JOURNAL OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE
J Tissue Eng Regen Med (2014)
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/term.1957

REVIEW

Using biomaterials to study stem cell mechanotransduction, growth and differentiation

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Abstract

Self-renewal and differentiation are two fundamental characteristics of stem cells. Stem cell self-renewal is critical for replenishing the stem cell population, while differentiation is necessary for maintaining tissue homeostasis. Over the last two decades a great deal of effort has been applied to discovering the processes that control these opposing stem cell fates. One way of examining the role of the physical environment is the use of biomaterial strategies that have the ability to manipulate cells without any requirement for chemical factors. The mechanism whereby cells have been found to respond to a mechanical stimulus is termed mechanotransduction, the process by which a mechanical cue (or alteration in cell spreading changing internal cellular mechanics, i.e. intracellular tension) is transduced into a chemical signal inside the cell, eliciting changes in gene expression. This can occur either directly, as a result of changes in the cell cytoskeleton, or indirectly through a series of biochemical signalling cascades. The main focus of this review is to examine the role of mechanotransduction in the differentiation and self-renewal of stem cells. In particular, we will focus on the use of biomaterials as a tool for examining mechanotrandsuctive effects on self-renewal and differentiation.

Received 22 October 2013; Revised 11 June 2014; Accepted 28 August 2014

Keywords **stem cell; self-renewal; differentiation; mechanotransduction; cell adhesion; integrins; topography; biomaterials**

1. Stem cells

There are different types of stem cells, including adult (e.g. mesenchymal stem cells, MSCs), embryonic (ESCs) and inducible pluripotent (iPSCs). Adult stem cells are derived from adult tissues and are more accessible. However, they are less potent than embryonic stem cells. ESCs are derived from embryos and are therefore associated with many ethical issues. Pluripotent stem cells can be produced following viral transfection of a terminally differentiated cell, using four key genes, *Oct4* (Pou5f1), *Sox2*, *cMyc* and *Klf4*. They provide us with the potential to address the issues of achieving increases in pluripotency and accessibility. However, they are also the subject of ethical issues (Takahashi *et al*., 2007).

Throughout different studies on stem cell differentiation in normal culture, the addition of differentiation factors into the medium is the accepted route; for example, dexamethasone addition to the culture medium induces osteogenic differentiation; insulin is used to induce adipogenic differentiation; and retinoic acid is used for neural differentiation (Pittenger *et al*., 1999; Jaiswal *et al*., 2000; Mbalaviele *et al*., 2000). However, over the last decade, researchers have shown that the sole use of nanotopography can induce differentiation without the need for supplements for specific media (Engler *et al*., 2006, Dalby *et al*., 2007c). This is an important issue when culturing cells with the potential to be transplanted into patients.

2. The stem cell niche

Schofield (1978) first reported the concept of a stem cell niche, where a stem cell may reside and associate with

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other cells types that help to regulate their behaviour and maintenance. The first stem cell niche to be identified, a germ-line stem cell niche in *Drosphila melanogaster*, was reported by Xie and Spradling (2000). Research has since identified niches associated with various stem cell types in mammals, such as haematopoietic, neural, skin and intestinal (Bjerknes and Cheng, 2001; Calvi *et al*., 2003; Doetsch, 2003; Zhang *et al*., 2003; Tumbar *et al*., 2004; Tavazoie *et al*., 2008). While a definitive stem cell niche associated with MSCs has not yet been identified, it has been proposed that MSCs reside within putative perivascular (Simmons, 2009) and endosteal (Bianco, 2011; Ehninger and Trumpp, 2011) niches. However, using these previously identified stem cell niches, it is possible to identify key factors which appear to be essential for maintaining the niche environment. Physical components include the interaction of stem cells with other cell types, the basement membrane and extracellular matrix (ECM), whilst intrinsic and extrinsic signalling from other cells within and outwith the niche, as well as neural and metabolic signalling, can also serve as regulators of self-renewal or differentiation (Li and Xie, 2005; Scadden, 2006).

2.1. Stem cell division

Proliferation, and therefore cell division, is an inherent part of self-renewal. However, the outcome of cell division can be different for both daughter cells. Stem cell division can be classed as either symmetrical, in which the stem cell divides to produce identical daughter cells, or asymmetrical, resulting in one stem cell and one differentiating cell (Morrison and Kimble, 2006; Wilson *et al*., 2008). It is the balance between symmetrical and asymmetrical cell division that can lead to either replenishment of the stem cell pool or maintenance of tissue homeostasis. *In vivo*, within the stem cell niche, the orientation of the mitotic spindle is thought to play a critical role in regulating symmetrical versus asymmetrical differentiation, with the relative positioning of daughter cells within the niche potentially resulting in different cell fates (Wodarz, 2005; Kanamori *et al*., 2008; Yamashita, 2009, 2010; Yamashita *et al*., 2010; Yadlapalli and Yamashita, 2013). *In vivo*, it is thought that stem cells undergo mainly asymmetrical differentiation, maintaining a constant stem cell pool while allowing for tissue replenishment. However, this propensity for stem cells to undergo asymmetrical cell division when cultured *ex vivo* leads to a diminishing stem cell population as the number of differentiating progenitor cells increases. This loss of the stem cell population limits the use of adult stem cells in particular as a therapeutic target where *ex vivo* expansion of the population is required following extraction from a patient (Banfi *et al*., 2000; Muraglia *et al*., 2000; Sherley, 2002; Siddappa *et al*., 2007; Sarugaser *et al*., 2009).

