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3D Biomaterial Microarrays for Regenerative Medicine: Current State-of-the-Art, Emerging Directions and Future Trends

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Three dimensional (3D) biomaterial microarrays hold enormous promise for regenerative medicine because of their ability to accelerate the design and fabrication of biomimetic materials. Such tissue-like biomaterials can provide an appropriate microenvironment for stimulating and controlling stem cell differentiation into tissue-specific lineages. The use of 3D biomaterial microarrays can, if optimized correctly, result in a more than 1000-fold reduction in biomaterials and cells consumption when engineering optimal materials combinations, which makes these miniaturized systems very attractive for tissue engineering and drug screening applications.

1. Introduction

Regenerative medicine holds great promise for treating patients with debilitating tissue damage resulting from traumas, diseases and aging.[1,2] The field of regenerative medicine can address these challenges by optimizing cell-biomaterial interactions for the biofabrication of artificial tissues.[3] In the past decade, smart and intelligent biomaterials that mimic the complex environment of native tissues have been developed.[4] However, the current approach to designing biomimetic biomaterials with appropriate physical, chemical, and biological properties is cumbersome, time consuming, costly, and inefficient. This is attributed to complex cell-matrix and cell-cell interactions, which are difficult to mimic under in vitro conditions. For example, the biological milieu of native tissues such as bone consists of several cellular and non-cellular components (**Figure 1**) including, collagen (type I), osteocalcin, osteopointin, bone morphogenetic proteins (BMPs), hydroxyapatite, and polysaccharides.[2,5] Through a conventional screening process, it is very difficult to recapitulate the native tissue microenvironment, resulting in a limited ability to design functional tissue structures. To address this critical issue, microarray technologies for high-throughput screening of multiplexed biomaterials have been developed.[6–9] The primary goal of these microarray platforms is to identify biomaterial combinations that can stimulate, direct, and reorganize cells into artificial tissues structures for regenerative medicine and drug screening applications.[10]

Despite exciting progress in multiplexed biomaterial screening, most of these studies have focused on cellular responses on 2D biomaterials surfaces.[10] However, under in vivo conditions, cells behave differently compared to in a 2D microenvironment,[11] and it is therefore it important to investigate the cell-biomaterial and cell-cell interactions in a 3D microenvironment to guarantee a successful therapeutic outcome. These interactions are able to recapitulate important in vivo conditions and thereby help us to design smart and bioresponsive materials to tailor the differentiation and commitment potential of encapsulated stem cells.[12] Since the structural, physical, and chemical properties of biomaterials play a major role in controlling cellular fate, the use of 3D microarray technology will significantly improve our efficacy in identifying the right material combinations for different tissue engineering approaches. Recent advancements in robotic microprinting have enabled the fabrication of 3D biomaterial microarrays for high-throughput screening of cellular fates within 3D microenvironments.[13–15]

Here, we highlight some of the recent advances in 3D biomaterial microarrays for regenerative medicine and drug screening applications. Various approaches to engineering 3D biomaterial microarrays are reviewed and their application to screening cell-matrix interactions in a 3D microenvironment is critically evaluated. We will also highlight cost effective microarray systems for in vitro drug testing. Finally, the emerging trends

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in designing cell-laden microarrays for examining cell fate within complex heterogeneous environments will be discussed. We will conclude by outlining some of the future prospects of the 3D microarray technology in regenerative medicine.

2. Direct and Indirect Fabrication of 3D Biomaterial Microarrays

The first biomaterial microarray was developed in 2004[6] and consisted of polymeric networks for seeding embryonic stem cells (ES's). This polymer-based biomaterial microarray was used for high-throughput identification of materials that supported ES growth. Later, biomaterial microarrays consisting of extracellular matrix proteins (ECM)[7,16] and peptides[9,17] were developed to evaluate cell-matrix interactions and the role of ECM components on stem cell fate. The 2D microarrays have revealed several regulators that can control and direct cell attachment, proliferation, migration and differentiation. However, cells under in vivo conditions experience a complex 3D microenvironment and thus, recent significant efforts have been directed toward engineering microarrays for screening cellular fates within 3D niches.[13–15,18,19] For example, a range of different ECM proteins, biomolecules, and drug-candidates can be entrapped within 3D biomaterial microarrays and the effect of these components on cellular behavior can be monitored in a combined approach. Such microarrays are directly manufactured through robotic spotting technology or indirectly through soft lithography techniques (**Figure 2**).

In direct manufacturing, cell-laden biomaterials are deposited with either a contact or inkjet printer. A contact printer uses a hollow pin to collet nanoliter volumes of precursor solution consisting of cells from a 384-well plate followed by surface deposition on a solid support.[20] Inkjet printing is based on a micronozzle that discharges the cell-laden precursor solution from a micronozzle onto a solid support.[19,21] Heat or piezoelectric forces can be used to discharge the precursor solution from the micronozzle tip to obtain a functional microarray.[21,22] Another approach to creating 3D biomaterial microarrays is to pipet cell-loaded precursor solutions onto superhydrophobic micropatterned surfaces with hydrophilic regions to create cell-laden microgel arrays.[23,24] These wettability contrast surfaces restrict the deposited cell-laden microgel solutions to the hydrophilic spots and thus result in the generation of microarrays. Some of the advantages associated with the direct manufacturing methods are the easy fabrication process, low cost, and high efficiency. However, some of the limitations of these techniques are a limited control over dispensed volumes and crosstalk between neighboring spots.[21] Both contact and inkjet printing have recently been used to fabricate cell-laden biomaterial arrays for investigating stem cell differentiation within combinatorial environments[13–15] and for high-throughput drug testing.[18,19]

