Replication of the 3D architecture of tissues

Biological tissues are ensembles of various types of cells and extracellular molecules. Functionality in tissues arises from their components (cells and extracellular molecules) as well as from the location of those components relative to each other. The organization of the constituents of a tissue is known as histoarchitecture. As cell culture reaches beyond flat, rigid surfaces, several approaches have been published that attempt to re-create in vitro the three-dimensional (3D) histoarchitecture found in vivo. In these approaches, researchers use scaffolding molecules (extracellular matrix, ECM) of natural or synthetic origin to support cell growth. Scaffolds harvested from tissues replicate precisely the in vivo ECM but they may be limited by its biologic variability. Conversely, synthetic scaffolds provide tailored, defined, repeatable ECM but lack the chemical signaling completeness provided by biological scaffolds. Here we will review methods for replicating in vitro the 3D histoarchitecture of live tissues, focusing on those approaches that use (or are compatible with) tissue-harvested scaffolds.

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The microenvironment of a cell comprises all the cues (stimuli) affecting the cell and includes attachment to neighboring cells, attachment to the structural molecules of the extracellular matrix (e.g., via integrins), molecules secreted by the cell itself or by other cells, nutrients, oxygen, mechanical stimuli (such as shear stress), UV light, and many others. In replicas of biological tissues, cells and microenvironmental cues are (i) integrated and (ii) organized according to the in vivo 3D histoarchitecture. These replicas of living tissues would behave similarly to the original tissues and could be used in therapeutic applications (regenerative medicine) and as realistic human models of tissues for the study of cell biology and for cell-based assays.
In conventional two-dimensional (2D) cell culture techniques, the cellular microenvironment is reduced to a flat, rigid substrate of plastic or glass (basal side of the cellular monolayer) and a volume of liquid culture medium with uniform concentration over the apical side of the monolayer. In living tissues, however, cells are exposed to a three-dimensional (3D) environment that consists of other cells, extracellular matrix, inhomogeneous concentrations of soluble molecules, and non-chemical stimuli, such as shear stress and ultra-violet (UV) light. Today, two main approaches coexist that have the same goal of replicating the 3D cellular microenvironment in tissues. The approaches differ in the type of porous material used as the three-dimensional scaffold in which cells are cultured: one set of techniques use synthetic polymers whereas the other set use biologically-produced matrices – i.e., natural biopolymers such as collagens or elastin, harvested from tissues, either in solution or as 3D structures (Fig. 1). The two approaches offer complementary advantages and limitations. First, synthetic scaffolds have a defined composition, oppositely to harvested matrices, which present variability from specimen to specimen (if harvested in the lab) or from batch to batch (if acquired). Definition of the composition of the cellular scaffold is important experimentally and for the approval of the clinical use of engineered tissues by health organizations. Second, synthetic scaffolds can be produced in large volumes whereas the production of scaffolds harvested from living organisms (e.g., humans, animals, algae) is limited by the availability of those organisms and of the scaffold of interest in those organisms. Third, synthetic scaffolds can be tailored chemically in order to present different functionalities such as (i) ligands that trigger specific pathways in the attached cells, (ii) bioresponsive groups that change the macroscopic characteristics of the scaffold (e.g., swelling or shrinking, gelation) in response to chosen environmental cues – e.g., nutrients, growth factors, antibodies, enzymes, whole cells – or (iii) photoinitiators that cause the scaffold to gelate or to degrade upon exposure to ultraviolet (UV) light. Harvested scaffolds, however, are minimally customizable since their composition is determined by the organism that produces the scaffold. Fourth, synthetic scaffolds become more similar to in vivo scaffolds as more characteristics (chemistry, mechanics, etc.) of the latter are integrated to the synthetic scaffold. The simpler the synthetic scaffold, the more different from the original. The challenge is to determine the maximum simplicity of the synthetic scaffold that results in cultured cells presenting the phenotype and differentiation state that those cells would have in vivo. Conversely, harvested scaffolds replicate closely the scaffold in vivo because they are ex vivo samples of living tissues. Last, different synthetic scaffolds need to be prepared for replicating the various types of extracellular matrices associated with each biological tissue and even with disease states or aging. On the contrary, biologically-produced scaffolds naturally replicate the variety of extracellular matrices as they are harvested directly from the tissue of interest.

Cell culture techniques that utilize 3D matrices – commonly known as 3D cell culture techniques – are an enormous breakthrough that moves cell culture away from plain, flat culture vessels. Cells cultured in a 3D environment present a phenotype that is different than that observed in cultures on flat, rigid substrates such as polystyrene or glass. From 3D matrices, cells receive mechanical support and, through binding of the cellular integrins, a variety of chemical signals (matrix-attached cues). In vitro models of breast cancer have shown that the evolution of the cellular cancer phenotype depends on the presence of a laminin-rich 3D scaffold. Also the mechanisms of cellular migration in 3D matrices differ from those in planar cultures. Given the specifics of each type of scaffold (synthetic or harvested from living tissues), choosing one type or another for 3D cell culture will depend on the application. In tissue engineering, the selection of a 3D scaffold for culturing cells may be dictated by the capability of producing that scaffold in large quantities. Tissue models, on the other hand, aim at replicating the complexity of living tissues as closely as possible. Harvested scaffolds are therefore better suited for tissue models because they provide a closer recapitulation of the in vivo ECM than synthetic scaffolds. In this review we will put the emphasis on applications of tissue modeling and therefore we will focus on the use of harvested scaffolds – collagen, glycosaminoglycans (GAGs), etc. or combinations of them.