2.2. Cell cycle control of self-renewal and differentiation

Whilst the molecular control of MSC self-renewal and differentiation is still not fully understood, *in vitro* studies examining various stem cell types have identified a functional link between self-renewal, the cell cycle and cell division (Walkley *et al*., 2005; Orford and Scadden, 2008; He *et al*., 2009). The cell cycle is made up of four distinct stages, growth phase 1 (G_1), synthesis phase (S), growth phase 2 (G_2) and mitosis (M). In addition, cells that are no longer undergoing division due to quiescence or senescence can exit the cell cycle and enter into G_0 phase. Progression of the cell cycle is tightly regulated by intrinsic checkpoints, which help to ensure the integrity of the genome as well as extrinsic mitogenic signals, with G_1 being split into two phases, an early mitogen-dependent and a late mitogen-independent phase (Elledge, 1996; Foster *et al*., 2010).

A role for the cell cycle in stem cell self-renewal first became evident when it was discovered that embryonic stem cells (ESCs) have a significantly shorter G_1 phase than other somatic cells (Savatier *et al*., 1994; White and Dalton, 2005; White *et al*., 2005; Becker *et al*., 2006). Further evidence for this includes observations that bone marrow haematopoietic stem cells (HSCs) maintain their cell population and preserve their self-renewal capabilities by continuously switching between states of quiescence and self-renewal, entering and exiting the cell cycle (Cheshier *et al*., 1999; Wilson *et al*, 2008). Furthermore, it was found that induced pluripotent stem cells (iPSCs) adopt a shortened G_1 phase, similar to ESCs, indicating that G_1 phase in particular may play a critical role in regulating self-renewal versus differentiation (Ghule *et al*., 2011). Adult stem cells are thought to utilize other mechanisms, such as quiescence, to evade passing through G_1, thereby protecting the population from the effects of mitogenic factors (Jaiswal *et al*., 2000). It is this requirement for mitogen-activated protein kinases (MAPKs) in both the progression through G_1 cell cycle phase and in the early onset of differentiation that makes self-renewing stem cells particularly susceptible to differentiation (Jaiswal *et al*., 2000; Zhang and Liu, 2002). In the case of MSCs *in vitro*, they are found to be a slowly proliferating population, with the onset on differentiation coupled with a decrease in proliferation and the upregulation of lineage-specific genes (Stein *et al*., 1990; Stein and Lian, 1993; Ullah *et al*., 2013).

3. Biomaterial control of stem cell self-renewal and differentiation

The role of mechanical cues *in vivo* can be assessed using biomaterials *in vitro*. In many of the studies listed below, the stem cells of choice have been MSCs. These are particularly useful, due in part to their accessibility but also

DOI: 10.1002/term

because their broad differentiation profile makes them ideal for use in clinical applications.

MSCs possess the capacity to self-renew and exhibit multipotency (Pittenger *et al*., 1999). They have the ability to differentiate into a number of cell types, including osteoblasts and adipocytes (Pittenger *et al*., 1999), myoblasts (Engler *et al*., 2006; Narita *et al*., 2008), chondrocytes (Muraglia *et al*., 2000), neural marker-expressing cells (Yim *et al*., 2007), fibroblasts and stromal cells (Caplan, 2009). However, the precise mechanisms underlying MSC self-renewal require further elucidation.

Biomaterials can be used to assess the effect of changing chemistry, topography or physical environment on cells. In recent years the effect of these parameters on the differentiation and self-renewal of stem cells has been studied (see review by Ingmar Schoen and Vogel, 2013).

3.1. Chemistry

The use of chemistry to pattern surfaces for culturing cells has many advantages. These include being able to define precisely the composition of the surface, such that the density or type of ligands presented can be tailored. This allows us to examine the role that individual ECM components play in regulating stem cell adhesion and differentiation. In addition, surfaces can be produced that alter cell shape on a single-cell basis, allowing for artefacts caused by differences in cell density and cell–cell contact to be eliminated. Surfaces can be produced using two techniques, microcontact printing (μCP), using self-assembled monolayers (SAMS), and SAMS presenting a maleimide group for immobilization of peptides such as RGD (Mrksich *et al*., 1996, 1997; Mrksich and Whitesides, 1996; Chen *et al*., 1997).

μCP was initially used to look at the effect of protein absorption on cell adhesion and other fundamental cell processes. However, a study conducted using μCP to alter stem cell density, and therefore the degree of cell spreading, was instrumental in demonstrating the influence of mechanical cues during differentiation (McBeath *et al*., 2004). This work showed that not only is stem cell differentiation and lineage commitment (osteogenic vs adipogenic differentiation) mechanically regulated, but also identified RhoA as a key mechanical transducer. More recent work using μCP to alter cell shape found that MSCs cultured on patterns of star and flower shapes were able to direct lineage commitment as a result of increased/decrease actomyosin contractility on the stars and flowers, respectively (Kilian *et al*., 2010). In this study the authors went on to propose that changes in cell shape not only alter cell contractility but also their responsiveness to extracellular signalling molecules.