Indirect manufacturing of 3D microarrays consists of two steps. First, microlithography is used to generate microwell arrays[25–27] from a biocompatible silicone rubber, polydimethylsiloxane (PDMS). In the second step, cells are seeded inside the microwells and stimulated into 3D cellular constructs for high-throughput studies.[26,28] The microwell arrays are not suitable for combinatorial biomaterial screenings, but are instead typically used to study the correlation between cell-construct geometry and cell differentiation.[27,29] Microfluidic devices that enable cost-effective screening of cell responses inside micro-bioreactors[30–32] have also been generated through indirect manufacturing techniques.[30,31,33] In one example soft lithography was used to fabricate twelve independent micro-bioreactors.[31] Human embryonic stem cell (hESC)-encapsulated hydrogels were crosslinked inside the micro-bioreactors and each bioreactor was exposed to a different media flow configuration. A correlation was determined between hESCs differentiation and the hydrodynamic shear forces. Indirect manufacturing provides advantages such as volume and geometry control of the deposited microgels. Moreover, the use of microwell arrays significantly reduces the crosstalk between neighboring biomaterial spots.[13] Recent studies have combined robotic printing technology

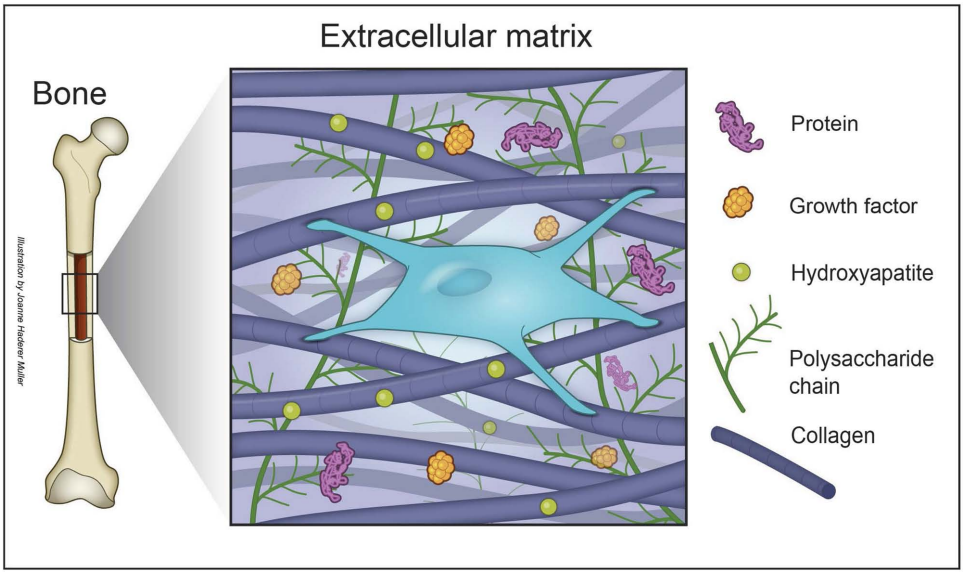

Figure 1. The combinatorial extracellular matrix (ECM) of bone. A range of biological, physical and topographical factors work synergistically to direct cell fate into predetermined tissue type. As an example, the ECM of bone tissue consists of proteins, growth factors, and minerals, as shown here.

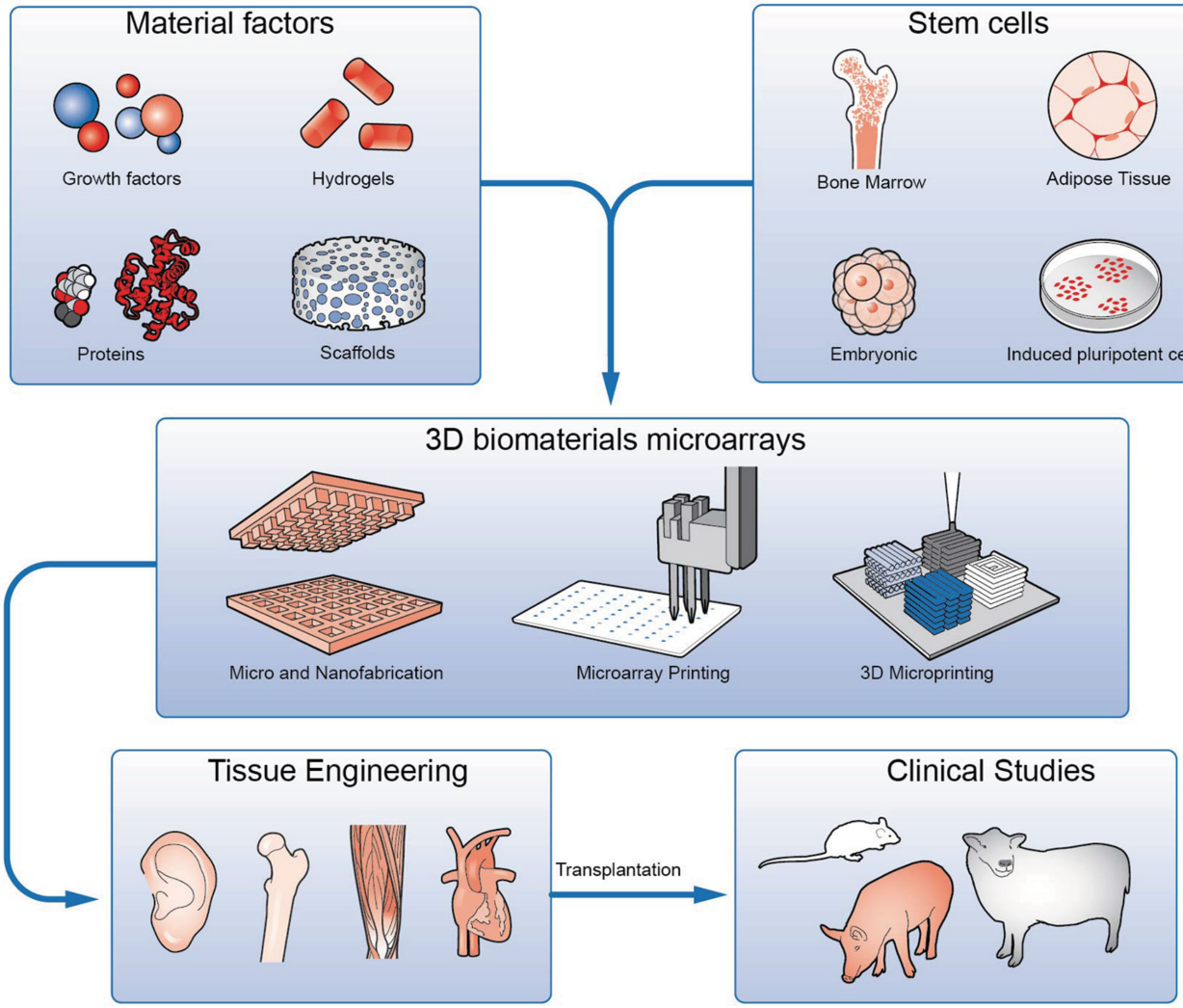

Figure 2. Cost-effective development of native-like 3D biomaterials using microarray technology. A range of material factors including soft hydrogels, hard scaffolds, proteins, and growth factors are combined with cells to create combinatorial cell-laden structures that can generate functional tissues. These native-like compositions are screened in a high-throughput matter to cost-effectively identify and select the best 3D biomaterials for engineering tissue transplants.

