stem cell
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REVIEW

Engineering a stem cell house into a home

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Abstract

In the body, tissue homeostasis is established and maintained by resident tissue-specific adult stem cells (aSCs). Through preservation of bidirectional communications with the surrounding niche and integration of biophysical and biochemical cues, aSCs actively direct the regeneration of aged, injured and diseased tissues. Currently, the ability to guide the behavior and fate of aSCs in the body or in culture after prospective isolation is hindered by our poor comprehension of niche composition and the regulation it imposes. Two- and three-dimensional biomaterials approaches permit systematic analysis of putative niche elements as well as screening approaches to identify novel regulatory mechanisms governing stem cell fate. The marriage of stem cell biology with creative bioengineering technology has the potential to expand our basic understanding of stem cell regulation imposed by the niche and to develop novel regenerative medicine applications.

Stem cells and their niche

Stem cells, in contrast to progenitor cells, harbor the unique ability to divide and generate additional stem cells (self-renew) and to produce progeny that differentiate into tissue-specific cells with defined physiological functions. These properties make embryonic stem (ES) cells, induced pluripotent stem (iPS) cells [1,2] and tissue-specific adult stem cells (aSCs) well suited for regenerative medicine applications. Nevertheless, the clinical use of ES cells, iPS cells, and aSCs for cell-based therapies is hindered by a number of critical hurdles. In addition to the ethical considerations associated with the generation of ES cells, cell populations derived from totipotent ES and iPS cells have the potential to generate teratomas upon transplantation if the fidelity and efficiency of differentiation and enrichment protocols are not ideal.

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aSCs are intrinsically wired to differentiate efficiently into cells from their tissue of origin. However, their relative infrequency in tissues and our limited understanding of the parameters regulating their differentiation and self-renewal currently precludes most aSC-based clinical applications. However, the medical potential of stem cells, specifically aSCs, can be realized by placing unprecedented emphasis on elucidating the mechanisms governing their behavior and fate.

aSC regulation is largely attributed to dynamic bidirectional interactions made with the tissue environment in the immediate vicinity of the cell, termed the 'niche' (Figure 1). First formally described in the fruit fly, *Drosophila* [3,4], the stem cell niche, or microenvironment, is composed of both biochemical (growth factors, cytokines, receptor ligands, and so on) and biophysical (matrix stiffness, topography/architecture, fluidity, and so on) factors that act singly and in concert to continuously modulate cell fate. Despite widespread recognition of its importance, our understanding of niche elements and their cell and molecular influence on aSCs is limiting. We can remedy this by adopting creative research approaches that allow systematic analysis of candidate niche factors and are amenable to screens to identify presently unrecognized niche elements. By advancing our understanding of stem cell niche regulation we can begin to envision regenerative medicine applications built on principles derived from fundamental niche biology.

Naturally derived (that is, collagen, fibrin, Matrigel™) and synthetic (that is, polyethylene glycol, polyacrylamide, nanofibers) biomaterials can be designed and patterned down to minute detail, offering the possibility to engineer stem cell niches and test effects of putative biochemical and biophysical features on stem cell fate in culture. Using biomaterials as a design framework, our understanding of niche composition and how components regulate stem cells is limited only by the imagination. In this review we will discuss two- and three-dimensional biomaterial approaches to deconvolve the niche and its regulatory effects, and we will provide several examples of clinical applications that may benefit from biomaterials research.