The mechanical regulation of epidermal stem cell terminal differentiation has also been well documented using μCP (Connelly *et al*., 2010, 2011). By altering parameters such as cell shape, ECM density and composition, the authors were able to deduce that terminal differentiation is reliant on cytoskeletal actin to mediate changes in cell shape over changes in ECM composition and density. In this case the authors found that levels of G-actin, as dictated by cell spreading on μCP surfaces, regulate the activity of serum response factor (SRF), a regulator of terminal differentiation. This is in contrast to MSCs, where cell shape changes in cytoskeletal tension, not G-actin levels, regulate differentiation (Connelly *et al*., 2010, 2011).

Using a second technique, SAMs have been developed to precisely mimic ECM composition and density. Initial studies using nanopatterns displaying the RGD ligand in various degrees of order and disorder examined the fundamentals of integrin binding and focal adhesion formation (Cavalcanti-Adam *et al*., 2007). As a result of this study, it was suggested by the authors that a key limitation in focal adhesion formation is in fact due to the limiting size of integrin-binding proteins, such as paxillin, as this creates a minimal lateral distance over which binding can occur.

More recently, work has shown the effect that the density and affinity of RGD ligands can have on influencing stem cell fate (Kilian and Mrksich, 2012). Using both a cyclic RGD ligand with a higher affinity for integrin binding, as well as a lower affinity linear RGD ligand, at both higher and lower densities, (Kilian and Mrksich, 2012) found that the lineage commitment of MSCs could be tailored to three distinct cell fates (osteo-, myo- and neurogenic), depending on the combination of RGD density and affinity. Together these studies highlight the role that mechanical cues play in regulating stem cell differentiation and, importantly, provide insight into how these mechanical cues elicit changes in gene expression and differentiation.

In terms of using 'pure' chemistry (surfaces with different chemical functionality) there have also been several major observations. For example, in relation to chemical information, different levels of complexity have been explored. Simple surface chemistry (OH, CH_3, COOH, NH_2) encourages deposition of the appropriate proteins from serum, which, in turn, dictates MSC differentiation (Curran *et al*., 2006). The same group, using precise patterning of CH_3-modified surfaces, also demonstrated retention of MSC markers and hence preservation of stem cell growth (Curran *et al*., 2010). Anderson *et al*. (2004) and Langer and Tirrell (2004) developed array-based methods to rapidly screen large libraries of chemical functionality for MSC differentiation potential. In 3D hydrogel scaffolds, remarkably simple chemical functionality was recently shown to induce controlled MSC differentiation, with hydrogels carrying *t*-butyl and phosphate functionalities giving rise to osteogenesis and adipogenesis, respectively (Benoit *et al*., 2008).

3.2. Topography

The use of topographically patterned surfaces to study mechanotransduction was first pioneered in the 1950s and 1960s. In these early studies, cells were shown to be

DOI: 10.1002/term

responsive to topographies such as grooves, with cells aligning to and along the grooves in what is known as contact guidance (Curtis and Varde, 1964; Weiss and Garber, 1952). Later, as techniques more commonly applied to the electronic industry became accessible, this made the production and variety of topographic surfaces more readily available. Since this early research, the size of the surface features that can be achieved has decreased dramatically, with features in the nanoscale range now commonly produced. The drive to produce increasingly smaller features is fuelled by an abundance of nanoscale features and proteins that make up the ECM, and to which cells readily come into contact within their native environment. Early research identified cellular interactions with topography to have an effect on cellular functions such as proliferation, morphology, adhesion and gene expression (Dalby *et al*., 2004, 2008; Milner and Siedlecki, 2007; Bettinger *et al*., 2008; Biggs *et al*., 2009; Yim *et al*., 2010). However, further research using stem cells showed that these changes in gene expression could ultimately influence the fate of stem cells (Dalby *et al*., 2007a, 2007b, 2007c; Yim *et al*., 2007).

Topography at the nanoscale level has been found to alter focal adhesion size and orientation (Biggs and Dalby, 2010; Tsimbouri *et al*., 2014). This has been found, in turn, to alter the cytoskeletal arrangement within the cell, altering cell shape and intracellular signalling. Using nanoscale-sized pits with altered geometries, MSCs were found to have increased focal adhesion size (Figure 1a, arrow) and an upregulation of osteogenic differentiation markers (e.g. osteopontin, *OPN*) when the nanopits were slightly disordered (Figure 2b). However, in a recent study the authors also found that when MSCs were cultured on ordered nanopits, MSCs instead underwent self-renewal and prolonged growth as multipotent stem cells (Figure 2a). This was found to correlate with a decrease in focal adhesion size and intracellular tension, indicating that self-renewal requires an intermediate level of cellular tension, whilst adipogenic and osteogenic differentiation require a lower and higher level,