with soft lithography to print cell-laden hydrogels loaded with small molecules inside microwells.[13,16,19,34] The combined platforms showed significant promise in multiplexed microarray studies with almost no spot-to-spot crosstalk.[13,19]

3. Biomaterial Microarrays in Stem Cell and Tissue Engineering

Stem cells hold enormous promise as a cell source for tissue engineering and regenerative medicine due to their unlimited self-renewal capacity and multipotency.[35] It is crucial to understand the interactions between biomaterials and stem cells in order to design and fabricate biomaterials that can direct lineage–specific differentiation. The commitment of stem cells to specific lineages is dependent on several instructive signals from their surrounding microenvironment such as basal nutrients, topographical cues,[36,37] mechanical cues,[38] and soluble or insoluble biological factors, among which growth factors, hormones, cytokines and proteins play a key role in determining cell fates.[2,5,39] There is therefore a need to understand the effect of the 3D microenvironment on stem cell fate in a combinatorial manner.[37,40,41] One avenue for meeting these challenges in stem cell bioengineering is the development of combinatorial 3D biomaterial microarrays for high-throughput identification of optimal tissue-engineering conditions.[10,14,42]

3.1. Cell-Laden Microgel Arrays

Soft hydrogels have attracted considerable interest as biomaterial candidates for tissue engineering due to their favorable characteristics such as high water content, biocompatibility, and injectability.[43] With the aid of robotic printing, cell-laden microgel arrays based on gelatin[14] (**Figure 3**a,b), collagen,[19] fibrin,[13] and alginate[15] have been fabricated. A range of ECM proteins such as fibronectin (FN), osteocalcin (OCN), laminin (LN), and collagen IV (Col IV) were embedded within

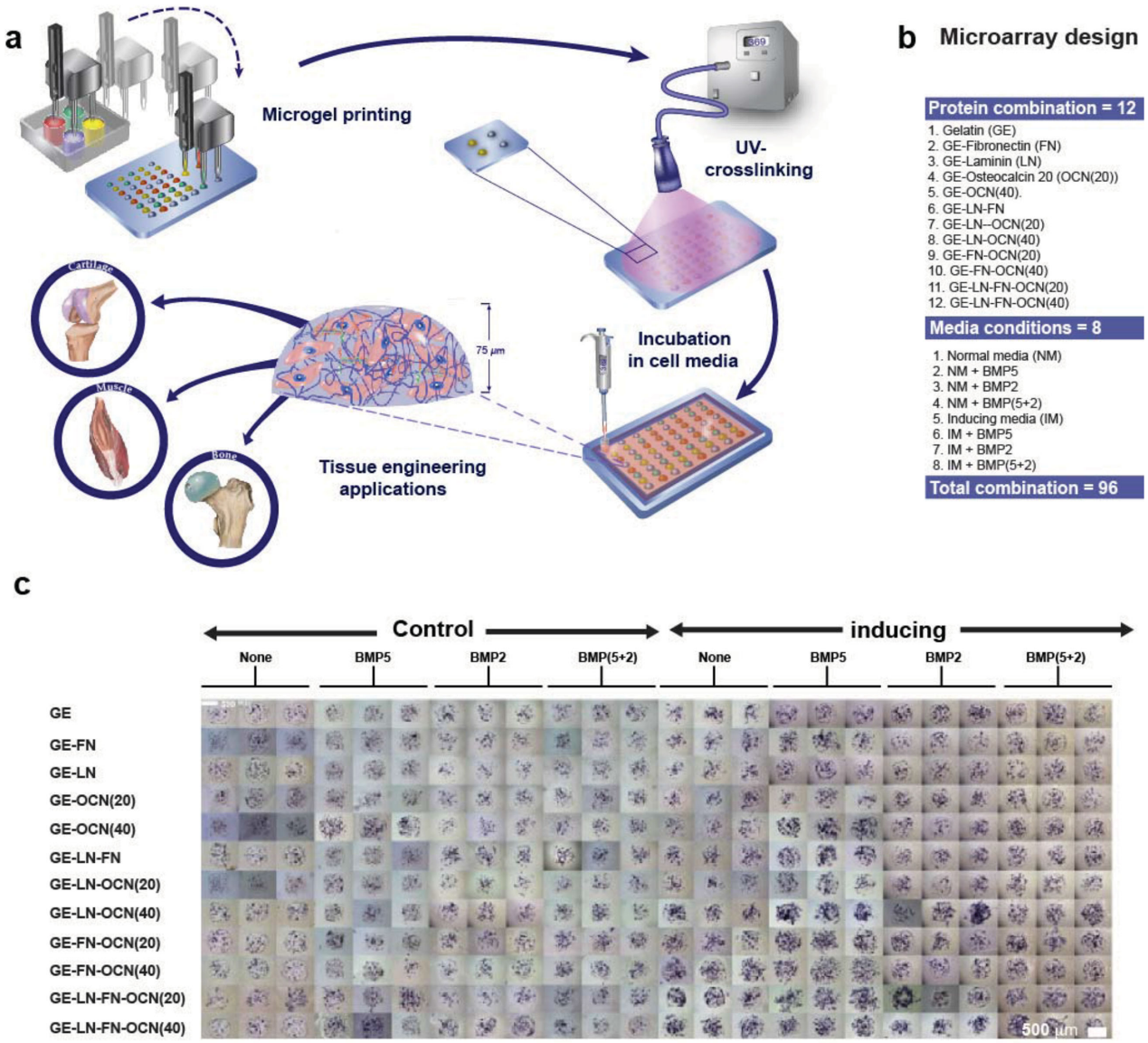

Figure 3. A cell-laden gelatin-based 3D microgel array. a,b) ECM proteins such as fibronectin, laminin and osteocalcin were encapsulated inside gelatin-based microgels together with hMSCs. c) By controlling the ECM environments within the microgels the osteogenic response of hMSCs can be fine-tuned as determined from ALP staining (blue). Adapted with permission.[14] Copyright 2014, Nature Publishing Group.

