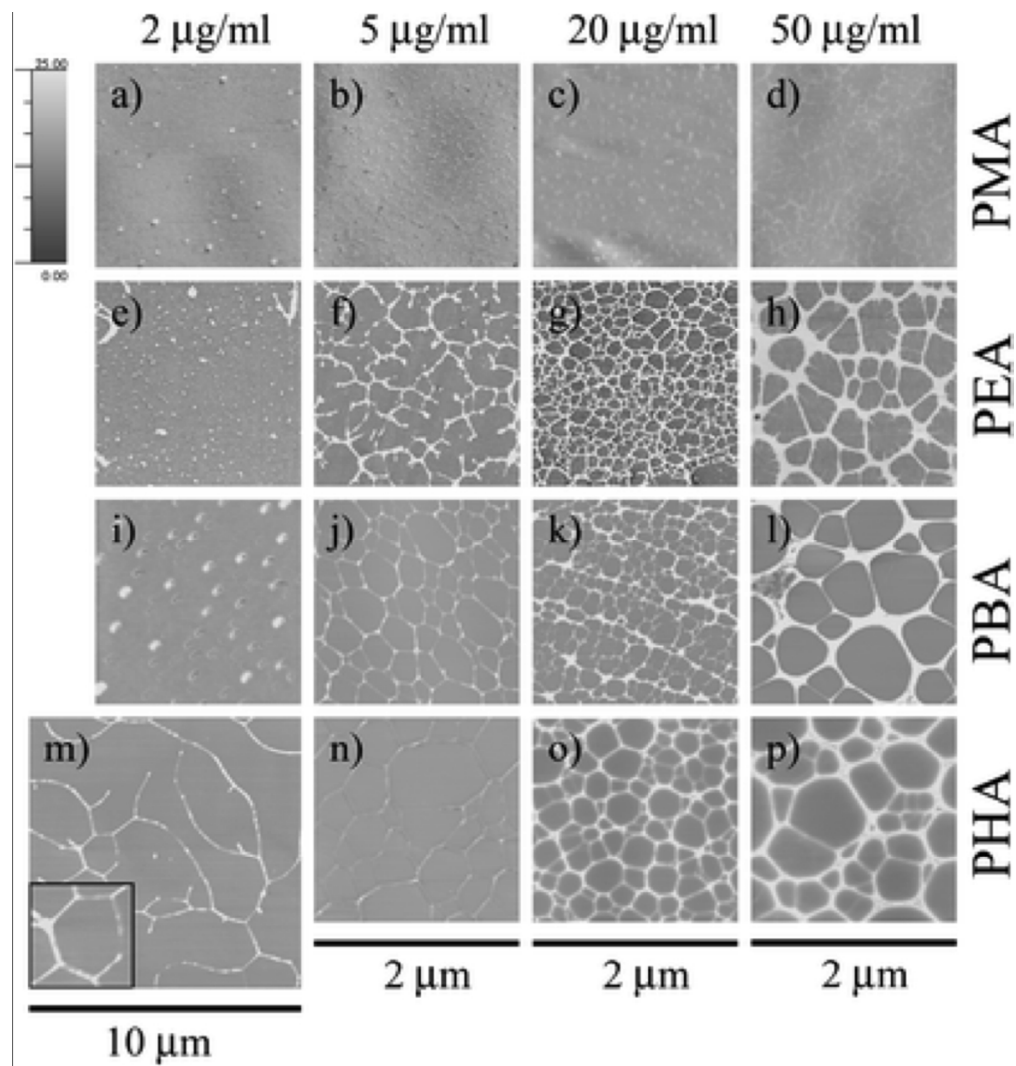


Figure 8: Protein adsorption on different materials
 Atomic force microscopy images of fibronectin adsorbed onto: poly(methyl acrylate), poly(butyl acrylate), poly(ethyl acrylate) and poly(hexyl acrylate) (PMA, PEA, PBA and PHA respectively) after 10 minutes of incubation in different solution concentrations. Images [120].