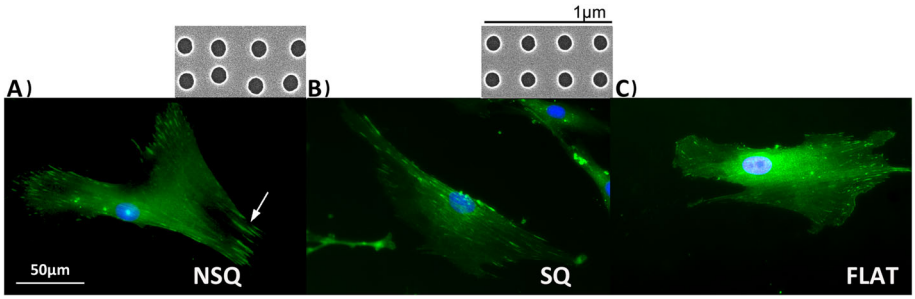

Figure 1. Fluorescent microscopy images of MSC morphology and attachment on nanopit substrates. (A) On disordered nanotopography [near square (NSQ), 120 nm diameter pits, 100 nm deep, average 300 nm centre–centre spacing in a square arrangement but with up to 50 nm placement error; left SEM], the MSCs were spread with large lamellae and displayed an elongated cell shape; cells were observed to develop super-mature adhesions (arrow). (B) On highly ordered nanotopography [square (SQ) as before, but with no placement error; right SEM], cells displayed less-spread morphology and adopted a stellate shape with smaller adhesions. (C) Similarly, on the flat control substrate, MSCs displayed an intermediate morphology/adhesion pattern: green, vinculin; blue, nucleus

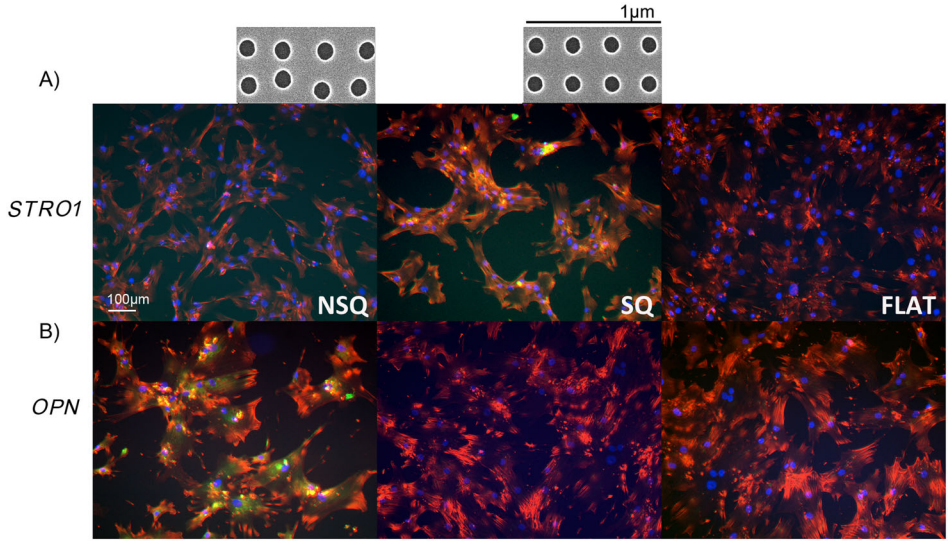

Figure 2. Fluorescent microscopy images of cell surface markers for MSC self-renewal/differentiation status on nanotopographies. (A) Only MSCs cultured for 4 weeks on the SQ surface express the MSC marker *STRO-1*. (B) The MSCs cultured on NSQ50 showed strong expression of the bone marker osteopontin (*OPN*). MSCs on planar controls did not express either of these genes after 4 weeks of culture: red, actin; green, STRO-1 or OPN; blue, nucleus

DOI: 10.1002/term

respectively (McMurray *et al*., 2011; Tsimbouri *et al*., 2012, 2014). This is in agreement with early work published by McBeath *et al*. (2004), as discussed previously.

Other studies using different topographic surfaces, such as carbon nanotubes, have also shown an induction of osteogenic differentiation (Oh *et al*., 2009). MSCs have also been proposed to undergo transdifferentiation, expressing markers associated with neural differentiation (Yim *et al*., 2007). Using nanogrooved surfaces, MSCs have also been shown to express neural markers in the absence of chemical induction factors. The authors further went on to show that phosphorylated FAK plays a critical role in transducing topographic signals for regulating cell fate. Whilst a large body of research has been published examining the response of MSCs to topography, other stem cell types are increasingly being investigated, such as embryonic and neural stem cells (Lim *et al*., 2010; Wang *et al*., 2011b; Chen *et al*., 2012; Kingham and Oreffo, 2013; Kingham *et al*., 2013; Qi *et al*., 2013). The ability to culture stem cells without the requirement for chemical supplements makes topography an attractive prospect for clinical applications in particular.

3.3. Elasticity

Tissues within the body exhibit different physical properties (Figure 3). The cells are entrenched inside a complex fibrous ECM. The mechanical properties of the ECM allow the tissues to function properly by regulating cellular processes, such as attachment and spreading, proliferation, migration and stem cell differentiation (Discher *et al*., 2005; Trappmann and Chen, 2013). Hence, the ECM can directly control many important biological processes, such as embryonic development and adult tissue homeostasis (Wozniak and Chen, 2009). ECM is also involved in the control of the pathogenesis of diseases such as cancer and fibrosis (Paszek and Weaver, 2004; Paszek *et al*., 2005; Georges *et al*., 2007).