these polymeric microgels to control and direct the differentiation of mouse ES Cells (mES Cells)[13] and human mesenchymal stem cells (hMSCs).[14] For example, the differentiation of hMSCs towards an osteogenic fate was investigated by incorporating ECM components such as FN, OCN, and LN within the gelatin-based microgels.[14] Enhanced expression of early osteogenic markers was observed due to synergistic contribution from different ECM components (Figure 3c). Specifically it was demonstrated that combinations of FN, OCN, and LN lead to the highest expression of alkaline phosphatase (ALP), an early osteogenic marker (Figure 3c). Moreover, one of the confounding factors that may influence the outcome in microarray analysis is crosstalk between different microgels. In order to overcome this, they used a macroscopic platform to recapitulate the results obtained in the microarray studies. They showed that the outcome from microarray analysis matched perfectly with the macroscopic counterpart, indicating that miniaturized cell niches are applicable as model systems for exploring 3D cell-matrix interactions.

In another approach to limit the potential spot-to-spot crosstalk, Ranga and co-workers[13] fabricated cell-encapsulated microwell arrays by combining both direct and indirect manufacturing techniques using robotic nanoliter liquid-dispensing technology. They showed that the 3D microgel matrixes supported mES growth and more than 1000 different microenvironments were simultaneously screened. They also showed that by controlling the matrix properties and cell-matrix interactions, it is possible to regulate self-renewal characteristic of ES cells. This is an effective technique for preventing crosstalk between microarray spots.

Cell-laden microarrays are also used to evaluate the effects of growth factors on stem cell fate. For instance, the influence of fibroblast growth factor-4 (FGF-4) and leukemia inhibitory factor (LIF) on mES cells was investigated.[13,15] The high-throughput analysis revealed significant up-regulation of LIF on neurogenic differentiation and mES proliferation, while the opposite effect was observed for FGF-4. Other studies have examined the effect of BMPs on hMSC's differentiation towards the osteogenic lineage.[14] Interestingly, the BMPs had an insignificant impact on hMSCs differentiation compared with ECM proteins.[14] These studies highlight that apart from cell-matrix interactions; the effects of growth factors, ECM components and cytokines can be investigated in a high-throughput manner.

To date, ECM components, growth factors, and small molecule libraries have mostly been used for 3D microarray analysis. However, the mechanical properties of biomaterials also play a significant role in directing cell fate. In order to investigate the effect of mechanical stiffness on cell fate in a high-throughput manner, Chatterjee and colleagues[44] monitored the differentiation of osteoblasts encapsulated within poly(ethylene glycol) (PEG) hydrogels with mechanical properties spanning from 10 kPa to 300 kPa. They showed that high compressive modulus (>225 kPa) of hydrogel promotes osteogenesis in 3D microenvironment. They were also able to identify and correlate the effect of matrix stiffness on cell fate and propose to engineer interface tissues with distinct mechanical properties. Another potential avenue for generating such gradient-like tissue interfaces is through multilayer printing (**Figure 4**a). Such multilayered microgel arrays are suitable for high-throughput screening of cell-laden hydrogels for interface tissues (Figure 4a). We believe that the development of new high-throughput technologies for studying stem cell behavior within multilayered materials would significantly advance the field of tissue engineering and regenerative medicine.

Another future avenue is to exploit the area of nanocomposite hydrogel materials, since such nanoengineered systems can