Engineering two-dimensional stem cell microenvironments

The native aSC niche is a three-dimensional entity, and ultimately the most representative culture model of any
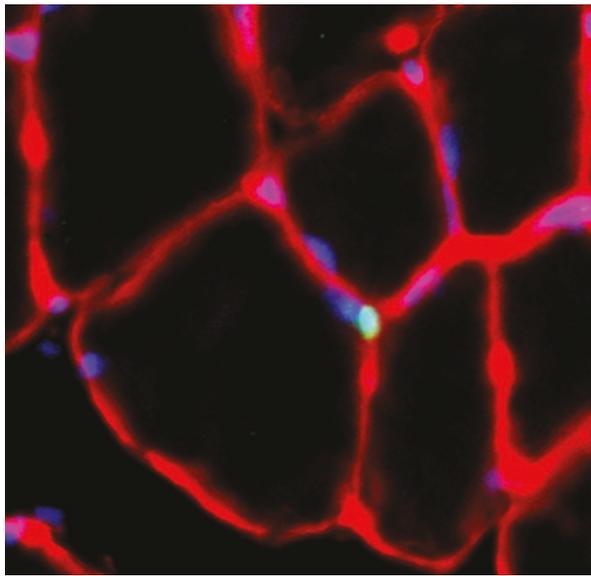
Figure 1. The satellite cell niche. Adult stem cells, such as skeletal muscle satellite cells, engage in bidirectional communication with the surrounding niche to maintain tissue homeostasis. Pax7 (green) expressing satellite cells receive direct biophysical and biochemical cues from the multinucleated (blue) skeletal muscle fibers (black) they sit on top of and the laminin (red) containing basement membrane with associated growth factors and cytokines surrounding each fiber and encasing the stem cell. This confocal image of a muscle cross-section further illustrates the architecture of the resting niche, which poses an additional level of regulation on stem cells.

tissue must reflect this detail. However, the effect of dimensionality on cells is complex to study and a means to do this has yet to be fully realized, making two-dimensional biomaterials approaches to deconstruct and study individual niche components particularly attractive. Extrinsic regulation of aSCs by niche elements - including cell-cell contact mediators, secreted signaling factors, extracellular matrix (ECM), substrate stiffness and topography, nutritional parameters (O_2, nutrients), pH, temperature, fluid flow, mechanical stress (that is, cyclic strain) and even gravity - can all be probed in two-dimensions to generate a modular toolbox of stem cell regulation that can be used in future three-dimensional niche reconstruction [5]. While our focus here is extrinsic stem cell regulation, it should be noted that intrinsic regulation is fundamentally important and typically both intrinsic and extrinsic regulation act in concert to modulate cell behavior [6]. In this section we will discuss several niche parameters and the approaches used to probe them in two dimensions using examples from the literature.

Exploring cell-cell interactions

Tissue regeneration requires resident aSCs to survey the status of the microenvironment and respond appropriately when alterations resulting from aging, injury or

disease are detected. In addition to changes incurred by the surrounding ECM or the influx of circulating factors from the vasculature, aSC behavior is guided through direct and indirect interactions with cells in close juxtaposition. Employing a biomaterials-based approach allows for fundamental insight into the spatial and temporal nature of aSC interactions with the surrounding support cells in the resting microenvironment and discovery of how those relationships change upon tissue insult.

Typically, co-culture of two or more cell types in a culture dish is used to study cell-cell interactions, though it is notoriously difficult to draw definitive conclusions about mechanism due to the complexity of the system. Rather than studying a heterogeneous mix of two cell types, clever biomaterials-based strategies were developed to generate isolated cell 'pairs'. Microfluidics technology [7] combined with patterning on polydimethylsiloxane (PDMS; a silicone polymer that can harden to a rubber-like material) to create an array of cell 'traps' and a three-step loading protocol, was used to create a grid containing hundreds of 'co-culture' replicates [8]. Spatially segregating the cell pairs enables the user to evaluate cell fate changes over time at the pair level. Physical isolation of two cell types can also be achieved using synthetic, polyethylene glycol (PEG) hydrogels or PDMS patterned with microwells [9-15]. Tunable PEG hydrogel provides the additional flexibility to interrogate cell pairs while altering additional microenvironmental parameters such as matrix rigidity and ECM/ligand identity, density or mode of presentation (that is, tethered or soluble). Importantly, these approaches are all amenable to high-throughput screening and time-lapse microscopy to assess co-culture effects on stem cell behavior and fate changes over time, such as division resulting in symmetric or asymmetric self-renewal, differentiation and changes in viability.

To investigate whether observed co-culture behaviors are contingent on the direct interaction of two cells or due to indirect paracrine effects, a co-culture approach utilizing two interlocking combs was developed [16]. In this paradigm each cell type is cultured on an individual silicon comb and cell behavior and fate are assessed while combs are interlocked or when separated at known micrometer scale distances. This biomaterials strategy can spatially resolve the distance of relevant cell-cell communications, but unlike the cell trap and microwell technology it is difficult to reliably study cell-cell interactions at the pair level and the approach is limited to adherent cell types.