The direct involvement of ECM in signal transduction through integrin receptors has been well studied (also mentioned in section 4). However, the physical properties of the matrix, such as its elasticity or stiffness, are equally important in the control of cellular processes (Discher *et al*., 2005; Vogel and Sheetz, 2006). Engler *et al*. (2006), in their effort to provide a new approach to direct stem cell fate, used techniques originally employed to study the effects of matrix elasticity on the growth and morphology of differentiated cells (Engler *et al*., 2006). They showed that a soft matrix of 0.1 kPa would support differentiation of MSCs into neuronal-like cells, a medium elasticity matrix of 11 kPa induced myogenic differentiation, and a stiff matrix of 34 kPa promoted osteogenic differentiation. Fu *et al*. (2010) used micromoulded elastomeric micropost arrays consisting of a gradient of different rigidity microposts and examined how MSCs cultured on these micropost arrays would respond to changes in micropost rigidity. They found that, in the presence of bipotential differentiation medium on rigid microposts, hMSCs showed osteogenic potential. In contrast, cells on soft microposts displayed adipogenic differentiation.

Moreover, the maintenance of the appropriate mechanical inputs from the ECM are required for maintaining the differentiated state of the lineage-committed cells and hence self renewal. Alcaraz *et al*. (2008) cultured mammary epithelial cells on different elasticity biomimetic surfaces and they used the expression of β-casein, a milk protein, as a marker of differentiation. They further showed that the cells on soft substrates ($\sim$100 Pa) maintained the expression of β-casein, whereas those on more rigid substrates ($>$250 Pa), stiffer than normal mammary tissue, showed a reduced β-casein expression, a sign of dedifferentiation or even tumourigenesis.

Furthermore, Gilbert *et al*. (2010), using a mouse model, tested the hypothesis that the elastic modulus of the cell microenvironment plays an essential role in muscle stem cell (MuSC) self-renewal and function in muscle regeneration. They reported that when MuSCs were cultured on substrates mimicking the rigidity of muscle tissue (12 kPa), they self-renew to generate stem cell progeny that can potentially repair damaged muscle when transplanted *in vivo*.

Recent work on matrix elasticity and downstream regulators has identified (Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ; also known as WWTR1) as nuclear transmitters of mechanical signals applied by ECM rigidity and cell shape (Dupont *et al*., 2011; Aragona *et al*., 2013). Their function is closely regulated by the Rho GTPase activity and tension of the actomyosin cytoskeleton generated upon cell adhesion to the ECM. The authors reported that their regulation is independent of the Hippo–LATS cascade. The authors further showed that YAP/TAZ activity is essential for the ECM stiffness-induced differentiation of MSCs and for the survival of endothelial cells regulated by cell geometry. Recent work by Yang *et al*. (2014) on MSC cultures on different-stiffness hydrogels revealed that stem

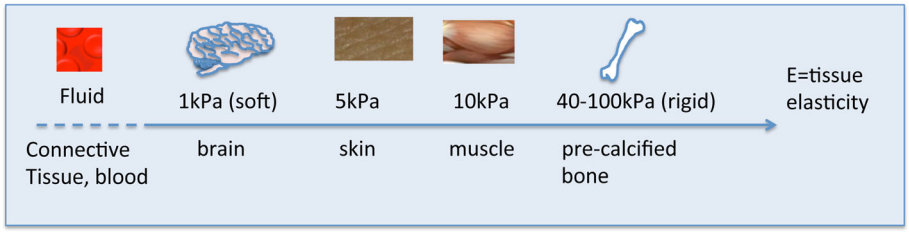

Figure 3. Tissue elasticity: solid tissues exhibit a range of stiffness, as measured by the elastic modulus, *E*

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cells have mechanical memory and that YAP/TAZ act as an intracellular mechanical stiffness sensor.

4. Cell–extracellular matrix adhesions

Adhesion of cells to the ECM is mediated via transmembrane integrins (Humphries, 1990). Integrin binding can occur via an outside-in or inside-out mechanism (Harburger and Calderwood, 2009). This creates a dynamic relationship whereby the ECM can transduce signals from the environment inside the cell or, conversely, signalling from inside the cell can result in remodelling of the ECM. For the purposes of this review, however, it is their role as mediators of outside-in signalling that is interesting, due to biomaterials now being designed to mimic various aspects of the extracellular environment. In particular, research that has identified a correlation between focal adhesion size and intracellular tension to regulating stem cell differentiation, using various biomaterial strategies, has been well documented (Goffin *et al.*, 2006; Dalby and Yarwood, 2007; Biggs and Dalby, 2010; Kilian *et al.*, 2010; Yim *et al.*, 2010). More recently, studies have also identified these to be key regulators of self-renewal (Gilbert *et al.*, 2010; McMurray *et al.*, 2011; Tsimbouri *et al.*, 2012). This has already been discussed earlier in this review.

The binding of integrins to ECM proteins is a complex process (Figure 4) and serves two functions: first, they form points of contact between the cell and a surface, allowing tension to be created; and second, as signalling transmitters, relaying information from the ECM to the cell. Although integrins themselves do not possess kinase activity, the binding of various integrin-binding molecules, such as focal adhesion kinase (FAK), leads to the activation of intracellular signalling cascades. These include extracellular signal-regulated kinase–mitogen-activated protein kinase (ERK–MAPK), a key pathway involved in regulating multiple cellular processes such as proliferation and differentiation (Miyamoto *et al.*, 1995; Zhu and Assoian, 1995).

Initial binding of integrins to ECM proteins results in changes in both their conformation and affinity resulting in integrin clustering and the formation of immature focal complexes (Kawakami *et al.*, 2001). The formation of these immature focal complexes and subsequent binding of actin linker proteins, such as vinculin and talin, result in actin stress fibre formation and increased focal adhesion size as the cell tries to counterbalance the internal forces that result from increased cytoskeletal tension.