166x175mm (150 x 150 DPI)

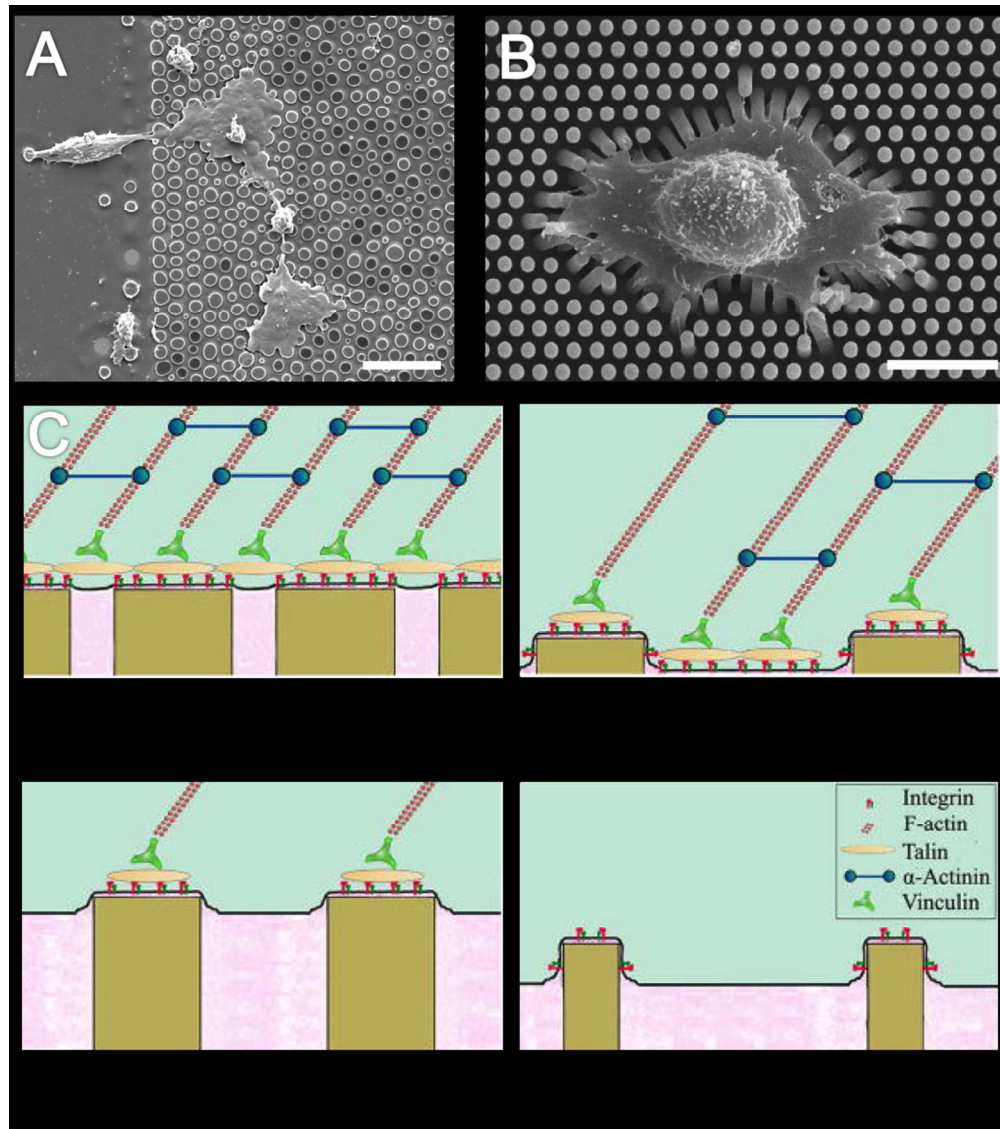


Figure 9: Topographic influences over cell morphology and adhesion
 Electron micrograph image (A) of fibroblast contact guidance on poly(styrene) pillars. Electron micrograph image (B) of a fibroblast grown on flexible polydimethylsiloxane pillars illustrate the tractional forces with which cells interact with the substrates on which they are grown. Schematic diagrams (C) illustrate the importance of topography feature dimensions for cell adhesion formation and frequency and cytoskeletal stresses. Scale bar is 20 μm (A) and 15 μm (B). Images A [126], B [122] and C [131].