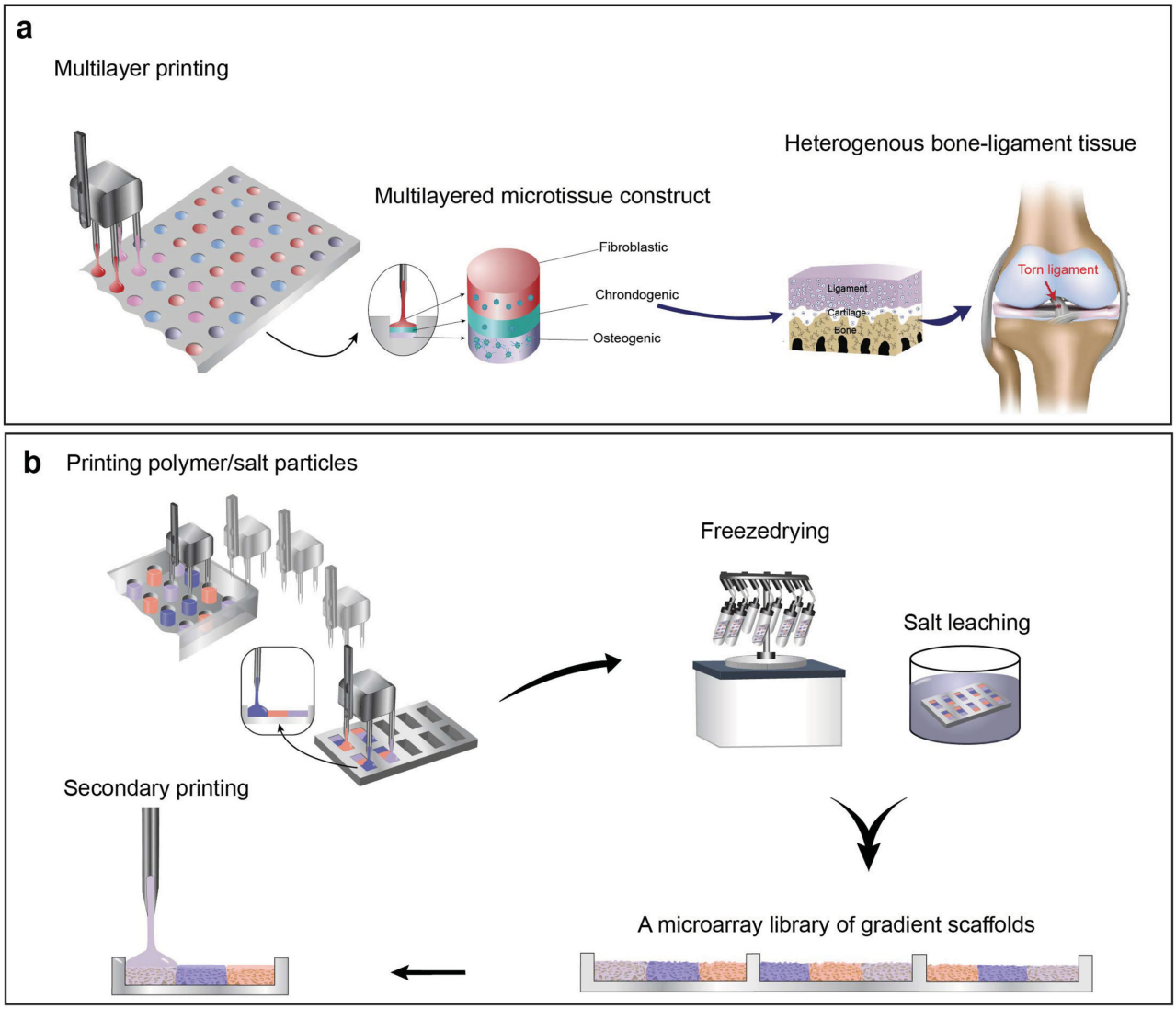

Figure 4. Multilayered cell-laden microarrays. a) Cost-effective development of multilayered cell-laden hydrogels for heterogeneous tissue regeneration. b) Multilayered scaffold microarrays with discrete osteogenic and chondrogenic cell-laden hydrogel regions for musculoskeletal regeneration.

present novel multifunctional properties based on harnessing the specific properties of nanocomposite components.[45] For instance, the incorporation of nanoparticles of suitable physical and chemical properties into hydrogel materials not only improves their mechanical properties but also can enhance the cell proliferation and cell differentiation.[46] Despite significant efforts in hydrogel microarray development, limited studies have focused on elucidating the role of nanocomponents on the cell/tissue behavior in microarray systems. Cell-laden microarrays can significantly help with the design and development of nanocomposite hydrogel materials.

3.2. Cell-Laden Scaffold Microarrays

Although hydrogels have been used for delivering signaling molecules to cells,[47] directing differentiation,[48] and facilitating tissue ingrowth,[49] their poor mechanical properties limit their application to non-load-bearing tissues.[50] Scaffolds fabricated from hard polymers have been investigated for the regeneration of mechanically stiff tissues such as muscle, bone and cartilage. Designing and optimizing these polymer components individually is both time consuming and expensive. Recently, scaffold microarrays are developed to perform cost-effective material development for musculoskeletal tissue engineering.[23,51,52] Scaffold microarrays can be generated through the deposition of polymeric solutions inside multiwell plates[52] or onto wettability contrast surfaces[23] followed by freeze-drying. Arrays consisting of chitosan,[23] alginate,[23] and tyrosine-derived polycarbonates[52] have been successfully manufactured and employed to investigate the effects of stiffness, material porosity and surface chemistry on the adhesion and viability of cells. We anticipate that the screening of cell differentiation inside such scaffold microarrays will accelerate the development of new scaffolding materials for musculoskeletal tissue engineering. 3D printed scaffold microarrays can also easily combine with cell-encapsulated microgels through a secondary printing step. The hybrid microarrays can be used for the high-throughput screening of tissue engineering constructs that share the biological and mechanical properties of musculoskeletal tissues. With the aid of new technologies that enable the manufacture of multi-layered cell-laden microarrays, the hybrid systems could create a new paradigm for regenerative medicine with significant advances in the development of off-the-shelf engineered tissues (Figure 4b).

4. Cell-Laden Microgel Arrays for High-Throughput Drugs Screening

The pharmaceutical industry is facing unprecedented productivity challenges due to the increasing cost of bringing a therapeutic drug to market. This is mainly attributed to the cost associated with in vitro and in vivo testing of drugs to determine their therapeutic efficacy and safety. Current challenges faced by the pharmaceutical industry include; a) limited understanding of the complex mechanisms that control drug mediated cell and tissue response, b) high toxicity of developed drug compounds in late clinical trials, and c) delayed approval of newly developed therapeutics by regulatory agencies.[42] Conventional drug screening utilizes a 2D culture system which is time consuming and expensive. It is therefore anticipated that further advances in regenerative medicine will hinge on techniques that enable high-throughput testing of drugs using human tissue models.

4.1. Toxicology Studies

The liver is the primary organ for drug metabolism and thus it is very sensitive towards pharmacological intervention.[53] Moreover, drug-induced liver injury is one of the leading causes of drug withdrawal from the market.[54] Therefore, most regulatory agencies require that the liver toxicity of potential drug candidates is thoroughly examined. High-throughput drug testing using liver cells has routinely been performed in the pharmaceutical industry by using multiwell plate assays.[55,56] Despite the application of multiwell-based platforms to drug development; such assays use large amounts of high-cost reagents and cells.[56] The use of microscale technologies to engineer 2D microwell platforms represents a great opportunity for cost-effective drug development. However, with 2D culture systems it is difficult to predict drug responses in 3D native tissues. To overcome this problem, cell-laden microgel arrays are used as a cost-effective toxicity screening of potential drug candidates.