4.1. Integrin-mediated mechanotransduction

As mentioned previously, the interactions between integrins and the ECM enable cells to generate tension and relay information from the ECM to the nucleus (Figure 5). This transformation of a mechanical signal into

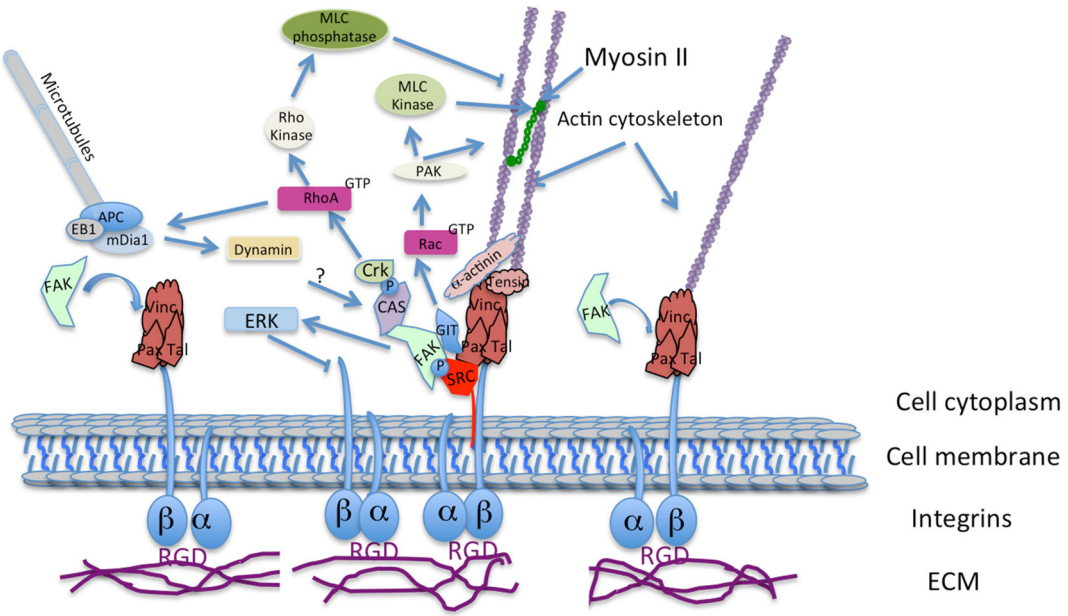

Figure 4. Focal adhesion formation/turnover. Immature or nascent adhesions (right) are linked to the actin cytoskeleton through a linking protein complex consisting of talin, vinculin and α-actinin. Signalling adaptors FAK and paxillin are also recruited to these focal complexes. Upon adhesion formation, signals are generated activating Rac, promoting actin polymerization and preventing myosin II coupling in the lamellipodium. These signalling cascades are required for the turnover of some adhesions as the cell moves. Some adhesions mature into focal adhesions (middle); during this process the focal complexes become larger and longer with the addition of new proteins, such as tensin. Some components, such as talin, vinculin and p130Cas, have conformations that are tension-sensitive. RhoA activation leads to focal adhesion formation and actin bundling, due to increased myosin II activity. Dynamin is involved in the internalization of the integrins and microtubule targeting, which may contribute to adhesion disassembly. Adapted from Vicente-Manzanares *et al.* (2005)

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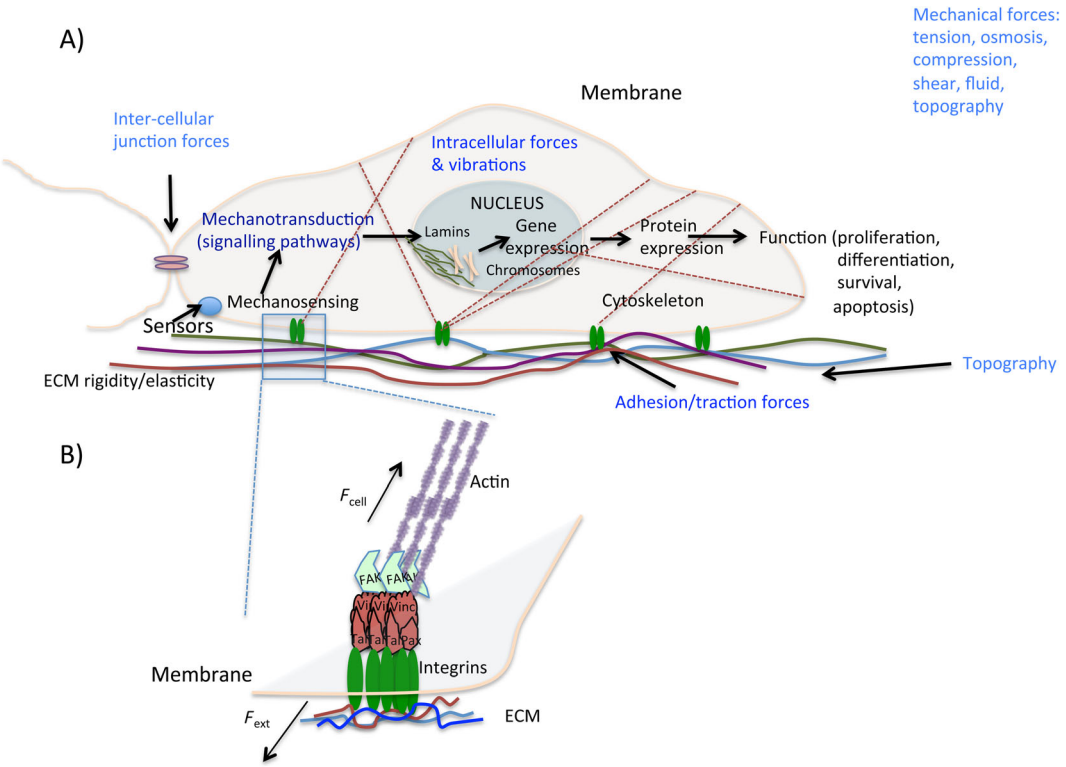

