We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 4

Substrates with Changing Properties for Extracellular Matrix Mimicry

Frank Xue Jiang

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/53547

1. Introduction

Cell-ECM interactions Fundamental to the success of using biomaterials in medical and health care applications, is the understanding of their interactions with biological tissues and systems. First step towards this end is the elucidation of cell-ECM interactions, which has attracted considerable interest in recent decade. Cellular decision-making process is driven by the internal genetic program and external factors comprising primarily other cells and extracellular matrix (ECM) via soluble factors and direct physical connections such as focal adhesion [1, 2]. Three key features of ECM have been identified of great significance in affecting cells, namely, chemical and biological composition, dimensionality (two- vs. three-dimensional), and physical properties [3-6]. These features can be sensed by cells via cell-ECM linkages, and the resulting signals subsequently follow intracellular pathways and trigger a cascade of events leading to alterations in gene expression and manifestation in phenotype. In contrast to the long recognized chemical composition and adhesive characteristics of the ECM, physical cues including topography, pore size, geometric patterns, and mechanical stiffness and their significance has just started being appreciated [7-10]. Whilst characteristics of ECM have profound effect on cells, cells may also actively exert impact on ECM by secretion of soluble factors or modify properties of ECM, or contribute to maintaining integrity or properties of ECM. At a larger scale, biological systems may actively interact with biomaterials to maintain or re-establish homeostasis.

Dynamic aspect of ECM To date, the majority of the substrates employed in cellular studies and other biological investigations have been of fixed mechanical stiffness and/or adhesive properties throughout cell culture. There is an increasing realization that a cell's microenvironment is dynamic and changing with time [11-13]. It is the case in both pathologic and normal tissues, at the tissue-implant interfaces, and during development and aging [14],

especially for load-bearing and mechanically active tissues (e.g., heart, cartilage, lung) [15]. Not only do these changes naturally occur, but there are also benefits associated with them from a tissue engineering viewpoint, as highlighted in the series of discussions in the March 2005 issue of MRS (Materials Research Society) Bulletin [16, 17] and later studies. Whitesides [18] and Mrksich [19] and their coworkers among a number of investigators pioneered the work on engineering cell growth by using dynamic substrates. Their work and later reports on differential cell responses to materials with different properties suggest that it is beneficial for biomaterials to have controlled changing properties [20]. These facts make it very desirable for the bio-mimetic materials to have the capability of undergoing controlled remodeling with respect to time. They also raised caution in interpretation of the observations made from the majority of the biological studies, where properties of the substrates (e.g., culture flask, Petri dish, and hydrogels) remain unchanged throughout the process.

The scope of this work A significant number of reviews are available on the changes in soluble factors of ECM that may affect cellular behavior (e.g., [21]) and particularly on the changing environment in bioreactors [22] (e.g., nutrients concentration, oxygen level, temperature). Thus, they are not covered in this review. Moreover, flow conditions and the resulting traction forces, and their effect on certain cell types including blood cells (i.e., endothelial cells and red blood cells) have also been intensively reviewed and hence are not discussed here. This is also the case for mechanical forces, strain and stress applied directly to the cells (e.g., [23, 24]) in load-bearing tissues such as bone, cartilage and lung (for reference, see, e.g., [25-27]).

Therefore, this review is focused on the latest studies and current knowledge of two- or three- dimensional substrates with changing or dynamic mechanical and adhesive properties, design and conditions to trigger and achieve designed dynamics, and the impact of in-situ change of these properties on cell behavior, which provides guideline for design of biomaterials for their applications in medical and healthcare applications. Note that mechanical stiffness and elastic modulus were used interchangeably in this work.

2. Dynamic nature of the cell microenvironment

Normal tissues The micro-environment within which cells reside in natural tissues undergoes constant synthesis and degradation [16, 17], and has long been recognized as dynamic and changing [25, 28]. Although the composition of tissues generally remain tightly controlled in maturation, ECM remodeling constantly takes place [25, 29, 30], particularly when under hormonal stimulation or stress responses (e.g., [31]). Cells actively participate in tissue remodeling by secreting and mobilizing ECM molecules [32]. Alterations in ECM composition may result in changes in cell adhesion and /or tissue stiffness [33, 34] which further stimulates cellular responses. For instance, laminin component involved in cell adhesion to ECM is variant due to dynamics in exogenous factors [35]; normal cartilage shows elevated stiffness [36]; and vocal fold tissue exhibits dynamic viscoelastic properties [37]. Some specialized cell types can experience fast adhesion and detachment from ECM [38]. Changing ECM can also modify cell-cell interactions, further affecting cell behavior [39].

http://dx.doi.org/10.5772/53547

Pathological tissues Diseased tissue may possess properties such as mechanical stiffness different from those of the normal tissue [40, 41]. As a typical example, it has been found that tumor cells display enhanced movement towards ECM with lower mechanical rigidity, which is interesting considering the general stiffening phenomenon of tumor tissues [42], and biomechanical characteristics of tissues play a crucial role in tumor development [41]. It has also been shown that during the surgical procedures such as radio-frequency (RF) ablation, tissue properties can be modified [43]. Moreover, changes in ECM composition and relative quantity of ECM molecules can be correlated to pathology. For instance, ECM composition change that occurs during sub-epithelial tissue remodeling proved associated with asthma [33]. ECM remodeling in diseased heart valves is correlated to myofibroblast contractility [44], and certain cell types such as valvular interstitial cells can be activated and contribute to further tissue remodeling [45]. Additionally, ECM remodeling affects tissue mechanical properties in addition to inflammatory responses [46]. Moreover, mechanical forces, as experienced in traumatic brain injury or even under normal conditions, could potentially cause protein aggregation, giving rise to various diseases including neurodegenerative diseases [47]. Furthermore, early investigation of properties of central nervous (CNS) tissue under impact yielded modulus values with considerable variation. As an example, Fallenstein and coworker reported storage modulus of human brain tissue of 0.6 ~ 1.1 kPa under sinusoidal shear stress input mimicking head impact [48].

Development and aging During development, synthesis and degradation of ECM is a controlled process (e.g., [8, 49]), and mis-regulation contributes to many forms of diseases [30] Particularly, the microenvironment for embryonic and adult stem cells is regulated both temporally and spatially [2, 34], and is involved in various developmental processes including responses to soluble factors, cell differentiation, and morphogenesis [12]. ECM in musculoskeletal and other tissues adapts to increasing mechanical requirements by altering the size of tissue components [50] during development. Structural dynamics of ECM components such as collagen, laminin, and fibronectin coincides with estrous cycle and developmental progression [51]. Besides development, aging is also accompanied by changing ECM composition and structures. For example, in connective tissues, aging has been reported to be associated with increase in type I collagen content and decrease in both type III collagen and proteoglycans content, and with collagen fiber disruption and unraveling [50].

Tissue-implant interfaces With the growing interest in developing biomimetic materials for tissue engineering applications, tissue-implant interfaces have been subject to considerable research effort. Previous reports showed that cells can actively modify ECM at the interfaces, and cause drastic changes in tissue or construct mechanics using fibroblast-populated construct and other biomaterials [52, 53]. The study by Lee and co-workers suggested that dynamic moduli of an alginate material may be due to the bioactivities of the chondrocytes encapsulated in the scaffold [54]. In a similar study, different substrate composition and architecture gave rise to distinct levels of modulus increase owing to chondrocytes responses [55]. To take another example, smooth muscle cells (SMCs) in contact with engineered arterial construct displayed distinctive responses in protein synthesis and consequently the mechanical properties of ECM were significantly different [56]. Additionally, biodegradable

materials used in various tissue engineering applications possess changing properties associated with specific degradation profiles.

Engineering advantages It has been suggested that temporal control over substrate or scaffold properties may entail great benefits in engineering cell growth. Among the notable examples is the stem cell differentiation and proliferation. A recent work showed enhanced hepatic functions from differentiated stem cells on softer substrates and improved expansion of undifferentiated cells on stiffer ones [57]. Therefore, it is promising to use stiffer substrates for optimal proliferations and subsequently soften them to gain better hepatic functions once differentiation completes. Langrana group also found that different neurite properties (e.g., axonal length and primary dendrite number) show differential preference towards substrate stiffness [58], suggesting the strategy of promoting nerve regeneration with scaffold of varying properties. Similar approach can be adopted to take advantage of differential cell responses (e.g., migration and functions) to adhesive properties.

The recurring indication from the above discussions is that *in vivo* ECM interacts with cells in many ways, and that the alteration in ECM composition or structures leads to changes in adhesive properties (hence cell adhesion) and/or mechanical properties. This potentially affects a variety of cell types and their properties and functioning, at different developmental stages, under normal or pathological conditions, or upon impact or injury. It also holds promises in offering novel approaches to tissue engineering applications. As a result, it is imperative to understand cellular responses to *changing substrate properties* for basic biology and biomimetic material (including biodegradable materials) design.