Lee and colleagues developed the first 3D high-throughput toxicology-screening platform in 2008[19] through the inkjet printing of cell-laden alginate pre-polymers onto a $BaCl_2$ functionalized substrate. The Ba^{2+} ions facilitated immediate gelation of the printed spots into 3D cell-laden matrixes and the drugs were delivered to the tissue-like matrixes by adding them to a secondary substrate and stamping the drug-substrate onto the cell-laden microarray. Human liver metabolism was mimicked by addition of the enzyme P450 to the cell-laden microarray. P450 facilitates the catalysis of drugs into metabolites, which are either more or less toxic than the drug itself.[53] Thus, drug-related enzymatic activity of P450 is an important indicator of liver toxicity. In total the toxicity of nine different drugs were screened and compared with values obtained through 96-well plate assays. The results showed no significant difference between the microarray and conventional assays, demonstrating that the scale reduction of the microarray platform did not influence the outcome of the microarray studies. In fact, cell-laden microarrays provide a better model for cancer growth and liver functions compared to conventional cell culture platforms.[57] This is partly attributed to the 1000-fold scale-down of the miniaturized microarray systems, which in turn helps to circumvent the inefficient oxygen transportation seen in conventional 3D cultures.[57]

In a similar study, Kwon et al. developed a 3D screening platform to investigate drug-induced liver injury by incorporating various drug-metabolism pathways in the cell-laden microarray.[58] Specifically, a complimentary arrangement of cell-laden micropillar structures and a microwell chip containing gene-carrying adenoviruses were assembled to transfect the cells (**Figure 5**). The platform was named "Transfected Enzyme and Metabolism Chip" (TeamChip) and could

deliver 84 gene combinations to the encapsulated cells. Each combination gave rise to the expression of a specified group of drug-metabolizing enzymes, and it was demonstrated that the TeamChip could reproduce liver metabolism profiles for several pharmaceutical agents. As a result of the controlled gene-expression of the cell-laden microarray, the TeamChip can also account for genetic polymorphism in the population. Therefore, the TeamChip is a promising platform for studying drugs that have high inter-individual variability. Moreover, this platform can reduce the overall cost associated with the development of patient-specific medicine for different population subgroups.

It is expected that 3D microarray technology will be able to overcome some of the existing limitations faced by the pharmaceutical industry by providing an alternative to expensive in vivo animal models and reducing the number of hepatotoxic drugs in late clinical trials. Moreover, after it becomes possible to miniaturize the in vitro liver-tissue model, an increase in the development of new drugs for regenerative medicine is expected.

4.2. Small Molecule Based Therapeutics

Small molecule-mediated restoration of dysfunctional organ functions has several advantages over tissue regeneration or artificial implants as it is minimally invasive and does not elucidate immune responses.[59] Moreover, in contrast to proteins or nucleic acid-based agents, small molecules are highly cell-permeable, cost-effective, chemically well-defined and easy to reproduce. Specifically, small molecules that can stimulate the regeneration of tissue to normal pre-injury are of tremendous interest from a pharmacological perspective.[59] Currently, there are several drugs on the market for treating tissue ailments including ischemic diseases,[60] neurodegeneration,[61] and musculoskeletal injuries.[62] However, most of these drugs focus on treating the symptoms of the disease, instead of restoring the pre-injury functions of the diseased tissue.

Cell-laden microarrays offer a great opportunity for developing therapeutic drugs in a cost-effective manner by screening the therapeutic efficacy of small molecules within native-like 3D environments. For example, tretonoin was delivered to alginate