Figure 5. Schematic diagram showing that mechanical forces stimulate cells through the activation of mechanosensors, some of which may be the receptors that respond to ligands. (A) Cells can be exposed to multiple types of forces, such as shear forces through fluid flow over the cell surface, tensile/traction forces through the ECM, and internal cytoskeletally generated contractile forces (actomyosin contraction, microtubule polymerization and depolymerization, osmotic forces). Signalling pathways are then activated through the sensors, leading to modulation of gene expression. Consequences of such mechanotransduction are modulations of protein expression and cellular functions such as proliferation and differentiation. Illustrated is a single cell attached to a complex ECM (multicoloured sheet) through the focal adhesions (square and inset). (B) Focal adhesion, showing the balance of external (F_{ext}) and internal (F_{cell}) forces in driving stress at a mechanosensor. Depicted are actin stress fibres (purple) anchored into focal adhesions (i.e. vinculin, talin, paxilin, focal adhesion kinase) that bind to the ECM through integrins (green). This balance of forces provides the stress necessary for mechanical sensing

a biochemical signal is known as mechanotransduction. The cytoskeleton is made up of various components that create a dynamic structure that not only provides a physical and biochemical link to the extracellular environment but also creates forces required for cell migration and maintaining cell shape. The three main cytoskeletal components are microfilaments, microtubules and intermediate filaments. It has been proposed that each of these components can work together to maintain cellular tensegrity, where cell shape is maintained through continuous tension (Ingber, 1997a, 1997b).

This cytoskeletal percolation provides non-homogeneity to the cytoplasm and hence provides the basis of conveyance of mechanical changes to the nucleus. The cytoskleton is attached to the nucleoskeleton, the lamins, via linkers of the nucleoskeleton and the cytoskeleton (LINC complexes) (Ostlund *et al.*, 2009). This provides direct mechanical connetection of the nucleus to focal adhesions via the cytoskeleton and from there into the extracellular environment to which adhesions are anchored.

Swift *et al.* (2013) used proteomic analysis of different human and mouse tissues to identify possible candidates involved in cell responses to changes in extracellular matrix (ECM) stiffness. They found that lamin A was more prevalent in stiff tissues and that this expression varied, depending on the tissue's stiffness. In addition, they observed that increased lamin A expression increased nuclear viscosity, rendering the nucleus more resistant to physical stress. It has further been proposed that direct mechanotransduction occurs as a result of changes in the cytoskeleton that can directly affect lamin-bound intermediate filaments at the nucleus, altering the spatial arrangement of lamin-bound chromatin and chromosome packing or positioning, subsequently altering gene expression (Maniotis *et al.*, 1997a, 1997b; Wang *et al.*, 2001, 2009; Dalby *et al.*, 2007a, 2007b; McNamara *et al.*, 2012; Tsimbouri *et al.*, 2013).

4.2. Integrins and the cell cycle

In addition to mitogenic factors, the cell cycle is found to be under regulation, again at G_1, by adhesion and mechanical cues. Early evidence through cell-spreading studies showed that the degree of cell spreading and intracellular tension are key factors in mediating the rate of proliferation (Curtis and Seehar, 1978; Folkman and Moscona, 1978). As a result of integrin binding, FAK becomes activated via phosphorylation (Kornberg *et al.*, 1992). This phosphorylation has been shown to be under

DOI: 10.1002/term

regulation by cellular spreading, with increased cellular spreading leading to higher levels of FAK phosphorylation. Conversely unphosphorylated FAK activates p190RhoGAP, an inhibitor of RhoA, leading to decreased actin polymerization and intracellular tension (McBeath *et al*., 2004). Phosphorylation of FAK is known to activate the ERK kinase pathway (ERK2) and the induction of cyclin D1, a critical regulator of the G_1–S cell cycle transition. In support of this, research using RGD nanopatterned surfaces with different spacing between the ligands found that disrupted integrin clustering decreased stable focal adhesion formation and cell spreading (Schlaepfer *et al*., 1998). Studies have also found that the activation of different integrins can have opposing effects, therefore indicating that the composition of the ECM plays a key role in mediating cell-cycle progression. In particular, integrin subunits α5 and α6, have been implicated in promoting cell cycle progression, whilst $\alpha 2\beta 1$ has been shown to decrease proliferation in certain cell types (Lavoie *et al*., 1996; Wang *et al*., 2011a, 2011b).