3. Types of dynamic substrates and stimuli

Here we consider two major classes of dynamic substrates that are based on self-assembled monolayer, or SAM, and hydrogels, as well as other types of substrates with surface or structural modifications. Since the focus of this work is on mimicking dynamic nature of ECM to examine cellular responses, those dynamic materials that are developed for other specific applications such as drug delivery [59] and do not involve changes that mimic dynamic ECM are beyond the scope of the review.

3.1. Self-assembled monolayer (SAM)

SAMs are formed by adsorption of molecules in solution or gas phase onto substrates in a spontaneous and organized fashion [60], and have emerged as an important candidate of materials in studying cellular responses to dynamic substrates [60, 61] where modifications could be made *in situ*. One of the major research focuses in this direction is to examine the effect of dynamic adhesive property of the substrate on cells, particularly by leveraging the ability to selectively capture or release cells upon application of a variety of stimuli (Table 1).

http://dx.doi.org/10.5772/53547

Type	Substrates	Properties changed and stimuli	Cell model	Observations and notes	Ref.
SAM	SAM incorporating O-silyl hydroquinone moiety	Adhesion on/off *Stimulus: electric potential*	3T3 fibroblasts	Modulation of cell adhesion and migration	[17, 61, 65]
	Electro-active quinine monolayer on Au	Adhesion on/off *Stimulus: electric potential*	3T3 fibroblasts	Selective release of adherent cells	[68]
	Azobenzene containing SAM on Au	Adhesion on/off *Stimulus: UV/visible light*	3T3 fibroblasts	Attachment and release of adherent cells Potential to control part of a single cell or groups of cells	[69]
Polymeric Hydrogel	MMP responsive polymer hydrogel network	Degradation of hydrogel *Stimulus: cell secreted MMP*	Human foreskin fibroblasts (HFFs)	Cell infiltration into the gel network with time	[74]
	Thermo-responsive polymer with photosensitive surface	Adhesion on/off *Stimulus: UV radiation and temperature*	CHO-K1 cells	Reversible control over cell adhesion Ability to control a population of cells	[72]
	poly(NIPAM-co-sodium acrylate) hydrogel films on rigid substrates.	Topographic change (swelling/de-swelling of gels) *Stimulus: temperature*	Porcine epithelial cells	Dynamic patterned substrates Reversible encapsulation of adherent cells	[73]
	DNA crosslinked PAM gels	Crosslinking density↑è Mechanical stiffness ↑, vice versa *Stimulus: ssDNA*	L929 & GFP fibroblasts	On dynamic substrate, L929 cells spread more than those on static stiff substrates (~23 kPa), while GFP fibroblasts respond differently to stiffening and softening of substrates Cell spreading and polarity (aspect ratio) respond differently to stiffness dynamics The range, starting point, and end point of change matter	[81, 83]
	DNA crosslinked PAM gels	Crosslinking density↓èstiffness ↓ *Stimulus: ssDNA*	Primary spinal cord cells	Neurite outgrowth respond to dynamic stiffness The trend in the response match that to the static stiffness except for primary dendrite length	[20]
	HA hydrogel	Crosslinking density change and ECM deposition è Mechanical stiffness change *Stimulus: hydrolysis or enzyme*	human mesenchymal stem cells (hMSCs)	Mechanical properties can be engineered with degradation Stiffness ↑ when degradation equals ECM deposition, and Stiffness ↓ at rapid degradtion Cellular responses to dynamic stiffness are different from static gels with the same initial or ending conditions	[78]

Type	Substrates	Properties changed and stimuli	Cell model	Observations and notes	Ref.
	Methacrylated HA hydrogel	UV exposure è stiffness ↑ *Stimulus: UV radiation and addition of reactive groups for*	hMSCs	Fate of hMSCs differentiate depends on the dynamics of stiffness change of substrates Adipogenic differentiation favored when cells is on the softer substrate long (stiffening at later times) Osteogenic differentiation when cells are on the stiffer substrate (stiffening at early times).	[79]
	Hydrogel based on PAM crosslinked by photosensitive reagent	Mechanical Stiffness (global or local)↓ *Stimulus: UV radiation*	3T3 fibroblasts	Stiffness decrease of 20-30% upon propose UV radiation Global stiffness decrease results in less spreading Localized softening to anterior and posterior area gives to differential responses	[76]
	PEG based hydrogel with photosensitive crosslinker	Mechanical Stiffness↓ Adhesive property change *Stimulus: UV radiation*	hMSCs and Valvular inter-stitial cells (VICs)	Valvular cell differentiation into myo-fibroblasts is inhibited by softening Good viability of hMSCs	[77]
Other types of substrates	Piezo-controlled substrate and AFM cantilever	Mechanical stiffness with cycling changes *Stimulus: stiffness clamp on AFM*	NIH 3T3	Apparent stiffness↑ leads to cells contraction rate↑ and contraction velocity↓ Changes took place instantaneously, and so did responses Responses were reversible, and consistent for same cell.	[84]
	Photo-active glass substrate with modifications	Adhesion on/off *Stimulus: UV radiation & pro- adhesive molecules*	HEK293, COS, NIH 3T3	Spatio-temporal control over cell adhesion Single cell control	[62]
	Substrates with photo-responsive caged peptides	Adhesion on/off *Stimulus: UV*	3T3 fibroblasts	Modifications of non-adhesive surfaces to adhesive ones	[63]
	PEG-modified ITO microelectrodes on glass substrates	Adhesion on/off *Stimulus: electric potential*	HepG2 (hepatic) and 3T3 cells, co-culture	Micro-patterned co-culture made possible	[85]
Promising materials*	Photo-crosslinked alginate hydrogel	Stiffness change; *Stimulus: light or hydrolysis*	Primary bovine chondrocytes	High survival rate for primary bovine chondrocytes Cellular responses to dynamic changes to be studied	[92]
	Gellan Gum hydrogel with both ionic crosslinking and	Stiffness, swelling, and degradation change *Stimulus: light or ion exchange*	NIH 3T3	Swelling and hydrolytic degradation vary with respect to crosslinking mechanism Stiffness may be changed quickly during photo-crosslinking process	[88]

Type	Substrates	Properties changed and stimuli	Cell model	Observations and notes	Ref.
	Methacrylated HA hydrogel with photo-crosslinker	Stiffening *Stimulus: UV radiation*	NIH3T3L HeLa Primary osteoblast	Good cell viability Cellular responses to dynamic changes to be studied	[80]
	PEG-based hydrogel incorporating CMP*	Softening *Stimulus: temperature or free CMP*	N/A	Cellular responses to dynamic changes to be studied	[90]
	PEG hydrogel (PEG vinyl sulfone crosslinked with PEG-diester-dithiol)	Softening *Stimulus: hydrolysis*	3T3 balb fibroblasts	Good cell viability in 3D gels Cellular responses to dynamic changes to be studied	[91]
	Resilin-like polypeptide (RLP) network crosslinked by THPP	Dynamic stiffness *Stimulus: oscillation*	N/A	Cellular responses to dynamic changes to be studied	[86]
	Thermo-reversible hydro-ferrogels (FGs)	Mechanical stiffness change *Stimulus: temperature change*	N/A	Cellular responses to dynamic changes to be studied	[89]

Note: ssDNA: single-stranded DNA; ↑ increase; ↓ decrease. *For 'Promising materials', most provides in vitro cyto-toxicity study, and cellular responses to dynamic properties remain to be investigated. N/A: not available

Table 1. A partial list dynamic substrates currently used in studying cell responses.

These stimuli, applied to initiate substrate dynamic, include light [62, 63], electricity [16, 17], pH, temperature, and others [16, 64] (Fig. 1). These approaches generally involve photochemical or electrochemical conversion, redox reactions, or stimulated configuration change of surface proteins, which leads to the attachment, detachment, shielding, or exposing of cell adhesion molecules, among which a popular choice is RGD peptide.

Mrksich group has been actively engaged in the development of SAM-based dynamic substrates by integrating surface chemistry, micro-patterning, and cell microenvironment engineering [17, 19, 61, 65, 66]. Based on an elegant design of SAM with electrochemically responsive group on a micro-patterned substrate, they first applied electrical stimulation to release 3T3 fibroblasts from designed areas on the substrate, and subsequently encouraged migration of neighboring cells to those areas with newly added adhesion molecules [65]. Refining this design by adding responsiveness to both negative and positive electric potentials, they demonstrated selective control over cell release [67] (Fig. 1C). Other groups have also engaged in the effort along this direction. By employing a hydroquinone terminated SAM based on re-

dox reactions, Chan and colleagues proposed a SAM on gold surface that enables attachment and release of cell adhesion molecules such as those with RGD motif [68], and selectively released adherent 3T3 fibroblasts bound through RGD motifs but not those adherent based on hydro-phobic interactions (Fig. 1A). Reversibility of cell adhesion is attractive in studying cellular responses and cell-ECM interactions [60]. As an example, a surface chemistry involving azobenzene capable of switching between two configurations was utilized to expose or hide adhesion sites (e.g., RGD motif) upon photochemical stimulation[69] (Fig. 1B). While the finding is interesting, the long exposure of cells to UV may be problematic despite the reported negligible impact of light with wavelength over 320 nm on cells [63].