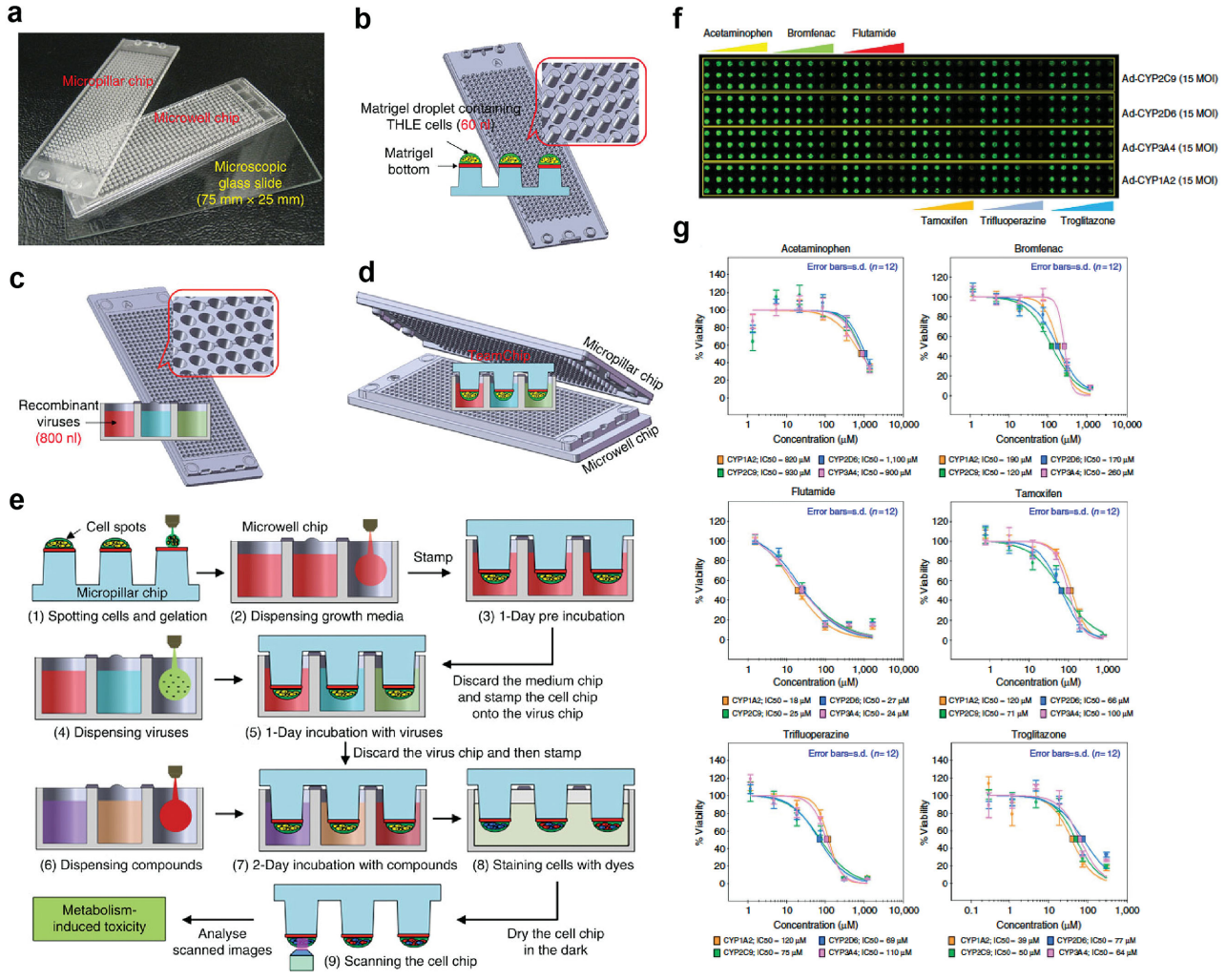

Figure 5. Toxicity screening of drugs in the presence of multiple metabolizing enzymes. a–e) A step-by-step guide to the fabrication of the TeamChip. f) Toxicology screening of six drugs in the presence of THLE-2 cells expressing several drug-metabolizing enzymes. g) Dose-response curves for the six tested compounds in (f). Adapted with permission.[58] Copyright 2014, Nature Publishing Group.

encapsulated mES cells by stamping the cell-laden microarray with a secondary array consisting of tretonoin.[15] The high-throughput analysis revealed a significant down-regulation of mES self-renewal ability in the presence of tretonoin. A similar method was used to deliver small molecules including retinoic acid and dexamethasone to differentiated neural progenitor cells.[18] The toxicity of the small molecules was studied in a high-throughput manner and compared to the effect of control compounds at relevant concentrations. Overall, their results demonstrated that neural progenitor cells can differentiate into neurons and glial cells within miniaturized 3D environments. It is anticipated that the high-throughput screening of neurogenic differentiation and the proliferation of microgel encapsulated cells in response to small molecules could accelerate the development of new drugs for neurodegenerative diseases. Other potential research directions are manufacture of multilayered hMSCs-laden microarrays for the cost-effective drug development of osteoarthritis treatment and miniaturized 3D co-culture systems, containing stem cells and endothelial cells, for the development of pro-angiogenic drugs against ischemic tissues.

5. Heterogeneous Cell-Laden Microarrays to Mimic Complex Tissue Structures

Dysfunctional tissues resulting from disease, trauma, and age affect millions of people worldwide. Most of these tissue ailments are complex in nature and involve multiple cells and tissue structures.[41,63] A number of tissue engineering approaches are developed to replace or repair such heterogeneous tissue structures, however difficulties in obtaining suitable transplants represents a significant challenge in treating these complex injuries.[41] Although it is anticipated that tissue engineering could solve this problem by manufacturing custom-made transplants, several challenges related to the manufacture of heterogeneous tissues still remains. The challenges include: a) understanding the effect of combinatorial factors that direct cell fate in a synergistic and antagonistic manner, and b) designing appropriate microscale geometries to mimic native tissue architectures.[41] It is therefore expected that further progress in tissue engineering will focus on combinatorial studies that can help identify appropriate architectural, cellular, and biological combinations to mimic some of the aspects of heterogeneous tissue structures. Microprinting of cell-laden hydrogels into 3D tissue-like constructs can be used to fabricate heterogeneous cell-laden microarrays for such complex microarray studies. With the aid of robotic microprinting of cell-laden microgels, specific tissue-like structures such as microvasculature networks[64] and multilayered tissue constructs[65] have been generated. Specifically, shear thinning and fast-gelling bioinks were stacked into microvasculature and multilayered tissue constructs at microscale resolutions.[64,65] This newly developed microprinting strategy offers an exciting framework for fabricating cell-laden microarrays for the high-throughput screening of cell behavior within heterogeneous environments.

In addition, microfabrication is also a feasible technique for developing heterogeneous cell-laden microarrays. In a recent study, sequential photolithography was used to design complex microgel arrays with precisely controlled hydrogel architectures including rectangular prisms, hemispherical, concentric and typescript patterns (**Figure 6**).[66] Through the sequential photolithographic crosslinking of cell-laden hydrogels, multiple cell types were sculpted into neural-like tissue microarrays enabling combinatorial studies of neurite growth within well-controlled architectures.[66] High-throughput screening of these neural-like tissue arrays could provide vital information about brain and spinal cord tissue development in a

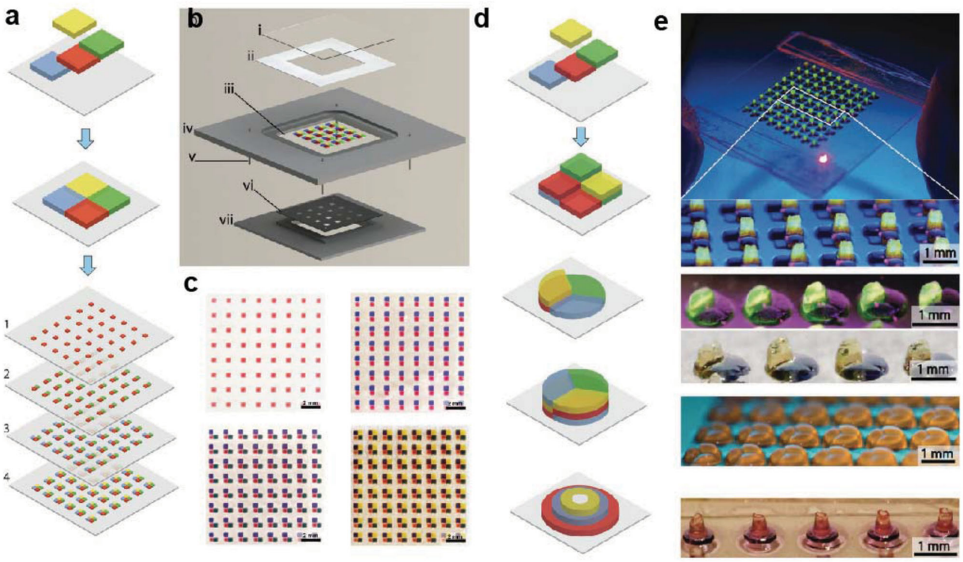

Figure 6. Microfabrication of tissue-like microarrays. a) A tissue microarchitecture consisting of four different building blocks composed of specific cellular and ECM composition. b,c) Generation of multilayered tissue constructs through layer-by-layer photomasking and mask alignment. d) Fabrication of heterogeneous tissue constructs with concentric, cubic, radial circular,and circular geometries. e) Side view of various multilayered and heterogeneous tissue microarrays. Adapted with permission.[66] Copyright 2013, John Wiley & Sons.

cost-effective manner. The combination of 3D microarray technology along with microfabrication technology is a promising new approach to engineering heterogeneous structures.