Elucidating cell-extracellular matrix communications

In addition to cell-cell interactions, aSC fate is modified by interactions with the ECM. Upon injury and aging or during disease progression the matrix composition is

dramatically altered, cryptic binding sites are exposed and aSCs can gain direct exposure to ECM ligands they were previously sheltered from. Identification of putative ECM ligands present in resting and activated tissue and their impact on aSC behavior and fate is enabled by recent advances utilizing robotic spotting to print single and combinations of ECM ligands as arrays and subsequently culture and follow the fate of exposed cells [17,18]. Using this type of unbiased throughput approach can greatly advance our basic understanding of cell regulation by the matrix in the niche as well as to provide a catalogue of matrix-mediated cellular outputs that can be used to direct stem cell fate.

Standard tissue culture protocols typically supplement growth factors and cytokines in the soluble media milieu, while in tissues these secreted morphogens are most commonly presented to cells tethered to the ECM [19]. Covalent attachment of secreted growth factors to biomaterial surfaces demonstrated improved stability of labile proteins and persistent signaling resulting in long-term maintenance of signaling without the requirement to supply additional protein [20-23]. In addition to protein stabilization, mode of ligand presentation (soluble versus tethered) was shown to have profoundly divergent effects on cell fate underlying the relevance of this distinction [20-22,24,25]. Studies investigating ligand presentation and assessing how the mode of presentation influences cell fate promise not only to advance our basic understanding of aSC regulation, but also to aid researchers in the smart design of culture conditions to promote a desired fate.

As described above, the ECM can directly modulate aSC behavior in the niche through direct receptor-ligand interactions. In addition, the density, fiber alignment and porosity of the ECM can impart spatial influence over cells to dictate cell shape, an aspect which is progressively gaining needed attention [26]. For example, cells cultured on micropatterned ECM islands with the same ligand density but with different surface area generate distinct spreading phenotypes resulting in marked cell shapes (rounded versus spread), which impose impressive influence over cell viability [27]. More recently, the molecular mechanisms and signaling pathways driving cell shape-mediated effects on stem cell populations have been described [28,29]. Importantly, during wound healing and disease progression, tissues undergo profound alterations in the identity and organization of the ECM, whose cellular and molecular effects are a topic of intense investigation. Niche architectural effects confer a unique dimension of aSC regulation by the ECM and warrant greater focus by stem cell researchers.
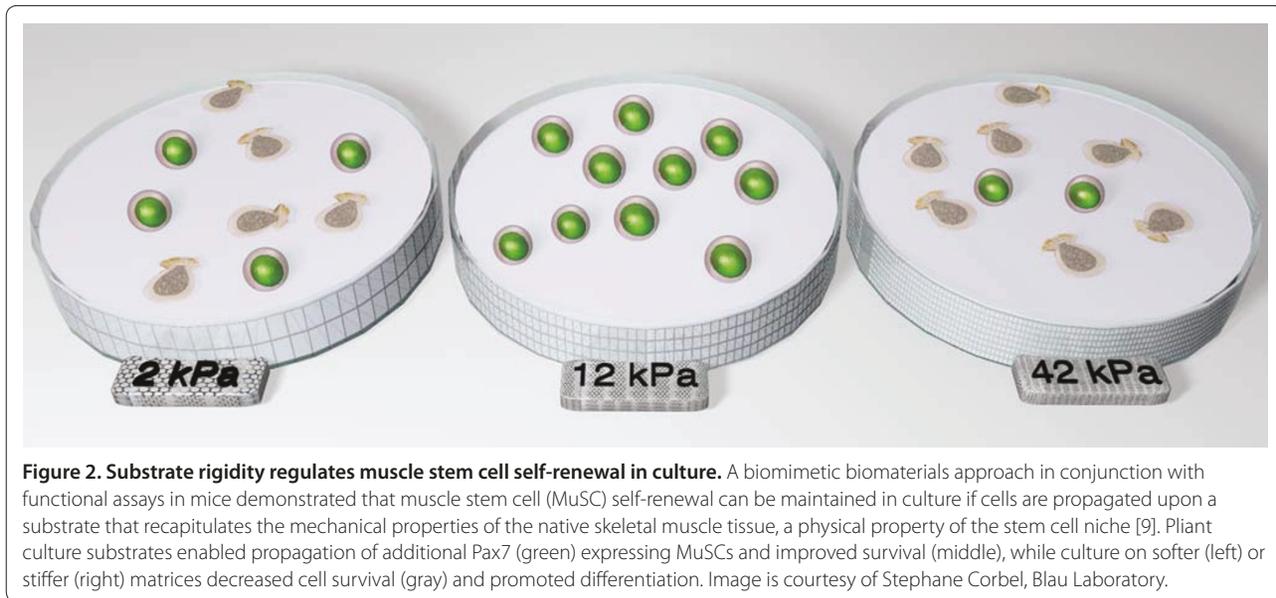
Investigating cell-matrix interplay