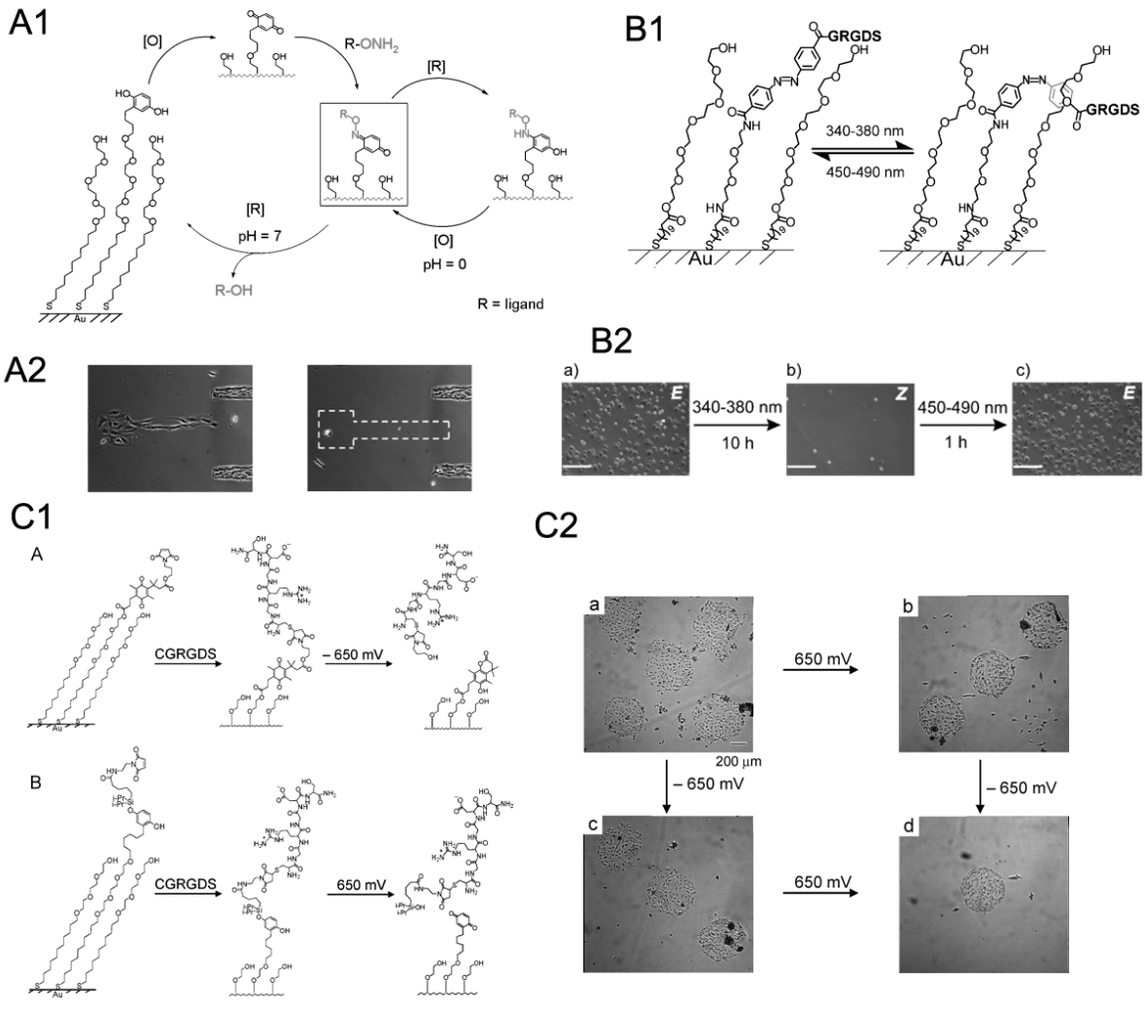

Figure 1. SAM-based dynamic substrates. (A) Schematic of the approach based on redox reaction (A1) by adjusting electrochemical potential, and cell detachment upon application of electric potential (A2). Extracted from [68]. (B) Schematic of altering configuration of azobenzene group under light of different ranges of wave length (B1) [69] and application to cell culture (B2) where NIH 3T3 fibroblasts initially adhere to adhesive surface (a) which was inhibited upon surface modification (b) followed by recovery of adhesion due to azobenzene configuration change (c). Extracted from [69]. (C) Illustration of a SAM that allows different modifications with positive and negative electric potential (C1) and its application in selective release of Swiss 3T3 cells (C2). Extracted from [67]. All with permission from publishers.

http://dx.doi.org/10.5772/53547

The above surveys part of the key advancement using SAM in modifying adhesion properties of the substrates mimicking those of natural cellular microenvironment. For a complete analysis of SAMs and their various applications, readers are referred to other reviews (e.g., [60, 70]). It suffices to point out that SAMs possess advantages in the precision (down to molecular level) of the control that can be applied in mechanistic studies [60, 66] of cell-ECM interactions, and are potentially useful for cell-based diagnostics among many applications. However, this approach has certain limitations. First, it mostly relies on coupling between electrical, chemical (including pH), mechanical, thermal, optical and biochemical (e.g., protein conformation) cues whose applicability under *in vivo* conditions is problematic. Next, the resulting changes in these studies are mostly of surface biochemical properties or of the presentation and biological activities of the surface ligands. Nevertheless, SAMs have greatly facilitated the probe and understanding of cell-ECM interactions and particular interplay between cells and ECM with dynamics in adhesive properties.

3.2. Polymeric hydrogels

Hydrogel materials are gaining popularity in the development of biomimetic materials, primarily due to the hydrated nature of natural ECM [14, 71]. Implantable hydrogel materials are increasingly being used in cardiovascular disease, nerve regeneration, and other conditions [59]. With careful design, hydrogel materials can have tunable materials properties, which have been demonstrated in a myriad of examples (Table 1). For instance, different than SAM-based approach, a polymer with both thermo- and photo-sensitivity was used to reversibly control adhesion of a group of cells [72]. Kim and colleagues took advantages of the thermo-responsive swelling behavior of copolymer between NIPAM and sodium acrylate, and created a hydrogel film that can be used to control cell encapsulation with surface topography [73]. Moreover, biomaterials responsive to the natural stimuli such as those experienced by biodegradable materials were found useful in mimicking biological events under physiological conditions, as illustrated in cell invasion to a MMP-responsive hydrogel scaffold [74]. This finding, among others, exemplifies the strategy of triggering material dynamic from bio-responsiveness to potential site- or disease-specific cues. The information from these studies is instrumental to the design of biodegradable materials in optimizing degradation profile for target cellular responses [75]. Naturally, in order to achieve desired outcome in adopting these strategies, it is important to gain thorough understanding of the natural environment, and minimize risks associate with biodegradable materials such as premature degradation, and potential toxicity of intermediate products from degradation.

Using a popular polyacrylamide hydrogel culture system with modifications that impart it with photo-sensitivity, Wang and colleagues [76] showed that upon UV induced substrates softening, spreading of 3T3 fibroblasts was hindered in contrast to that under static conditions (Fig. 2A). More interestingly, localized softening at anterior and posterior of cells yielded differential cellular morphology and migration responses [76]. Meanwhile, a PEG based polymer (PEGA) crosslinked by photosensitive crosslinker (PEGdiPDA) has been developed by Kloxin et al. [77], and used to lower gel stiffness upon UV exposure, which resulted in de-activation of myofibroblasts (Fig. 2B). Although UV radiation is preferentially avoided,

these methods made possible high precision in applying changes of cellular mechanical microenvironment, and potentially allow creation of dynamic stiffness gradient.

