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Material control of stem cell differentiation: challenges in nano-characterization

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Recent experiments have revealed that stem cells respond to biophysical cues as well as numerous biochemical factors. Nanoscale properties at the cell–matrix interface that appear to affect adherent stem cells range from matrix elasticity to porosity-dependent matrix tethering and geometry of adhesive linkages. Some stem cells can also remodel their immediate environment to influence phenotype, but this depends on matrix-material properties such as covalent bonding and soft versus hard materials. Efforts to combine both matrix instructions and active cell feedback are required to properly direct stem cell behavior. Comparisons to tissues will be increasingly key and have begun to reveal remodeling of nuclear factors that influence epigenetics.

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Introduction

Within the last ten years, stem cell approaches have become conceivable or even realized for each of the roughly 200 differentiated cell types in humans. Since most tissue cells are anchorage dependent — meaning that adhesion to a solid is necessary for cell survival, it is only sensible that the physicochemical nature of the support can influence stem cell fate. Substrate stiffness, geometry, porosity, and topography are now understood to influence stem cells, perhaps as much as biochemical factors [1]. Molecular pathways of cellular mechanotransduction that ultimately affect both cell phenotype and genotype are slowly becoming clear. Matrix stiffness-dependent lineage commitment of stem cell has been suggested to involve YAP/TAZ, which are transcription factors previously known to influence proliferation, whereas newer evidence suggests they are also nuclear mechano-transducers regulated by Rho GTPase and cytoskeletal tension independent of the canonical Hippo pathway [2•]. More recently, polymer physics type scaling between tissue stiffness and the expression levels in primary tissue of the nuclear envelope structural protein, Lamin-A, has been reported to co-regulate YAP, among other transcription and epigenetic factors [3]. The results suggested that increased matrix rigidity (due to greater collagen in high stress tissues) leads to nuclear stiffening and greater DNA protection as a homeostatic response in all tissue cell types, including stem cells [3•,4]. More pathways are likely to emerge as the field gains insight and control over micro/nano-environments as analyzed in vivo or applied to stem cells in culture.

Virtually every organ in the body contains resident stem cells or progenitors that contribute to organ homeostasis or repair. Exploiting stem cells for regeneration of damaged tissue has spurred research into their multipotentiality as well as immunocompatibility. Therapeutics is limited in part by *in vitro* cell expansion as well as materials issues that include the design of biocompatible scaffolds for co-transplantation. Since many stem cells are anchorage dependent, injection *in vivo* should work best if the cells adhere quickly and adequately, but it is clear in many trials with mesenchymal stem cells (MSCs) that the vast majority of injected cells die rather than contribute to tissue [5]. Recapitulating the various stem cell niches *ex vivo* is extremely challenging as it likely involves spatiotemporal regulation of biostimuli that extend to extracellular matrix architecture. Nonetheless, understanding the niche *in vitro* might help in translation to *in vivo* [6].

In this review, we describe recent advances as well as challenges in the material control of stem cell multipotency and lineage commitment. By 'stem cell', we refer to cited studies on MSCs (mesenchymal), NSCs (neural), ESCs (embryonic), iPSC (induced pluripotent), epidermal stem cells, etc., but we emphasize the generality merely with 'stem cell' and encourage the interested reader to seek the primary literature for specific stem cell types. We attempt to highlight in more detail how the field is beginning to formulate materials design rules for stem cell cultures down to the nanoscale in terms of fabrication and/or physical characterization. Approaches are crudely – split into soft materials such as hydrogels that are as soft as most tissues or else hard materials in which the softest thing in culture is the cell. Remarkably, there seem to be ways — i.e. rules — to manipulate 'boundary conditions' in order to fool cells into responding to a hard material in a manner similar to (but not exactly the same as) that on a much softer material, and *vice versa*.