Imagine pulling a string to turn on or off a lamp. Typically the string is attached to something stationary and stiff allowing you to generate resistance and activate the switch. Imagine instead that the string is attached to something soft like putty; the more you pull the string, the more the soft putty will stretch preventing force generation or activation of the light bulb. Adherent cells are constantly assessing their microenvironment by making contact with and pulling at the ECM. Cells pulling on adhesion ligands attached to a stiff as opposed to a soft matrix experience cytoskeletal reorganization resulting in distinct intracellular signaling that can profoundly alter cell fate [30-32]. Thus, the mechanical properties of the niche, a biophysical cue, add yet another level of regulation imposed by the ECM.

First demonstrated using immortalized cell lines [33], the ability of matrix stiffness to regulate cell fate is now widely accepted. In a groundbreaking study exploring the impact of substrate rigidity on stem cell fate, mesenchymal stem cells were shown to differentiate into bone, muscle or brain when cultured on polyacrylamide substrates mimicking the mechanical properties of each tissue [34]. Since then, a similar biomimetic approach to tune the culture substrate to the stiffness of the endogenous tissue has been used to encourage lineage-specific differentiation to additional multipotent stem cells, such as neural progenitors, and to culture ES and iPS cell colonies long term without loss of stemness in the absence of the fibroblast feeder layer [35-37]. Notably, soluble factors present in culture media typically act together with the culture matrix to regulate cell fate and these interactions should be considered when drawing conclusions. Also, in contrast to standard tissue culture plastic, porous matrices (polyacrylamide, PEG) permit diffusion of soluble molecules to both the apical and basal cell surfaces, and decoupling the effects of substrate stiffness from bidirectional diffusion is still a challenge.

Unlike ES and iPS cells, prospectively isolated aSCs, such as skeletal muscle satellite cells, are notoriously difficult to expand in culture due to their natural inclination to differentiate upon exposure to rigid tissue culture plastic [38]. Satellite cells were first identified by electron microscopy according to their anatomic location and described as a mononucleated cell that resides atop multinucleated postmitotic skeletal fibers and beneath a thin basement membrane (Figure 1) [39]. Despite the current knowledge that satellite cells are responsible for the remarkable ability of postnatal skeletal muscle tissue to regenerate in response to injury, aging and disease [38,40-46], surprisingly little is known about the components of the niche or the extrinsic regulation imposed by the niche on satellite cell fate. However, recently developed strategies to prospectively isolate satellite cells to relatively high purity [38,41-46] in conjunction with robust *in vivo* functional assays of muscle stem cell fate [9,46] render the satellite cell ready for interrogation in culture.

Figure 2. Substrate rigidity regulates muscle stem cell self-renewal in culture. A biomimetic biomaterials approach in conjunction with functional assays in mice demonstrated that muscle stem cell (MuSC) self-renewal can be maintained in culture if cells are propagated upon a substrate that recapitulates the mechanical properties of the native skeletal muscle tissue, a physical property of the stem cell niche [9]. Pliant culture substrates enabled propagation of additional Pax7 (green) expressing MuSCs and improved survival (middle), while culture on softer (left) or stiffer (right) matrices decreased cell survival (gray) and promoted differentiation. Image is courtesy of Stephane Corbel, Blau Laboratory.