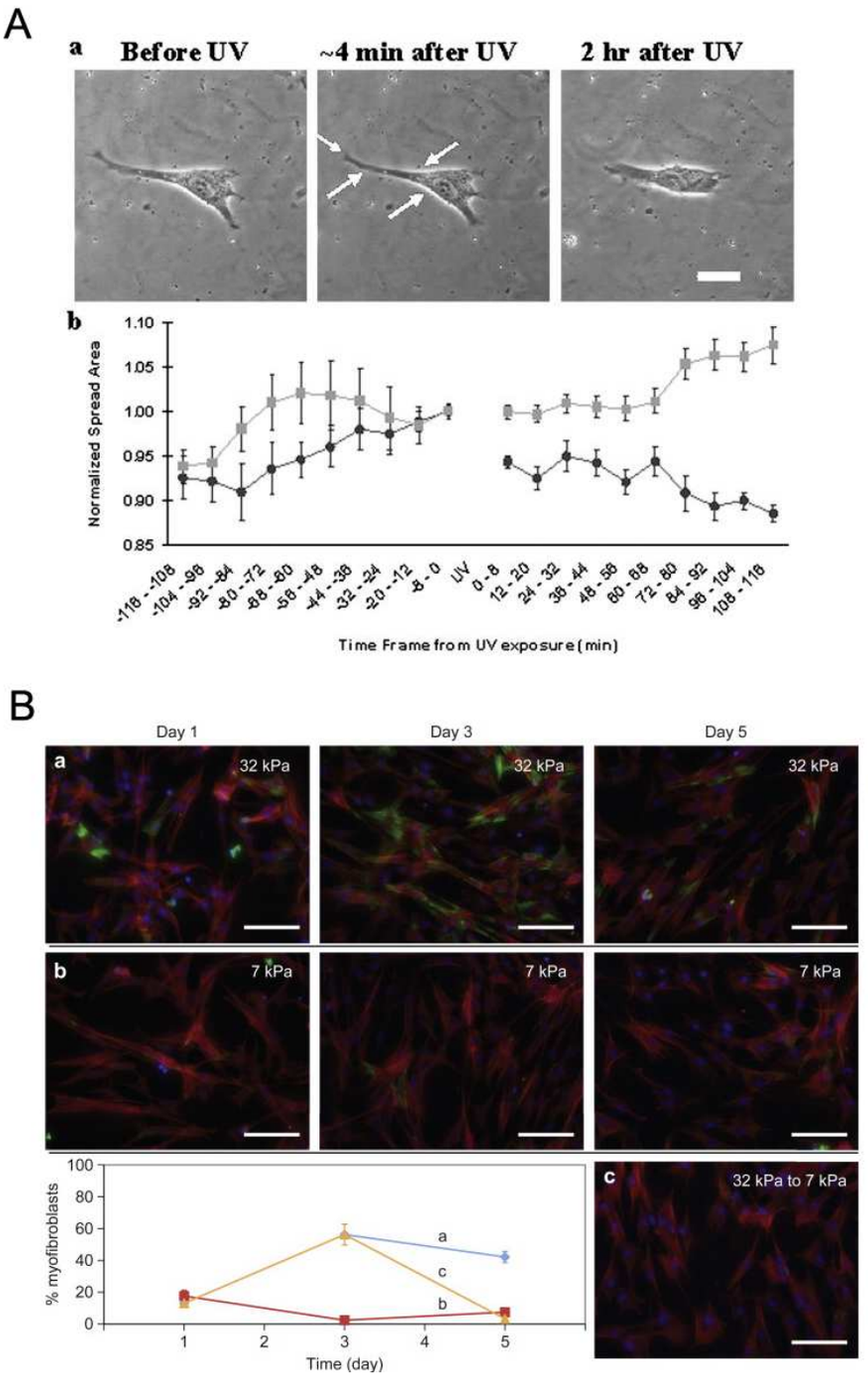

Figure 2. Photosensitive hydrogels and the study of cellular responses. (A) On a polyacrylamide hydrogel with photosensitive crosslinker, NIH 3T3 cells contract as indicated by projection area in response to UV-induced substrate softening. Extracted from [76]. (B) Valvular interstitial cells (VICs) on a PEG based hydrogel with photosensitive crosslinker displayed de-activation when UV radiation triggered substrate stiffness decrease. Extracted from [77]. Both with permission from publishers.

http://dx.doi.org/10.5772/53547

Similar observations were made by Burdick group for human mesenchymal stem cells (hMSCs) by using hyaluronic acid (HA) hydrogel degradable from hydrolytic and enzymatic reactions [78] (Fig. 3A). Very recently, a new material platform has been constructed by this group [79] and others [80] where the stiffness of a methacrylated hyaluronic acid hydrogel is increased via addition of photo-initiator and UV light exposure. In response to elevated stiffness, human mesenchymal stem cells (hMSCs) spread more and exert greater traction forces in hours (almost one magnitude of difference), and the rate of stiffness elevation dictates fate of cell differentiation towards adipogenic (slower) or osteogenic (faster) lineage. Their work highlighted that cellular behavior on dynamic gels is not the same as that on static gels with same initial or final properties, underlining the significance of dynamics in gel properties. This has been echoed in the concurrent work [81], where, for instance, the fibroblasts on 100% crosslinked hydrogels demonstrated different morphology from that on 100% crosslinked gels modified from 50% gels. Therefore, it is conceivable that the previous state of the cells and their ECM is also among the determinants of their current state, and that time dimension of ECM is of great importance.

Factors other than environmental conditions (e.g., light, pH, temperature) can also be delivered to stimulate dynamics in substrate properties. Incorporating DNA as crosslinker, Jiang and colleagues have developed a hydrogel system for cell attachment where mechanical properties of the substrates can be altered *in situ* in a controlled fashion when the cell culture is present [20, 81]. These DNA crosslinked hydrogels may also be designed to be potentially responsive to bio-stimuli, such as temperature or enzymes. Two representative cell types were chosen for the study of cell responses to dynamic substrate: fibroblasts whose sensitivity to mechanical cues is well documented, and neurons whose mechanosensing capability has recently just started being appreciated. The reports [20, 81] offered evidence that both cell types do respond to dynamic alternations in the mechanical characteristics of ECM, and suggest that the *alternations* in the mechanical stiffness may be involved in disease progression (Fig. 3B). It has been shown that the stiffness change resulting from pathological processes, may also aid in further progression of diseases [82].

The same material system was employed by Previera and co-workers, and they firstly proved the dual mechanical stimuli, namely strain and stiffness drop, during the dynamic processes, and secondly contrasted cell behavior to stiffness decrease with that for hardening of the substrates [83]. On hardening gels (from 12 kPa to 22 kPa), cells spread more than those on static substrates of higher stiffness (22 kPa), whereas on softening ones, they have greater spreading area than that on either starting or ending stiffnesses. In these studies, cell responses are determined by the range of rigidity change (due to crosslinking density), starting and ending rigidity, and specific cell properties (e.g., projection area vs. aspect ratio and protrusion for fibroblasts). The stress generation may also be involved in affecting cell behavior [83].

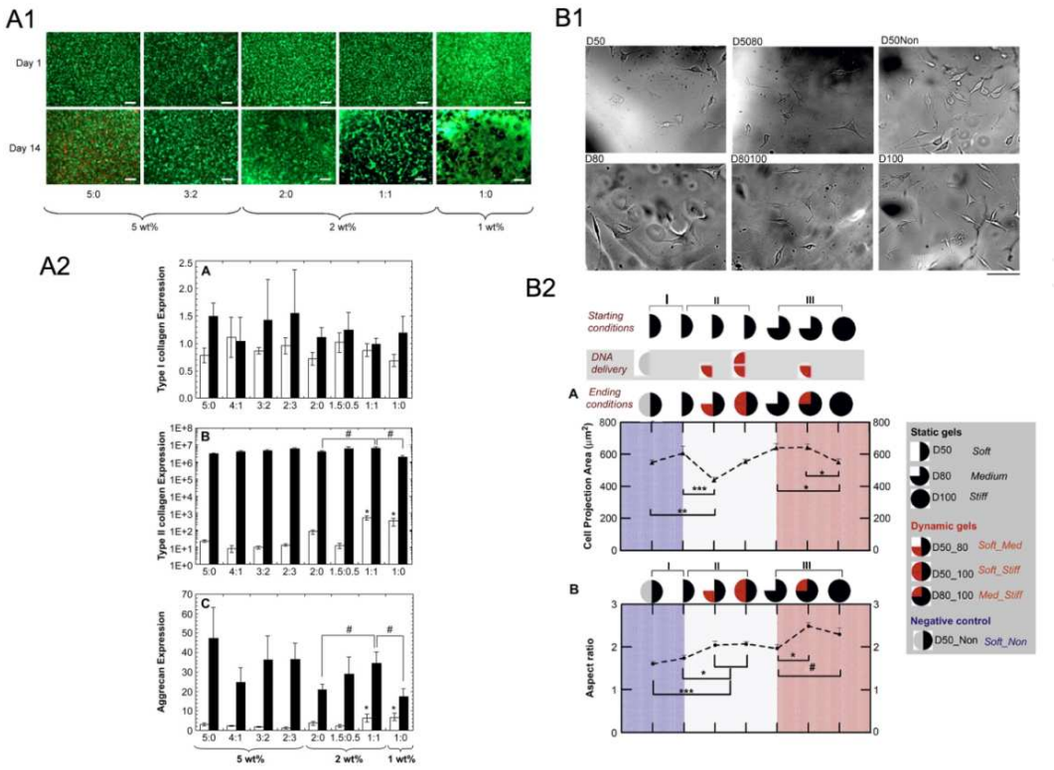

Figure 3. Substrates with dynamic mechanical stiffness and their application in cell culture studies. (A) Live/deal cell staining of human mesenchymal stem cells (hMSCs) in a hyaluronic acid hydrogel (A1) and the analysis of gene expression of type I/II collagen and aggrecan revealed that aside from type I collagen, both type II collagen and aggrecan exhibited an elevated level of expression on dynamic gels from the static ones. Extracted from [78]. With permission from publisher. (B) L929 fibroblast growth in a DNA crosslinked hydrogel with dynamic stiffness from crosslinking density change (B1) and the quantification of spreading area and aspect ratio (B2) showed that dynamic gels are significantly different from their static counterparts. Extracted from [81].