Soft matter control

Tissue stiffness or elasticity is dictated by the extracellular matrix (ECM). Even a few minutes of incubation with collagenase can soften a tissue dramatically [3•]. ECM is comprised a network of fibrous proteins, such as collagens, that are crosslinked in a homophilic or heterophilic manner. A hierarchical polymeric network of variable density allows for a broad range of characteristic microelasticities for tissues: brain [7] and fat [8] are hundreds of Pascals in stiffness whereas cartilage [9] and pre-calcified bone [10] are dozens of kiloPascals or even stiffer on larger length scales. Precise regulation of physical properties of the ECM seems to match and couple to the applied mechanical forces that contribute to specific cell differentiation programs in adult tissue and likely in the embryo. A differential cell response to both ECM elasticity and dimensionality (i.e. 2D vs 3D) — termed ECM mechanosensing — has been observed *in vitro* through various materials approaches, particularly with natural [11] and synthetic [12] hydrogels. Naturally derived polymers such as silk [13], collagen and hyaluronic acid matrices [14] are currently used as delivery vehicles for cell transplantation. Synthetic scaffolds are chosen based on properties that range from biostability or biocompatibility to biodegradability and porosity. Inert synthetic hydrogels are used *in vitro* for studying cell behavior such as migration, proliferation, and differentiation. Indeed, due to the chemistry that can sometimes be very simple, physical parameters such as elastic and viscous moduli can be precisely tuned to mimic biological tissues.

Synthesis, functionalization and characterization

The basic components for polymer hydrogel synthesis are a monomer, a crosslinker, and an initiator of polymerization. The ratio and concentration of monomer and crosslinker are varied to achieve desired viscoelastic properties, perhaps to mimic a normal or diseased tissue or perhaps to be distinct from a tissue. For example, a myocardial infarct stiffens twofold to threefold relative to normal heart tissue (~12–20 kPa) [5]. Rheological methods provide measures of a material's complex modulus or stiffness (G^*) composed of both an elastic modulus (G') and a viscous modulus (G''). These can be measured as a function of frequency of oscillatory shear with a rheometer, and one typically considers that the 1 Hz beating of the heart is close to the high frequency limit of cell biological relevance. Solid tissues are mostly elastic, with G' values ranging from 0.1 to 100 kiloPascals [10]. Material-dependent cell responses are thus strongly influenced by the elastic component of a hydrogel, at least when G'' is two orders of magnitude lower than G'. Viscous matrix effects on cell morphology are nonetheless interesting based on recent examples in the literature [15,16].

Control of hydrogel chemistry can extend to spatiotemporal control of polymerization [17] and micropatterning [18]. Non-uniform substrates might, for example, mimic a heterogeneous cell microenvironment, but in such a case, rheological measurements must be done at the cellular scale. One particularly attractive method is atomic force microscopy (AFM): a cantilever probe reflects a laser onto a photodiode detector that measures small variations in cantilever deflection as it indents a substrate. For such heterogeneous substrates, an AFM cantilever can probe and create a viscoelastic map along a preset path. For example, we recently examined 'durotaxis', which is a phenomenon in which a cell migrates toward increasing matrix stiffness [19,20,21], and so we and others made hydrogels with stiffness gradients and used AFM to measure the steepness of those gradients [20,21].

Most hydrogels require some form of functionalization to promote favorable cell–material interactions. This can be done by linking cell-adhesive moieties into the polymer backbone, via functional side group chemistry. The well-known integrin-binding tripeptide RGD can be incorporated into a methacrylated polymer backbone (e.g. methacrylated polyethylene glycol) via a Michael-type addition reaction between thiol (from a cysteine moiety in the cell-adhesive peptide) and methacrylate groups [18]. Large matrix proteins like fibronectin and collagen can also be covalently crosslinked into an amine-containing hydrogel backbone via a heterobifunctional crosslinker that contains a primary amine-reactive succinimidyl ester and a photoactivatable nucleophilic azide (e.g. sulfo-SANPAH) [22]. Conventional matrix functionalization of hydrogel systems involves copious coverage of the cell–material interface with cell-adhesive molecules, to ensure that cell attachment is not adhesion ligand-limited and that any differential cellular response is due to physical properties of the matrix.

Advances in soft matter research

Whereas past studies of the cell–material interface have focused on the effects of relatively homogeneous and weakly varying materials on stem cells, recent efforts have begun to address some aspects of matrix micro/nano-heterogeneity. Tools that allow non-invasive *in situ* measurements of cell–material interaction at small scales could ultimately clarify governing principles for cell–material interface design. Fabrication approaches are equally important as they should allow for systematic nanoscale control of substrate topography and functionalization. A great deal of effort needs to be spent in (1) understanding how matrix ligand is presented at the interface, (2) how a cell adheres and applies ligand-dependent and stiffness-dependent traction forces to a material, and (3) how a cell remodels or secretes adhesion-relevant molecules or other factors presented at its interface.

Insight into the first two issues above has been obtained from integrin clustering that occurs when a cell exerts traction forces in response to stiff matrix. Huebsch *et al.* [23••] found that increasing matrix resistance to adhesion