To investigate the role of matrix rigidity on satellite cell fate, freshly isolated and FACS (fluorescence activated cell sorting) enriched muscle stem cells (MuSCs) were cultured on PEG hydrogels with differing mechanical properties but constant ligand density [9]. Timelapse videos of MuSC clonal division within microwells were automatically analyzed using the Baxter algorithm and revealed improved survival when MuSCs were cultured on substrates that mimic the mechanical properties of skeletal muscle tissue. Noninvasive bioluminescence imaging of luciferase-expressing MuSCs transplanted intramuscularly into mice after culture on hydrogels of varied stiffness demonstrated that culture on a muscle biomimetic substrate provides the optimal condition to maintain 'stemness' long term (Figure 2). Further, an *in vivo* functional assay showed definitively that MuSCs cultured on pliant hydrogel could self-renew in culture while those propagated on plastic lost self-renewal potential in as few as 2 days. Critical to the conclusions drawn in these studies is the use of freshly isolated aSCs in combination with functional assays in mice to validate all culture observations; an experimental paradigm that sets the bar for future applications of biomaterial approaches to study stem cell fate.

In conclusion, two-dimensional biomaterial approaches are exceptionally well suited to study the cellular and molecular mechanisms governing stem cell fate regulation by the immediately opposing niche as well as the greater surrounding microenvironment. Tunable synthetic polymer platforms offer the flexibility to study stem cell fate in response to simple or complex combinations of putative niche parameters. In addition, these systems are highly amenable to time-lapse microscopy analysis and

with recently developed strategies to automatically analyze cell behavior and lineage relationships, it is now feasible to evaluate the vast amounts of data generated by such studies [9,11,47,48]. The success of two-dimensional biomaterials approaches to study stem cell regulation in culture is contingent on the availability of markers and/or behaviors that accurately predict stem cell fate *in vivo* [49]. Transgenic reporter animals used for prospective isolation of aSC populations can be used to dynamically assay stem cell fate in real time and are particularly advantageous. Without robust, simple readouts it is difficult to perform high-throughput analysis of aSC populations to screen for novel biochemical and biophysical features that regulate stem cell fate and further refine the resting, aged, injured and diseased niches. Nevertheless, by implementing two-dimensional biomaterials-based approaches to study aSC regulation, we are likely to expand our current diagnostic capabilities, enable *in vivo* modulation of aSC populations, and develop strategies to expand aSCs in culture for use in cell-based therapies.

Engineering three-dimensional stem cell microenvironments

In contrast to two-dimensional tissue culture approaches, many aSCs are embedded within a complex, instructive three-dimensional matrix, often in intimate contact with additional cell types and in proximity to nutrient and oxygen-delivering vasculature. While two-dimensional approaches enable well controlled interrogation of single putative niche elements on cell fate, the focus of three-dimensional tissue engineering is to reconstruct the complex architecture of stem cells within a three-dimensional matrix to achieve a physiologically relevant

structure. Of course, this goal is highly complicated, but by comparing to and extending the design principles established in two-dimensional studies, three-dimensional material biology has the greatest potential to impact our understanding of *in vivo* tissue function. As there are several excellent reviews describing the current technical advances in the relatively nascent field of three-dimensional tissue model generation [50-54], here we will focus on the challenges and potential of three-dimensional matrix biology.

Challenges of three-dimensional culture models

Three-dimensional biomaterials to encapsulate stem cells and investigate niche-mediated effects come with a number of design challenges absent in two-dimensional culture that must be overcome prior to use of the materials by the biological community. A first design concern is the nutrient and oxygen requirements of fully encapsulated stem cells [55,56]. Hydrogel systems with the flexibility to optimize matrix porosity can easily meet this design challenge and provide adequate energy requirements to maintain viability. A second criterion to consider is the mechanism of polymer polymerization. While natural matrices and some synthetic polymer systems spontaneously interact over time to form a three-dimensional network, other synthetic hydrogel matrices rely on chemical or photo-initiators to achieve polymer crosslinking and have potentially toxic effects on encapsulated cells. An additional challenge inherent to synthetic three-dimensional scaffolds is the need to design strategies permitting cell migration after encapsulation. This has been successfully achieved through incorporation of matrix metalloproteinase or other proteolytic cleavage sequences into the polymer sequence [57]. An added benefit of polymer design is the ability to design scaffolds that permit migration of specific cell types based on whether or not they secrete certain enzymes. A final design challenge is development of three-dimensional polymer matrices that permit independent tuning of biophysical and biochemical parameters allowing three-dimensional culture optimization on a cell type basis. Extending this to permit matrix tunability over time in a spatial and temporal manner has the potential to enable exquisite study of stem cell fate changes as they may occur during disease progression [58]. Through the careful design and thoughtful characterization of the parameters described above it is now possible to produce biomaterials that promote long-term survival, proliferation and differentiation of stem cells in three dimensions.