4.3. Integrins and stem cell division

Integrins and other cell adhesion molecules play an important role in determining cell polarity, orientation of the mitotic spindle and, ultimately, the plane of cell division (Toledano and Jones, 2009; Marthiens *et al*., 2010). During stem cell division, the plane of cell division is thought to be an important factor in regulating whether the stem cell undergoes symmetrical or asymmetrical division. In a study by Thery and Bornens (2006), the spatial distribution of the ECM was shown to play a crucial role in determining the plane of cell division. In a similar study, Toyoshima and Nishida (2007) also used micropatterning to dictate the orientation of the mitotic spindle. However, in this study the authors also showed that when β1-integrin was blocked, cells lost the ability to orientate their spindles, highlighting a role for individual integrins in regulating stem cell division. Kosodo *et al*. (2004) similarly found that when integrin binding is blocked in neural stem cells, this creates a shift in the plane of cell division, resulting in a switch from asymmetrical to symmetrical cell division.

Within the stem cell niche, however, the orientation of cell division is important for dictating cell fate (Siller and Doe, 2009; Yamashita 2009, 2010; Yamashita *et al*., 2010). In the case of stem cells, as discussed previously, cell division can result in either symmetrical or asymmetrical outcomes, whereby mechanical cues may also dictate the plane of cell division, as demonstrated by Fink *et al*. (2011). *In vivo*, this asymmetry may result in the differential exposure of daughter cells to chemical factors. Indeed, the role of asymmetrical exposure to factors such as wnt3a was demonstrated by Habib *et al*. (2013), who found that spatially restricted exposure to wnt3a can result in asymmetrical outcomes for dividing stem cells.

5. Mechanotransduction and the primary cilium

The primary cilium is a microtubule-based organelle, which forms as a compartment protruding from the cell membrane into the extracellular space. It has been shown to function as a mechanosensor, most notably in the kidney, where fluid flow within the kidney bends the cilium, allowing entry of Ca^{2+}, and as a signalling hub for key developmental pathways, such as hedgehog, non-canonical wnt, PDGF and calcium signalling (Kiprilov *et al*., 2008; Clement *et al*., 2009, 2013; Schneider *et al*., 2010; Lancaster *et al*., 2011; Wann *et al*., 2012). Formation of the cilium is tightly coupled with the cell cycle, with ciliogenesis occurring during G_0–G_1, and cilia disassembly occurring at the onset of late G_1–S phase (Christensen *et al*., 2008). As the G_1 cell-cycle phase is known to play a critical role in mediating stem cell differentiation, it is therefore not surprising that reducing the rate of cilia disassembly, thereby delaying entry into S phase, has been found to correlate with an increase stem cell differentiation (Kim *et al*., 2011; Li *et al*., 2011). In MSCs and other stem cells, the primary cilium has also recently been shown to play a role in both mechanotransduction and chemically induced differentiation (Clement *et al*., 2009; Tummala *et al*., 2010; Hoey *et al*., 2012). In addition, ciliogenesis has been shown to be responsive to changes in cell shape-induced cytoskeletal changes. In a study using micropatterns of different sizes designed to alter the degree of cell spreading, cilia prevalence and length were found to decrease under increasing cellular spreading (Pitaval *et al*., 2010). These studies highlight an emerging role of the primary cilium in multiple cellular processes and begin to provide us with an understanding of the mechanisms which regulate its structure and function.

6. Conclusions and further directions

6.1. Dynamic surfaces

With the development of new technologies, a more sophisticated set of biomaterials are being created – those with the ability to spatially and temporally alter the environment of the cell *in vitro*. The development of dynamic surfaces is an exciting step towards creating multifactorial environments that better mimic the changing chemistry, geometry, mechanics or topography of the *in vivo* extracellular environment. By combining techniques used in engineering and chemistry, materials have been created that can:

- Alter substrate stiffness through changes in pH or hydrogel composition (Gillette *et al*., 2010; Yoshikawa *et al*., 2011). Surface topography can be tuned using thermally activated surfaces or oxidizable polymers, and can be used to alter topography down to submicrometre scales (Le *et al*., 2011; Ebara *et al*., 2012).

DOI: 10.1002/term

- Switch surface chemistry, by revealing cell adhesion ligands using either photo- or electrosensitive protecting groups (Yeo *et al*., 2001; Yeo and Mrksich, 2006; Wirkner *et al*., 2011). In addition, creating a cleavable linker enables the release or detachment of cell adhesion ligands (Todd *et al*., 2007, 2009).

The use of such techniques will help create surfaces that provide cells with appropriate cues in a temporal manner to enable the study of stem cells in a dynamic *in vitro* system – more akin to the dynamic *in vivo* niche.

7. Summary

We are at an exciting point of scientific endeavour where stem cells are becoming better understood and the possibility for their exploitation in regenerative therapy is eagerly anticipated. We propose that biomaterials may play an vital role in accelerating our understanding of stem cells, through providing appropriate *in vitro* control of stem cells without recourse to the use of complex media containing cocktails of soluble factors to drive differentiation or try to control growth. Use of surface chemistry, nanoscale topography and tuneable stiffness has been used to understand MSC adhesion/differentiation requirements, and we now move to, for example, dynamic surfaces able to turn on stem cell functions on demand – as in the niche. The techniques are also being applied to other stem cell types that will further increase their relevance to developing stem cell-regenerative therapies.

Conflict of interest

The authors RJM, MJD and MPT declare that there is no conflict of interest.

Acknowledgements

This study was supported by grants from BBSRC (grant number BB/G008868/1) and a Wellcome Trust ISSF fellowship awarded to M.P.T.

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DOI: 10.1002/term