5.1. In Vivo High-Throughput Testing of Biomaterials

Clinical trials are a crucial step towards probing the foreign-body response and toxicity of newly developed biomaterials. However, testing the clinical potential of biomaterials requires a significant number of animals, which limits the number of biomaterials tested under in vivo conditions. By implanting biomaterial microarrays, a large number of biomaterial combinations can be investigated using fewer animals. This high-throughput clinical testing offers a major advancement in biomaterial development and a great ethical improvement in the field of biomaterials science. In a recent study, a microarray chip containing 36 different scaffolds was implanted in Wistar rats.[67] The microarray chip was used to study the immune response towards the implanted biomaterials in a cost-effective manner. The inflammatory response of the individual scaffolds was accessed after implantation (up to 7 days) by examining the recruitment of macrophages and lymphocytes to the respective scaffolds. The implanted micro-scaffolds elucidated different inflammatory responses depending on their physical and chemical properties. Moreover, through a histology analysis of the tissue surrounding the microchips it was evident that the inflammatory response in each scaffold was unique and independent between the implanted scaffolds. Although the in vivo high-throughput testing of biomaterials is a very promising approach, additional experiments are needed to further validate this system.

6. Conclusion and Future Directions

Tissue regeneration and maturation involves numerous chemical, physical, and biological events of tremendous complexity. The symphony of these events controls and directs cell function, and facilitates the formation of tissue architectures which are important for the development of functional tissues. The ultimate goal of tissue engineering is to recreate such heterogeneous environments for engineering artificial tissues to replace or regenerate dysfunctional tissue. 3D biomaterial microarrays can significantly aid in the identification of such magical formulations in the shortest possible time, by enabling the high-throughput screening of cell-matrix, cell-ECM, and cell-drug interactions within heterogeneous tissue-like environments. Such 3D heterogeneous cell-laden microarrays hold remarkable promise for investigating cell-cell and cell-matrix interactions within a range of different physical, biological, and geometrical settings in a cost-effective manner (**Figure 7**a). It also enables in vitro screening of tissue

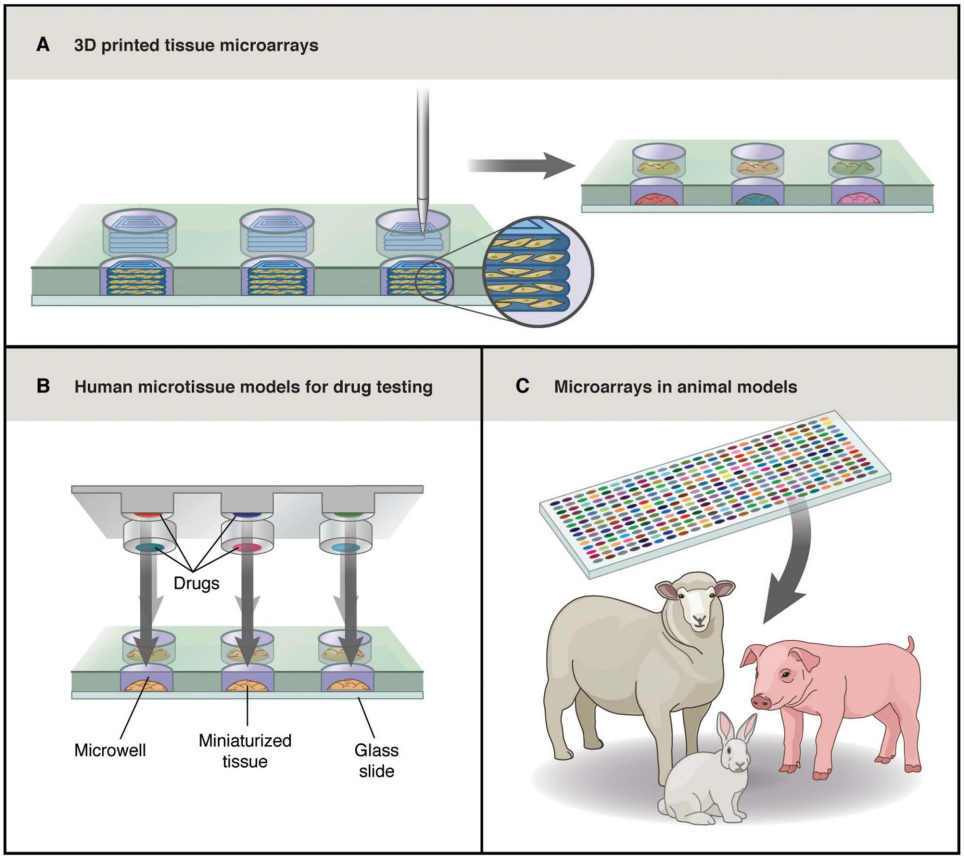

Figure 7. Emerging trends in 3D biomaterials microarrays. a) 3D printed tissue microarrays for cost-effective development of new tissue engineering strategies. b) Printed tissue microarrays for high throughput and in vivo like drug testing. c) Implanting microarray chips in animal models.

responses to newly developed drugs (Figure 7b). It is therefore anticipated that heterogeneous cell-laden microarrays can significantly advance the field of regenerative medicine through a better understanding of tissue regeneration and in vivo drug responses.

Another future avenue is the ability to create biomaterial microchips (Figure 7c) that can be implanted clinically for cost-effective screening of the host response to new biomaterials. Earlier biomaterial microchips[67] used relatively big scaffolds (~2 mm in diameter), thus limiting the number of scaffolds that can be tested in an animal. Moreover, this study was also limited to the foreign-body response towards implanted biomaterials. It would be interesting to investigate whether a biomaterial microchip can also be used to examine the tissue regeneration potential of scaffolds in a high-throughput manner. A further downscaling of such implantable microchips could lead to a rapid breakthrough in tissue engineering and bring the field closer to meeting its clinical potential.

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