Establishing the effects of dimensionality

One of the most exciting research areas enabled by three-dimensional biomaterials technology is the ability to determine the behavioral and molecular effects of dimensionality. While standard two-dimensional approaches essentially define the apical and basal surface of the cultured cells, three-dimensional culture provides a situation wherein the cell actively directs its own polarity. By comparing cell behavior in three dimensions to that in two dimensions it is feasible to probe the influence of dimensionality on cultured cells. However, it is critically important to consider the limitations of the system employed, asan observed effect could be due to a constraint in the culture system and not dimensionality *per se*. For example, a difference in cell behaviour or function may be confounded by a lack of appropriate growth factor and nutrient diffusion through three-dimensional biomaterials. Culture systems designed to overcome this common diffusion barrier in the three-dimensional culture setting are needed to draw meaningful conclusions about the effects of dimensionality on cell fate [59].

Recent studies exploring the effect of dimensionality on cell behavior and fate have revealed several surprising findings. For example, a comparison of breast tumor cells lacking or re-expressing *HOXA9*, a novel breast tumor suppressor gene, exhibited no difference in cell growth when assayed in two dimensions, but when the cells were embedded within a three-dimensional reconstituted basement membrane (mimicking the *in vivo* micro-environment) distinct differences in proliferation were observed [60]. These studies underscore the importance of studying cells in the context of a three-dimensional tissue-like structure in order to fully realize the effects of a genetic (intrinsic) alteration. Further, when reconstructing a three-dimensional stem cell microenvironment it should not be assumed that observations made in two dimensions will necessarily translate into a similar effect in three dimensions. Often additional tweaking of biophysical and biochemical parameters in three dimensions is necessary to optimize a desired stem cell behavior [19,36,61]. Arguably, one of the most interesting dimensionality-related discrepancies arose from studies on cell migration. Until now, models of cell migration were derived from two-dimensional studies of cell motility and led to an understanding that migration is intimately linked to the formation of distinct sites of cell attachment containing paxillin, vinculin, actin, focal adhesion kinase as well as other structural and signaling molecules necessary for focal adhesion formation and force generation. However, in three dimensions it was noted that migration occurs in the absence of distinct focal adhesion formation and the characteristic molecules observed in focal adhesion aggregates in two dimensions (paxillin, vinculin, and so on) were found diffusely localized throughout the cell during three-dimensional movement [62]. Similar comparisons of two-dimensional

behaviors in three-dimensional culture systems may reveal similar discrepancies and contribute to our understanding of how dimensionality regulates stem cells.

Potential of three-dimensional matrix biology

Three-dimensional biomaterials enable reconstruction of physiological models of tissue matrix scaffolds and their accompanying cell types in both homeostatic and disease states [19]. Not only can they be used to expand our basic knowledge of stem cell regulation by the microenvironment, but these models can also facilitate identification of therapeutics targeting the stem cell niche to treat aged, injured and diseased tissues. While it is unreasonable to expect three-dimensional models to mimic the native tissue down to molecular detail, by recapitulating certain fundamental physiological functions, such models can be used to study how perturbations to systems such as the human airway wall, the lung or liver effect specific functional outcomes to investigate the efficacy and mode of action of novel and currently prescribed medications [63-65]. In addition, these models can be used to test the toxicity of drugs intended for use in patients. Finally, three-dimensional biomaterials can be expected to play a substantial role in directing tissue regeneration or even act as replacement tissues as described in the following section.