3.3. Other types of materials

The approach of employing polymeric hydrogel to study dynamic changes has certain limitations, one of which is the coupling of mechanical stiffness and forces (e.g., [83]). To address this concern and others, different from the approach by using SAM or polymeric hydrogel, AFM based method put forth by Webster and co-workers [84] probed cellular response to instant step change in stiffness excluding influence from stress or strain in the substrates (Table 1). It has been confirmed that indeed individual 3T3 fibroblasts are able to sense and respond to the stiffness in a scale of seconds as demonstrated in traction rate and contraction velocity [84]. However, this approach is most likely with inherent limitation in mimicking natural cell environment while remains an interesting tool in probing cellular responses to instantaneously change in stiffness. Additionally, this approach is applicable mostly to cells with dynamic morphology.

Common cell culture substrates (e.g., glass coverslip) modified with common photo-cleavable agents (NPE-TCSP) were shown to be useful for controlling cell adhesion selectively and temporally [62]. In this method, target areas were first irradiated to remove BSA known to

http://dx.doi.org/10.5772/53547

prevent cell adhesion, and then pro-adhesive molecules (e.g., fibronectin) were added, followed by cell seeding. It is useful to study dynamics in interactions between single cells. Petersen et al. [63] used light to stimulate photosensitive surface modification resulting in uncovering of the RGD motif upon release of a caging group (Fig. 4A). In doing so, adhesion of 3T3 fibroblasts was first inhibited and then encouraged, although this process is not reversible. With a sequential activation of adhesive sites upon application of electric potential, a recent study [85] demonstrated the utility of substrates with ITO (indium tin oxide) microelectrodes modified with poly(ethylene glycol), or PEG, in co-culture of two cell types (hepatic cells and fibroblasts) in a controlled manner (Fig. 4B).

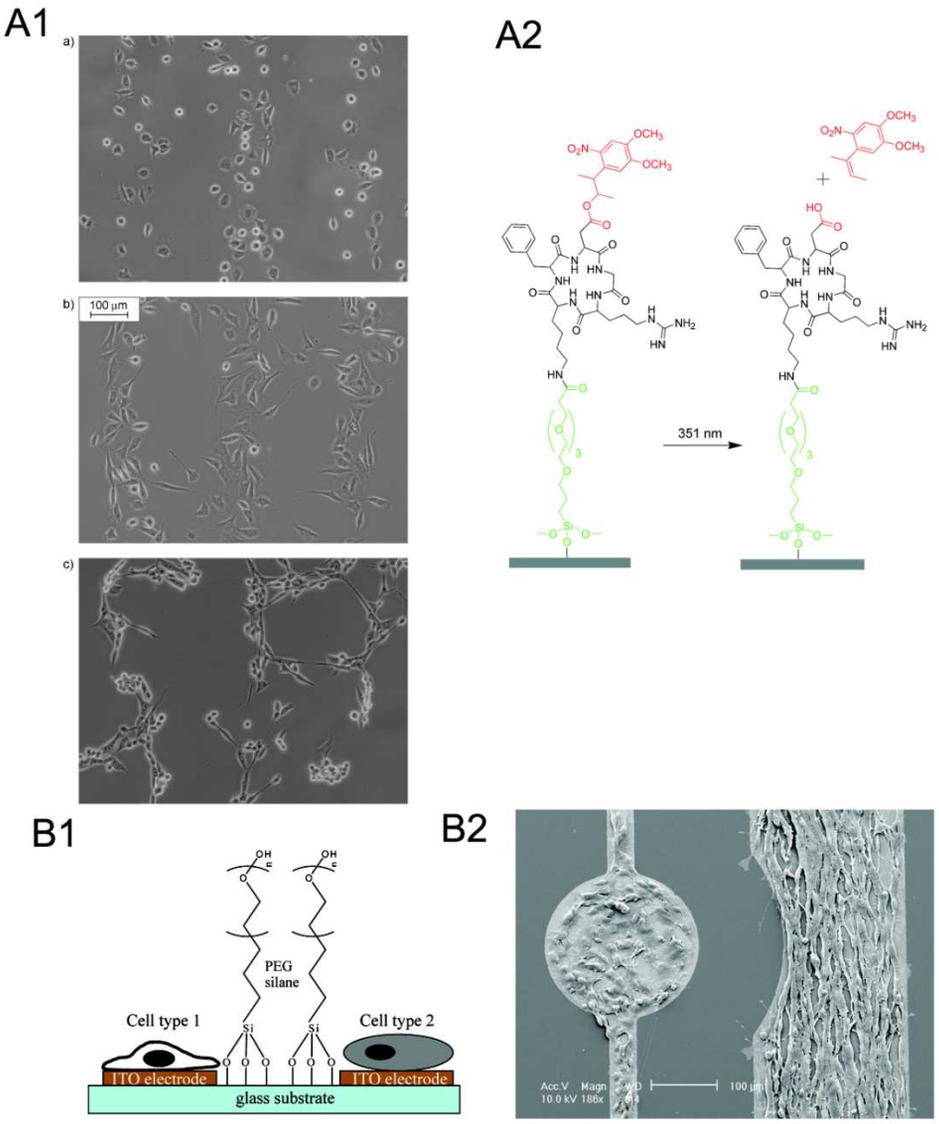

Figure 4. Cellular behavior in responses to other substrates with dynamic properties. (A) 3T3 fibroblasts grown in areas of patterned stripes (A1) generated from UV radiation based on the surface chemistry involving a caging group (A2). Extracted from [63]. (B) With PEG-modified ITO microelectrodes on glass substrates (B1), co-culture of two different cell types, HepG2 (hepatic) and 3T3 cells, was made possible. Extracted from [85]. Both with permission from publishers.

3.4. Promising substrates

By applying an oscillation to a resilin-like polypeptide network crosslinked by THPP (β-[Tris(hydroxymethyl) phosphino] propionic acid (betaine), Li and colleagues were able to observe dynamic mechanical stiffness of the gels varying with regard to oscillatory frequency mimicking the load from human vocal tissues [86] Oscillatory shear induced stiffening and softening of the collagen network might also serve as good substrates for mimicking cellular microenvironment particularly that in mechanically active tissues [87]. Ion concentration may be another stimulus to allow for temporal modification of hydrogel properties as exchange of ions between monovalant and divalant cations [88], and further work is needed to confirm it. Temperature-dependent substrate softening has been demonstrated by Krekhova et al. [89] and 3D complex with temperature-mediated crosslinking hence mechanical properties has been proposed by Stahl et al. [90], while applicability of these systems in mimicking cellular microenvironment remains to be seen. Zustiak et al. [91] reported mechanical stiffness drop, from approximately 1 kPa and at different rate, along with degradation of a poly(ethylene glycol), or PEG, hydrogel which might serve as not only drug delivery vehicle but also biomaterial construct, and they have offered preliminary evidence of good viability of 3T3 balb fibroblasts on the hydrogel substrate. Similarly, rigidity change from ~180 kPa to tens of kPa in 3-week period of degradation from a photo-crosslinked alginate hydrogels based on alginate methacrylation were presented by Jeon et al [92], and cyto-toxicity has been found to be low. In summary, these substrates holds promises as substrates with modifiable properties *in situ*, and need to be carefully tuned and evaluated for use as substrates with dynamic properties (Table 1). Other materials responsive to various stimuli including pH, temperature, and biochemical factors for a variety of applications, including can be found in the earlier reviews [64, 70, 93], and thus are not discussed in detail here due to the focus of the current analysis.

4. Design considerations and outlook

4.1. Dynamic properties of the substrates

As indicated in the discussions in Background and Motivation, the progression in changes of ECM properties is also critical in addition to changes *per se* in light of the observations in normal and pathological tissues, development and aging, and potential engineering benefits. Towards this end, rate of change (e.g., gradual vs. abrupt), range of change (e.g., small perturbation vs. drastic modifications), and change profile (e.g., monotonic increase vs. fluctuation) characterizing the nature of changes and their impact on cellular processes are subject to research effort, apparently adding to the complexity of the problem (Table 1). Take biodegradable material (e.g., [94]) as an example. It would be relevant to understand how mechanical and adhesive characteristics evolve with degradation and how the degradation profile affects the changes in the cellular micro-environment. Experimental design along this line may include, for example, different rates of release of RGD motif decreasing adhesiveness while keeping the same range of change (e.g., half of the total RGD presenting sites), or

http://dx.doi.org/10.5772/53547

altering the range of change while maintaining the same rate of change. Furthermore, it is not clear at this point whether cellular responses to opposite changes (e.g., increase vs. decrease in adhesiveness or rigidity) of substrate properties are symmetric, thus their behavior to one direction of dynamic alterations may not be a reliable predictor of that to the opposite changes.