191x216mm (150 x 150 DPI)

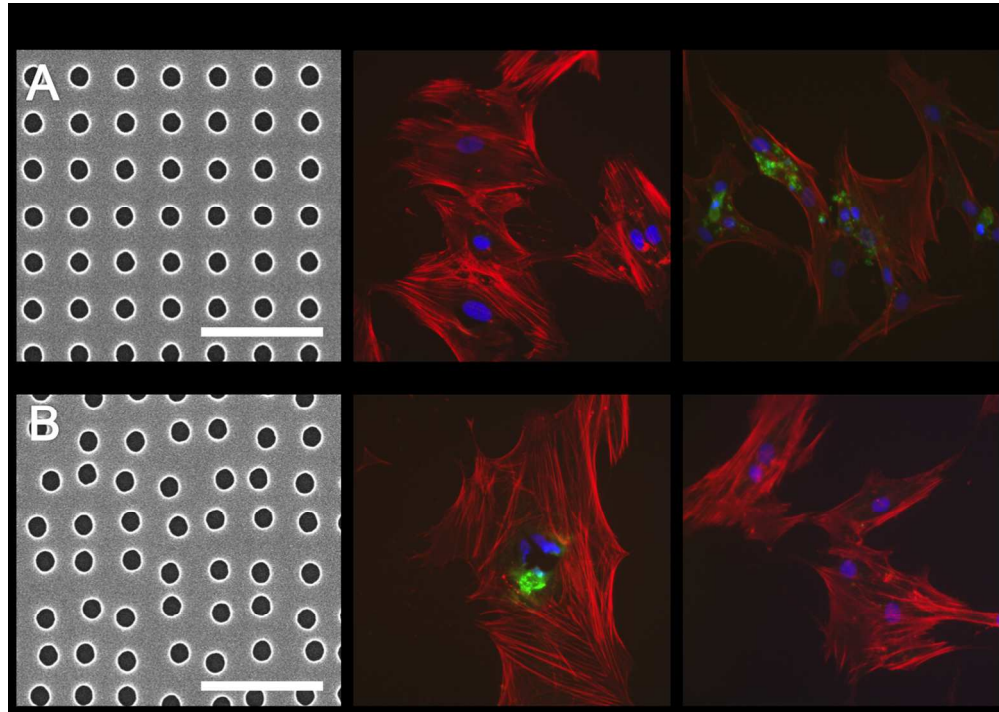


Figure 10: Topographic influences over stem cell differentiation/multipotency
Scanning electron microscopy (left hand side of A and B) and fluorescence microscopy (central and right hand side images of A and B) images of MSCs cultured on substrates patterned with 120 nm diameter nanopits (100 nm deep) with ordered (A) and disordered (B) layouts. In the fluorescent images: nuclei are highlighted in blue, actin cytoskeleton in red and the osteogenic marker osteopontin (A) or the pluripotency marker STRO-1 (B) in green. Scale bars are 750 nm.

236x168mm (150 x 150 DPI)

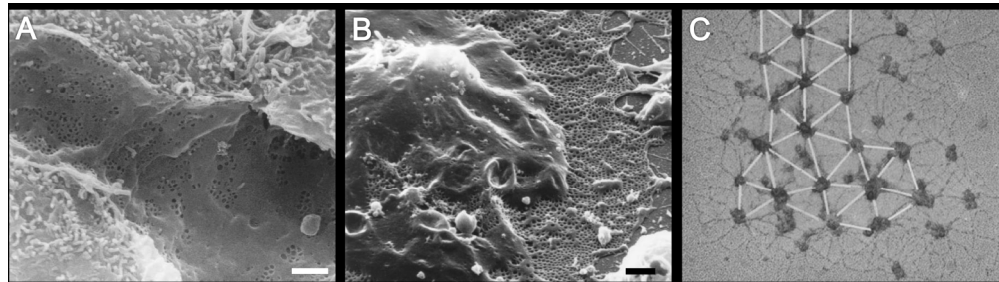


Figure 11: The in vivo bone marrow stem cell niche
Electron microscopy images showing (A) fenestrae in the rat sinusoidal epithelium (B) a liver sinusoidal endothelial cell after treatment with latrunculin highlighting large fenestrated areas and (C) type 10 chicken collagen. Scale bars = 1 (A), 2 μm (B) and 200 nm (C). Images A and B [152] and C [153].

372x103mm (150 x 150 DPI)