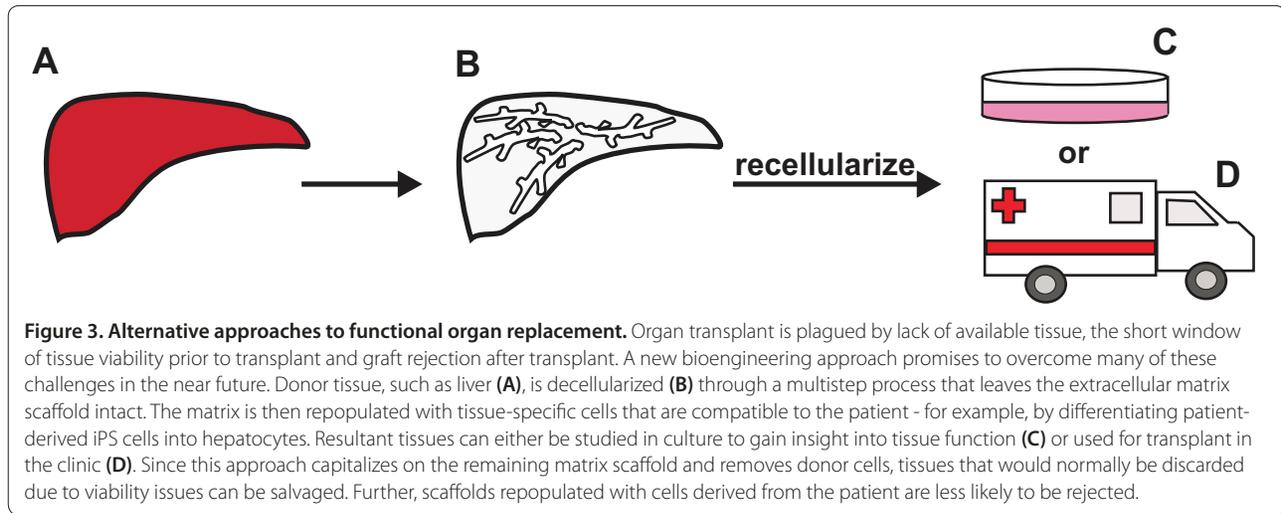## Clinical translation of engineered microenvironments

The integration of bioengineering approaches with stem cell biology has the potential to substantially change the practice of medicine as we know it today. While hematopoietic cell transplantation therapies have been used in the clinic for more than a decade to resolve blood malignancies, most solid tissues are precluded from treatment with cell-based therapies to regenerate defects and restore function. Several complicated factors lend to this discrepancy, but the lack of suitable strategies to expand isolated aSCs or to robustly differentiate ES or iPS cells into a single tissue-specific lineage is a major limitation to the progress of cell-based therapies. Using two-dimensional or three-dimensional biomaterials approaches, it is realistic to imagine that in the near future we will identify simple strategies based on smart design principles to expand aSCs and direct ES and iPS cell fate, enabling cell-based regenerative therapeutics.

After injury, or as result of aging or disease, the homeostatic microenvironment can undergo substantial remodeling and reconstruction and, consequently, render the environment ill-instructive for resident tissue-specific aSCs. For example, it is hypothesized that extrinsic changes to the satellite cell microenvironment prevent effective skeletal muscle regeneration rather than intrinsic changes to the satellite cell itself during aging [66].

As an alternative to cell based therapies, studies suggest that simply providing an instructive cell-free scaffold to artificially modify the microenvironment and direct the aSCs residing in tissue could prove useful to regenerate damaged tissue [67]. This approach was first developed and utilized in the repair of critical sized defects in bone through the use of allogeneic demineralized bone matrix, a US Food and Drug Administration approved product, and has now been extended to many other tissue types [68,69]. For example, cell-free scaffold-based strategies are already used in the clinic to repair open skin wounds on war victims [70]. By focusing on biochemical and biophysical parameters governing stem cell fate decisions (that is, directed migration, proliferation, differentiation, and so on), materials impregnated with signaling molecules designed for release in a temporally and spatially regulated manner are a viable option to modulate cell fate and promote repair over time within the intact patient [71].

Regenerative medicine using cell-free scaffolds relies on the patient's own cells to migrate into and repopulate the acellular scaffold (Figure 3). To overcome this potential challenge, strategies combining synthetic or natural matrices repopulated with cell types required for long-term function of the replacement tissue are being developed. For example, large cartilage defects resulting from injury or aging are notoriously difficult to repair. Use of a nanofibrous scaffold seeded with human mesenchymal stem cells (which evade the immune response) demonstrated the ability of a bioengineering approach to repair large cartilage defects in swine while restoring smooth cartilage at the surface and withstanding use-associated compression force [72]. Similarly, corneal function was restored in patients afflicted by debilitating burns using autologous limbal stem cells embedded in fibrin gels [73].