4.2. Potential effect on cell-cell interactions

Changes in adhesive or mechanical properties of ECM can stimulate cells, which, in response, secrete soluble factors and ECM molecules, and this further impacts neighboring cell types. Additionally, some cell types such as neurons may use other cells (e.g., astroglia) as substrate [95], and stiffness change of 'underlying' cells per se due to ECM stimulation may give rise to further alternations thanks to cell-cell interactions. For instance, during asthma, ECM stiffening contributes to stiffness increase of airway smooth muscle (ASM) cells, which potentially affects other cell types in the close proximity [33].

4.3. Design parameters for biomaterials and outlook

The design parameters of dynamic substrates from current studies are summarized in Table 2, which includes, but are not limited to, material system to consider (e.g., SAM or polymeric hydrogel materials), nature of change (mechanical stiffness or adhesion), rate of change (e.g., transient or gradual change, controllability of the rate of change), range of change (e.g., at different stiffness range) as well as potential issues in further investigations and applications to medical and healthcare applications. If the interest is in understanding the cellular behavior to mechanical stiffness alone, then an AFM based approach might be more attractive [84] as others will involve stress or strain as part of the stiffening or softening process. If precise control over stiffness range is desired, the DNA crosslinked PAM hydrogel system will serve the purpose better [20, 58, 81]. Polymeric hydrogel materials with controllable degradation profile and hence mechanical stiffness dynamics during degradation (e.g., [88]) will serve the purpose best when biodegradable materials are applied. Some of the material systems do offer unique benefits such as reversible property change or without using environmental factors (by applying oscillation, crosslinker, or ssDNA).

Meanwhile, there are inherent limitations to each of the material system under discussion (Table 2). UV exposure generally causes concern to its impact on cellular activities despite the findings of little impact from a number of studies based on a range of biological assays. Under physiological conditions, application of certain cues (e.g., ssDNA, light, or ion) might be too difficult or it might be greatly limited (e.g., temperature triggered changes). However, it is still possible to find ways to apply these cues with careful design. For instance, ssDNA design based on pre-screening using BLAST search against targeted specie or tissue type may minimize the chance of interfering with normal biological activities. Local heating/cooling may be carefully applied to induce dynamic changes to achieve cellular responses. Three dimensional system may better mimic natural cellular micro-environment than their 2D counter parts.

Stimuli	Material system	Nature of change	Range of change	Rate of change	Invasiveness of stimulus and potential issues	Ref.
Ion	Ion-crosslinked GC hydrogel	Stiffness	~22 to ~17 kPa (with chemical crosslinking)	N/A	Under physiological conditions, divalent ions exchanged by mono-valent ones	[88]
Light	Hydrogels based on PAM crosslinked by photosensitive agents	Softening	Stiffness: 5.5~7.2 kPa	Approximately 0.5~0.6 kPa/ min	UV exposure for 3 min UV radiation with low energy density Depth of penetration and limit on dose	[76]
	Methacrylated HA hydrogel	Stiffening Irreversible change	Stiffness: ~3 to ~30 kPa	Approximately 9 kPa/hr (short term); 2 kPa/day (long-term)	UV exposure for a few min Potential toxicity of photoinitiator Depth of penetration and limit on dose	[79]
	Photo-crosslinked methacrylated Gellan Gum hydrogel	Stiffness; Swelling Hydrolytic degradation	Stiffness: a few kPa to 22 kPa (by physical crosslinking)	Approximately 20 kPa/ min	UV exposure for one min Depth of penetration and limit on dose	[88]
	Methacrylated HA hydrogel with photo-crosslinker	Stiffness; Irreversible change	Stiffness: 1.6 to 3.8 kPa; 3-12 kPa	Approximately 0.1 or 0.3 kPa/min (during gelation)	UV exposure for a few min Potential toxicity of photo-initiator Depth of penetration and limit on dose	[80]
	PEG based hydrogel with photosensitive crosslinker	Stiffness↓ Adhesive property Irreversible change	N/A	N/A	Depth of penetration and limit on dose	[77]
DNA	DNA crosslinked PAM system	Stiffening & softening, potentially coupled with strain/stress Reversible change	Stiffness: ~5.9 to 22.9 kPa Stress > 0.5 Pa	Up to 8.5 kPa/ day	No differentiation in cellular responses between forces, stress, and stiffness Potentially interference from DNA with bio-activity (e.g., as anti-sense DNAs), and potential issue with DNase BLAST search against target specie & tissue type	[20, 81, 83]
AFM/ stiffness clamp	Piezo-controlled substrates and AFM stiffness clamp	Instantaneous change in stiffness Unidirectional	Stiffness: 3.6 to 90 nN/μm	Step change (instantane-ously)	Applicable only to cells with dynamic morphology	[84]
Hydro-lysis	Photo-crosslinked alginate hydrogel	Softening due to degradation	Stiffness: ~25 to ~180 kPa	7-8 kPa/ day	In sample preparation (with cells), UV exposure for 10 mins	[92]
	HA hydrogel	Stiffening & structure change	Stiffness: e.g., ~5 to 30 kPa for one case	0.7 kPa/ day	Dense crosslinking may impede cellular growth limited by diffusion& concentration of radicals	[78]
	PEG hydrogel (PEG vinyl sulfone	Softening due to degradation	Stiffness: from ~1 kPa-3 kPa to very low	From ~900 Pa/day to 500 Pa/day	Good cell viability Hydrogel degraded in 16 hours	[91]

Stimuli	Material system	Nature of change	Range of change	Rate of change	Invasiveness of stimulus and potential issues	Ref.
	crosslinked with PEG-diester-dithiol)					
Temp-erature	Thermo-reversible hydro-ferrogels (FGs)	Stiffening due to structural transition	Stiffness: ~28-24 kPa for 2ºC change at 37ºC	A few kPa for 1ºC of temperature change	Temperature change needs to be defined to be relevant to cell culture	[89]
	PEG-based hydrogel incorporating CMP*	Stiffness change Due to temperature and free CMP	Stiffness (indirect measurement)	N/A	Temperature change needs to be defined to be relevant to cell culture Bio-compatibility of free CMP	[90]
Oscilla-tion	Resilin-like polypeptide based elastomer	Stiffness change due to oscillation	Storage modulus between 0.5 and 10 kPa	Highly dynamic	Strain/stress that is associated with oscillation	[86]

Note: N/A: not available; This is a partial list of the current work under examination.

Table 2. Design considerations in constructing dynamic substrates mimicking extra-cellular matrix (ECM).

A few new material system have been identified with the potential as dynamic cell culture platform as well as choice of biomaterials (Table 1). Many of them have demonstrated good cyto-compatibility, and investigation of impact of *in situ* changes to cells will be desired.

5. Concluding remarks

There is an increasing recognition of the discrepancy between static nature of the current cell culture substrates or scaffolds and the dynamics in ECM in natural or diseased tissue, during development and aging, or at tissue-scaffold interfaces. This has motivated the development of materials with controlled changing properties that mimic those of ECM. An array of stimuli, including environmental factors (temperature, pH, light, electrical potential) and non-environmental cues including enzyme and DNA, have been implemented to trigger dynamics in a number of material platform such as SAMs, polymeric hydrogels and other substrates with surface chemistry and modifications.

To date, most of the effort along this line has been devoted to *in vitro* models, and *in vivo* studies of the effect of dynamic tissue properties on cellular behavior are still rather limited, which awaits further development in cell biology and proper tools such as imaging techniques [12, 14, 29].

Understanding the interplay between cells and the extracellular matrix (ECM) including its dynamic aspect is fundamental to biology, development, aging and pathology, and can aid in the design of biomaterials. Ultimately, the system enabling both spatial and temporal control [96] of cells would be most relevant in terms of bio-mimicry and tissue

engineering applications. Some of the potential directions include creating dynamic adhesive gradient to guide cell migration or neurite outgrowth at desired time point, constructing scaffolds with suitable mechanical rigidity to inhibit glia cell growth (thus hinder scar formation) while promoting nerve regeneration with compliance gradient, and developing dynamic platform for stem cell harvesting and differentiation for cell-based therapies.

Acknowledgments

The helpful discussions and advice from Langrana group at Rutgers University, New Jersey, USA as well as previous collaborators are greatly appreciated.

Author details

Frank Xue Jiang

Address all correspondence to: Frank.Jiang@unilever.com

Unilever Research & Development, Shanghai, P.R. China

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the Unilever, its management or employees. The authors have no financial interest in this publication and receive nothing in exchange for providing this review.

References

[1] Keatch RP, Schor AM, Vorstius JB, Schor SL. Biomaterials in regenerative medicine: engineering to recapitulate the natural. Curr Opin Biotechnol 2012;23:579-582.