A major challenge in the clinic is the availability of donor tissue for transplantation into patients with critical organ failure. A tissue-engineering approach based upon the principle of designing stem cell microenvironments that incorporate the cell types, signaling cues and structure required for long-term physiological function and incorporation in a living patient has the potential to substantially reduce the current reliance on organ donors to provide tissues to patients in critical need. Though generation of functional three-dimensional organs is an extraordinary challenge, several research groups are actively pursuing this goal and the literature is already repleat with successes. To overcome the challenge of lost bladder function in young patients afflicted with disease rendering malfunction, researchers utilized a bioengineering approach to construct collagen scaffolds in the likeness of the human bladder. To ensure proper long-term function and to reduce the possibility of tissue

Figure 3. Alternative approaches to functional organ replacement. Organ transplant is plagued by lack of available tissue, the short window of tissue viability prior to transplant and graft rejection after transplant. A new bioengineering approach promises to overcome many of these challenges in the near future. Donor tissue, such as liver **(A)**, is decellularized **(B)** through a multistep process that leaves the extracellular matrix scaffold intact. The matrix is then repopulated with tissue-specific cells that are compatible to the patient - for example, by differentiating patient-derived iPS cells into hepatocytes. Resultant tissues can either be studied in culture to gain insight into tissue function **(C)** or used for transplant in the clinic **(D)**. Since this approach capitalizes on the remaining matrix scaffold and removes donor cells, tissues that would normally be discarded due to viability issues can be salvaged. Further, scaffolds repopulated with cells derived from the patient are less likely to be rejected.

rejection, the engineered bladders were seeded with urothelial and muscle cells isolated from the patient prior to transplantation. Follow-up studies 2 years following transplantation concluded that the bioengineered bladders had not only maintained architecture, but were also still fully functional in the patient recipients [74]. Organ transplantation is typically accompanied by use of immune suppression treatment to reduce the incidence of immune rejection. To improve transplantation success, several researchers are adopting a bioengineering approach that entails decellularizing donor tissue (to remove the major histocompatibility complex (MHC) component) with a gentle, multistep detergent treatment that leaves the matrix scaffold intact and permits recolonization with patient derived cells. This approach has been used successfully to treat a patient suffering from broncho-malacia (loss of airway function). Transplant of a decellularized donor trachea repopulated with epithelial cells and chondrocytes from patient-derived mesenchymal stem cells led to successful long-term repair of the airway defect and restoration of mechanical properties [75]. Finally, a recent study demonstrated the possibility of using a bioengineering approach to construct corporal tissue to facilitate penile reconstruction. In a multistep, dynamic process the three-dimensional corporal tissue was engineered from a naturally derived collagen matrix reseeded with autologous cells and transplanted into rabbits with excised corpora. Amazingly, the bioengineered phallus was structurally similar to the native tissue and function was demonstrated by successful impregnation of female rabbits with the engineered tissue [76]. Together these examples exemplify the potential impact that material science will have on the treatment of human disease in the not so distant future.

Conclusion

Both two-dimensional and three-dimensional biomaterials approaches are changing the way scientists think about the stem cell microenvironment and are providing strategies to regulate the fate of prospectively isolated stem cells in culture and of stem cells residing in intact tissues. More importantly, current biomaterials technologies and the inevitable future technological advances in the field provide a novel toolbox for stem cell biologists to investigate the impact of niche biochemical and biophysical properties in unprecedented ways. These engineering approaches can be extended to all prospectively isolated stem cell populations for the purpose of elucidating the mechanisms governing their regulation.

To accelerate the impact of biomaterials towards the treatment of human disease, it is essential to incorporate *in vivo* functional assays as a standard practice to validate observations made in culture. Furthermore, by placing more emphasis on human stem cells and their niche regulation, we can advance the translation of material-based therapeutics from the bench to the bedside. Bioengineering approaches to study the stem cell microenvironment have the potential to revolutionize regenerative medicine by providing physicians with tools to regulate resident aSC behavior (that is, self-renewal, differentiation, migration) in patients, cells for cell-based therapies, and perhaps even bioengineered organs to replace defective tissues. Ultimately, the active collaboration of engineers, biologists, physicians, chemists, computational scientists and physicists towards the goal of understanding the niche, how it regulates stem cell fate and how it changes with aging, injury and disease will allow us to harness this knowledge and generate novel regenerative medicine therapeutics.

Abbreviations

Competing interests

Authors' contributions

Acknowledgements

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