[2] Brizzi MF, Tarone G, Defilippi P. Extracellular matrix, integrins, and growth factors as tailors of the stem cell niche. Curr Opin Cell Biol 2012;(Epub).

[3] Cukierman E, Pankov R, Stevens DR, Yamada KM. Taking cell-matrix adhesions to the third dimension. Science 2001;294:1708-1712.

[4] Geiger B. Cell biology. Encounters in space. Science 2001;294:1661-1663.

[5] Discher DE, Mooney DJ, Zandstra PW. Growth factors, matrices, and forces combine and control stem cells. Science 2009;324:1673-1677.

[6] Page H, Flood P, Reynaud EG. Three-dimensional tissue cultures: current trends and beyond. Cell Tissue Res 2012;(Epub).

[7] Benjamin M, Hillen B. Mechanical influences on cells, tissues and organs - 'Mechanical Morphogenesis'. Eur J Morphol 2003;41:3-7.

[8] Mammoto T, Ingber DE. Mechanical control of tissue and organ development. Development 2010;137:1407-1420.

[9] Tee SY, Bausch AR, Janmey PA. The mechanical cell. Curr Biol 2009;19:R745-748.

[10] Zhu C, Bao G, Wang N. Cell mechanics: mechanical response, cell adhesion, and molecular deformation. Annu Rev Biomed Eng 2000;2:189-226.

[11] Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. Cold Spring Harb Perspect Biol 2011;3.

[12] Rozario T, Desimone DW. The extracellular matrix in development and morphogenesis: A dynamic view. Dev Biol 2010;341:126-140.

[13] Xu R, Boudreau A, Bissell MJ. Tissue architecture and function: dynamic reciprocity via extra- and intra-cellular matrices. Cancer Metastasis Rev 2009;28:167-176.

[14] Goody MF, Henry CA. Dynamic interactions between cells and their extracellular matrix mediate embryonic development. Mol Reprod Dev 2010;77:475–488.

[15] Godier AF, Marolt D, Gerecht S, Tajnsek U, Martens TP, Vunjak-Novakovic G. Engineered microenvironments for human stem cells. Birth Defects Res C Embryo Today 2008;84:335-347.

[16] Lahann J, Langer R. Smart Materials with Dynamically Controllable Surfaces. MRS Bulletin 2005;30:185-188.

[17] Mrksich M. Dynamic Substrates for Cell Biology. MRS BULLETIN 2005;30:180-184.

[18] Abbott NL, Gorman CB, Whitesides GM. Active Control of Wetting Using Applied Electrical Potentials and Self-Assembled Monolayers. Langmuir 1995;11:16-18.

[19] Yousaf MN, Houseman BT, Mrksich M. Using electroactive substrates to pattern the attachment of two different cell populations. Proc Natl Acad Sci U S A 2001;98:5992-5996.

[20] Jiang FX, Yurke B, Schloss RS, Firestein BL, Langrana NA. Effect of dynamic stiffness of the substrates on neurite outgrowth by using a DNA-crosslinked hydrogel. Tissue Eng Part A 2010;16:1873-1889.

[21] Peerani R, Zandstra PW. Enabling stem cell therapies through synthetic stem cell-niche engineering. J Clin Invest 2010;120:60-70.

[22] Dawson E, Mapili G, Erickson K, Taqvi S, Roy K. Biomaterials for stem cell differentiation. Adv Drug Deliv Rev 2008;60:215-228.

[23] Haq F, Keith C, Zhang G. Neurite development in PC12 cells on flexible micro-textured substrates under cyclic stretch. Biotechnol Prog 2006;22:133-140.

[24] Nicodemus GD, Bryant SJ. Mechanical loading regimes affect the anabolic and catabolic activities by chondrocytes encapsulated in PEG hydrogels. Osteoarthritis Cartilage 2010;18:126-137.

[25] Brown L. Cardiac extracellular matrix: a dynamic entity. Am J Physiol Heart Circ Physiol 2005;289:H973-974.

[26] Jung Y, Kim SH, Kim YH. The effects of dynamic and three-dimensional environments on chondrogenic differentiation of bone marrow stromal cells. Biomed Mater 2009;4:055009.

[27] Tschumperlin DJ, Boudreault F, Liu F. Recent advances and new opportunities in lung mechanobiology. J Biomech 2010;43:99-107.

[28] Gorstein F. The dynamic extracellular matrix. Hum Pathol 1988;19:751-752.

[29] Dallas SL, Chen Q, Sivakumar P. Dynamics of assembly and reorganization of extracellular matrix proteins. Curr Top Dev Biol 2006;75:1-24.

[30] Daley WP, Peters SB, Larsen M. Extracellular matrix dynamics in development and regenerative medicine. J Cell Sci 2008;121:255-264.

[31] Shi YB, Fu L, Hsia SC, Tomita A, Buchholz D. Thyroid hormone regulation of apoptotic tissue remodeling during anuran metamorphosis. Cell Res 2001;11:245-252.

[32] Sivakumar P, Czirok A, Rongish BJ, Divakara VP, Wang YP, Dallas SL. New insights into extracellular matrix assembly and reorganization from dynamic imaging of extracellular matrix proteins in living osteoblasts. J Cell Sci 2006;119:1350-1360.

[33] An SS, Kim J, Ahn K, Trepat X, Drake KJ, Kumar S, et al. Cell stiffness, contractile stress and the role of extracellular matrix. Biochem Biophys Res Commun 2009;382:697-703.

[34] Reilly GC, Engler AJ. Intrinsic extracellular matrix properties regulate stem cell differentiation. J Biomech 2010;43:55-62.

[35] Chen ZL, Indyk JA, Strickland S. The hippocampal laminin matrix is dynamic and critical for neuronal survival. Mol Biol Cell 2003;14:2665-2676.

[36] Coles JM, Zhang L, Blum JJ, Warman ML, Jay GD, Guilak F, et al. Loss of cartilage structure, stiffness, and frictional properties in mice lacking Prg4. Arthritis Rheum 2010;62:1666-1674.

[37] Kutty JK, Webb K. Tissue engineering therapies for the vocal fold lamina propria. Tissue Eng Part B Rev 2009;15:249-262.

[38] Siu MK, Cheng CY. Dynamic cross-talk between cells and the extracellular matrix in the testis. Bioessays 2004;26:978-992.

[39] Hui EE, Bhatia SN. Micromechanical control of cell-cell interactions. Proc Natl Acad Sci U S A 2007;104:5722-5726.

[40] Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. Science 2005;310:1139-1143.

[41] Yu H, Mouw JK, Weaver VM. Forcing form and function: biomechanical regulation of tumor evolution. Trends Cell Biol 2011;21:47-56.

[42] Zaman MH, Trapani LM, Sieminski AL, Mackellar D, Gong H, Kamm RD, et al. Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. Proc Natl Acad Sci U S A 2006;103:10889-10894.

[43] Bharat S, Techavipoo U, Kiss MZ, Liu W, Varghese T. Monitoring stiffness changes in lesions after radiofrequency ablation at different temperatures and durations of ablation. Ultrasound Med Biol 2005;31:415-422.

[44] Walker GA, Masters KS, Shah DN, Anseth KS, Leinwand LA. Valvular myofibroblast activation by transforming growth factor-beta: implications for pathological extracellular matrix remodeling in heart valve disease. Circ Res 2004;95:253-260.

[45] Rabkin-Aikawa E, Farber M, Aikawa M, Schoen FJ. Dynamic and reversible changes of interstitial cell phenotype during remodeling of cardiac valves. J Heart Valve Dis 2004;13:841-847.

[46] Dobaczewski M, Gonzalez-Quesada C, Frangogiannis NG. The extracellular matrix as a modulator of the inflammatory and reparative response following myocardial infarction. J Mol Cell Cardiol 2010;48:504-511.

[47] Hachiya NS, Kozuka Y, Kaneko K. Mechanical stress and formation of protein aggregates in neurodegenerative disorders. Med Hypotheses 2008;70:1034-1037.

[48] Fallenstein GT, Hulce VD, Melvin JW. Dynamic mechanical properties of human brain tissue. J Biomech 1969;2:217-226.

[49] Latimer A, Jessen JR. Extracellular matrix assembly and organization during zebrafish gastrulation. Matrix Biol 2010;29:89-96.

[50] Silver FH, DeVore D, Siperko LM. Invited Review: Role of mechanophysiology in aging of ECM: effects of changes in mechanochemical transduction. J Appl Physiol 2003;95:2134-2141.

[51] Yamada O, Todoroki J, Takahashi T, Hashizume K. The dynamic expression of extracellular matrix in the bovine endometrium at implantation. J Vet Med Sci 2002;64:207-214.

[52] Marquez JP, Genin GM, Pryse KM, Elson EL. Cellular and matrix contributions to tissue construct stiffness increase with cellular concentration. Ann Biomed Eng 2006;34:1475-1482.

[53] Bellows CG, Melcher AH, Aubin JE. Contraction and organization of collagen gels by cells cultured from periodontal ligament, gingiva and bone suggest functional differences between cell types. J Cell Sci 1981;50:299-314.

[54] Lee B, Han L, Frank EH, Chubinskaya S, Ortiz C, Grodzinsky AJ. Dynamic mechanical properties of the tissue-engineered matrix associated with individual chondrocytes. J Biomech 2010;43:469-476.

[55] Appelman TP, Mizrahi J, Elisseeff JH, Seliktar D. The differential effect of scaffold composition and architecture on chondrocyte response to mechanical stimulation. Biomaterials 2009;30:518-525.

[56] Crapo PM, Wang Y. Physiologic compliance in engineered small-diameter arterial constructs based on an elastomeric substrate. Biomaterials 2010;31:1626-1635.

[57] Li L, Sharma N, Chippada U, Jiang X, Schloss R, Yarmush ML, et al. Functional modulation of ES-derived hepatocyte lineage cells via substrate compliance alteration. Ann Biomed Eng 2008;36:865-876.

[58] Jiang FX, Yurke B, Firestein BL, Langrana NA. Neurite outgrowth on a DNA crosslinked hydrogel with tunable stiffnesses. Ann Biomed Eng 2008;36:1565-1579.

[59] Ulijn RV, Bibi N, Jayawarna V, Thornton PD, Todd SJ, Mart RJ, et al. Bioresponsive hydrogels. Materials Today 2007;10:40-48.

[60] Robertus J, Browne WR, Feringa BL. Dynamic control over cell adhesive properties using molecular-based surface engineering strategies. Chem Soc Rev 2010;39:354-378.

[61] Mrksich M. Using self-assembled monolayers to model the extracellular matrix. Acta Biomater 2009;5:832-841.

[62] Nakanishi J, Kikuchi Y, Takarada T, Nakayama H, Yamaguchi K, Maeda M. Photoactivation of a substrate for cell adhesion under standard fluorescence microscopes. J Am Chem Soc 2004;126:16314-16315.

[63] Petersen S, Alonso JM, Specht A, Duodu P, Goeldner M, del Campo A. Phototriggering of cell adhesion by caged cyclic RGD peptides. Angew Chem Int Ed Engl 2008;47:3192-3195.

[64] Mendes PM. Stimuli-responsive surfaces for bio-applications. Chem Soc Rev 2008;37:2512-2529.

[65] Yeo WS, Yousaf MN, Mrksich M. Dynamic interfaces between cells and surfaces: electroactive substrates that sequentially release and attach cells. J Am Chem Soc 2003;125:14994-14995.

[66] Mrksich M. A surface chemistry approach to studying cell adhesion. Chem Soc Rev 2000;29:267 - 273.

[67] Yeo WS, Mrksich M. Electroactive self-assembled monolayers that permit orthogonal control over the adhesion of cells to patterned substrates. Langmuir 2006;22:10816-10820.

[68] Chan EW, Park S, Yousaf MN. An electroactive catalytic dynamic substrate that immobilizes and releases patterned ligands, proteins, and cells. Angew Chem Int Ed Engl 2008;47:6267-6271.

[69] Liu D, Xie Y, Shao H, Jiang X. Using azobenzene-embedded self-assembled monolayers to photochemically control cell adhesion reversibly. Angew Chem Int Ed Engl 2009;48:4406-4408.

[70] Nandivada H, Ross AM, Lahann J. Stimuli-responsive monolayers for biotechnology. Progress in Polymer Science 2010;35:141-154.

[71] Gillette BM, Jensen JA, Wang M, Tchao J, Sia SK. Dynamic hydrogels: switching of 3D microenvironments using two-component naturally derived extracellular matrices. Adv Mater 2010;22:686-691.

[72] Edahiro J, Sumaru K, Tada Y, Ohi K, Takagi T, Kameda M, et al. In situ control of cell adhesion using photoresponsive culture surface. Biomacromolecules 2005;6:970-974.

[73] Kim J, Yoon J, Hayward RC. Dynamic display of biomolecular patterns through an elastic creasing instability of stimuli-responsive hydrogels. Nat Mater 2010;9:159-164.

[74] Lutolf MP, Lauer-Fields JL, Schmoekel HG, Metters AT, Weber FE, Fields GB, et al. Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: engineering cell-invasion characteristics. Proc Natl Acad Sci U S A 2003;100:5413-5418.

[75] Baker BM, Nerurkar NL, Burdick JA, Elliott DM, Mauck RL. Fabrication and modeling of dynamic multipolymer nanofibrous scaffolds. J Biomech Eng 2009;131:101012.

[76] Frey MT, Wang YL. A photo-modulatable material for probing cellular responses to substrate rigidity. Soft Matter 2009;5:1918-1924.

[77] Kloxin AM, Benton JA, Anseth KS. In situ elasticity modulation with dynamic substrates to direct cell phenotype. Biomaterials 2010;31:1-8.

[78] Chung C, Beecham M, Mauck RL, Burdick JA. The influence of degradation characteristics of hyaluronic acid hydrogels on in vitro neocartilage formation by mesenchymal stem cells. Biomaterials 2009;30:4287-4296.

[79] Guvendiren M, Burdick JA. Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics. Nat Commun 2012;3:792.

[80] Hachet E, Van Den Berghe H, Bayma E, Block MR, Auzely-Velty R. Design of Biomimetic Cell-Interactive Substrates Using Hyaluronic Acid Hydrogels with Tunable Mechanical Properties. Biomacromolecules 2012;(Epub).

[81] Jiang FX, Yurke B, Schloss RS, Firestein BL, Langrana NA. The relationship between fibroblast growth and the dynamic stiffnesses of a DNA crosslinked hydrogel. Biomaterials 2010;31:1199-1212.

[82] Huang S, Ingber DE. Cell tension, matrix mechanics, and cancer development. Cancer Cell 2005;8:175-176.

[83] Previtera ML, Trout KL, Verma D, Chippada U, Schloss RS, Langrana NA. Fibroblast morphology on dynamic softening of hydrogels. Ann Biomed Eng 2012;40:1061-1072.

[84] Webster KD, Crow A, Fletcher DA. An AFM-based stiffness clamp for dynamic control of rigidity. PLoS One 2011;6:e17807.

[85] Shah S, Lee JY, Verkhoturov S, Tuleuova N, Schweikert EA, Ramanculov E, et al. Exercising spatiotemporal control of cell attachment with optically transparent microelectrodes. Langmuir 2008;24:6837-6844.

[86] Li L, Teller S, Clifton RJ, Jia X, Kiick KL. Tunable mechanical stability and deformation response of a resilin-based elastomer. Biomacromolecules 2011;12:2302-2310.

[87] Kurniawan NA, Wong LH, Rajagopalan R. Early stiffening and softening of collagen: interplay of deformation mechanisms in biopolymer networks. Biomacromolecules 2012;13:691-698.

[88] Coutinho DF, Sant SV, Shin H, Oliveira JT, Gomes ME, Neves NM, et al. Modified Gellan Gum hydrogels with tunable physical and mechanical properties. Biomaterials 2010;31:7494-7502.

[89] Krekhova M, Lang T, Richter R, Schmalz H. Thermoreversible hydroferrogels with tunable mechanical properties utilizing block copolymer mesophases as template. Langmuir 2010;26:19181-19190.

[90] Stahl PJ, Romano NH, Wirtz D, Yu SM. PEG-based hydrogels with collagen mimetic peptide-mediated and tunable physical cross-links. Biomacromolecules 2011;11:2336-2344.

[91] Zustiak SP, Leach JB. Hydrolytically degradable poly(ethylene glycol) hydrogel scaffolds with tunable degradation and mechanical properties. Biomacromolecules 2010;11:1348-1357.

[92] Jeon O, Bouhadir KH, Mansour JM, Alsberg E. Photocrosslinked alginate hydrogels with tunable biodegradation rates and mechanical properties. Biomaterials 2009;30:2724-2734.

[93] Mano JF. Stimuli-Responsive Polymeric Systems for Biomedical Applications. Advanced Engineering Materials 2008;10:515-527.

[94] Kim S, Chung EH, Gilbert M, Healy KE. Synthetic MMP-13 degradable ECMs based on poly(N-isopropylacrylamide-co-acrylic acid) semi-interpenetrating polymer networks. I. Degradation and cell migration. J Biomed Mater Res A 2005;75:73-88.

[95] Lu YB, Franze K, Seifert G, Steinhauser C, Kirchhoff F, Wolburg H, et al. Viscoelastic properties of individual glial cells and neurons in the CNS. Proc Natl Acad Sci U S A 2006;103:17759-17764.

[96] Yousaf MN. Model substrates for studies of cell mobility. Curr Opin Chem Biol 2009;13:697-704.

IntechOpen

IntechOpen