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Fig. 1 Summary of methods for replicating the architecture of living tissues (1) by patterning cells on harvested scaffolds, (2) by patterning cells in harvested scaffolds, (3) by self-organization of cells in harvested scaffolds, and (4) by seeding cells on decellularized scaffolds.
The microenvironment found in living tissues, however, is complex and comprises more elements (other cells, soluble molecules, and physical cues) than just the extra-cellular matrix (ECM). All these constituents (cues) of the physiological milieu are not randomly distributed in living tissues but the location of each constituent is accurately defined by the tissue architecture and results in dramatically inhomogeneous microenvironments. Works by Dressler and Piddini et al. are illustrative examples of the complex histoarchitecture in the mammalian kidney and in the wings of fruit flies (Drosophila) during development, respectively. Realistic in vitro tissue models, therefore, need (i) to integrate and (ii) to position precisely the diverse cues of the in vivo microenvironment. Here we will review methods (Fig. 1) that replicate in vitro the architecture of living tissues using bottom-up (sections 1 – 3) and top-down (section 4) techniques and ranging from the micropatterning of cells and scaffolds to the self-organization of cells in patternless scaffolds. In particular, we will focus on those methods that allow the integration of harvested scaffolds with additional cues such as other cells, soluble molecules, and physical cues.

**Histoarchitecture replicated by culturing cells on harvested scaffolds**

Extracellular matrices harvested from living tissues were first integrated into cell culture simply by coating the culture vessels with a layer of harvested ECM in solution. After allowing the ECM to gel, cells were plated on the layer of ECM. This technique originated results that demonstrated the relevance of the ECM in cellular biology. The analysis of the many harvested ECMs available under a variety of conditions is a daunting effort that would benefit from techniques offering high throughput. Micropatterning and microspotting techniques make it possible to fabricate arrays of hundreds to thousands of micrometric islands that contain different ECMs on a surface of few squared millimeters. These techniques make it possible to culture cells on flat layers of 3D scaffolds, which replicate the matrix-attached cues of living tissues but fail to integrate other elements of the tissue architecture, such as inhomogeneous concentrations of soluble cues or the 3D (not flat) architecture of the tissue.

Inserts are cup-like vessels with a porous bottom that fit into the wells of standard tissue-culture well plates. Typically, the porous membrane of the insert is coated with a thin layer of ECM and cells are seeded on that layer of ECM. When two different solutions fill the insert and the well (where the insert sits), a gradient of concentration of soluble molecules arises due to diffusion across the ECM layer and the porous membrane. Inserts allow the generation of inhomogeneous soluble environments across layers of 3D scaffolds and as a result, the use of inserts is widespread in tissue culture. Inserts, however, are limited in that the gradient of concentration is not stable – but it changes as the concentrations in the insert and the well change by means of diffusion – and in that the translucency of the membrane lessens the quality of the cell imaging. A microfluidic version of standard inserts consists of a membrane sandwiched between two individually-addressable microchannels. The membrane in this microdevice is amenable to cell culture similarly to the membranes of inserts. The delivery of a different solution to each microchannel generates a gradient of concentration of soluble molecules across the membrane. In comparison to inserts, this microfluidic configuration makes it possible to refill constantly the channels above and below the membrane and results in a stable gradient of concentration across the membrane. Additionally, Huh et al. used the top channel to deliver liquid plugs to the cells on the membrane in order to study the effects of mechanical stresses on the pulmonary epithelium.

Because of the micrometric dimensions of biological cells (diameters of few to tens of microns), methods derived from microfabrication technologies have been successfully adopted for the in vitro replication of the tissue architecture. In addition to techniques that use ultraviolet (UV) light to shape (i.e., photopatterning) synthetic 3D scaffolds, molding techniques are available to shape scaffolds that undergo a liquid-to-gel phase change, such as harvested matrices—collagen, fibrin, etc. In one group of techniques, the liquid ECM filling a chamber (typically, a microchamber) is brought to gelation. As a result, the gelled ECM retains the planar shape of the chamber and shrinks in height. The difference in height between the ECM construct (i.e., structure of gelled ECM) and the microchamber makes it possible to deliver liquid solutions over the construct, which remains in the chamber. In another group of techniques, stamps made of poly(dimethylsiloxane) (i.e., PDMS) and pressed against liquid ECM, produced microstructured layers of ECM gel and arrays of micrometric constructs. After shaping the scaffold, cells were seeded on the surface of the structured gel construct, as schematized by route 1 (Fig. 1). Using this technique, Nelson et al. developed a model of the mammary gland consisting of grooves of different shapes molded in collagen. They used the model to study the branching morphogenesis of mammary epithelial tubules.

**Histoarchitecture replicated by patterning cells within harvested scaffolds**

Techniques based on seeding cells on a surface are inherently two-dimensional and rely on cellular self-organization and migration to form a 3D architecture that presents cells distributed throughout the whole volume of the scaffold – note that in the next section we will cover cellular self-organization as a route to replicate in vitro the 3D architecture of living tissues (route 3 in Fig. 1). To avoid possible limitations arising from seeding cells on surfaces, some techniques mix the cells with the liquid scaffold (for instance, a solution of collagen) prior to bringing the scaffold to gelation – as schematized in route 2 (Fig. 1). Some authors have demonstrated that dielectrophoresis and optical tweezers can be used for organizing cells in suspension.
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According to complex 3D patterns, after the scaffold gels, cells become encapsulated and retain their 3D pattern even in the absence of dielectrophoretic or optical traps. Alternatively, microchannels made of cells in gel (or cell-laden microchannels, as some authors call them) were fabricated by casting a suspension of cells and biopolymer63-64. These channels, however, consisted of one type of cell and one type of biopolymer only. We will now review methods developed for building constructs that consist of several types of cells and biopolymers. These methods can be divided in three main groups depending on the mechanism used for creating the multi-element construct: (i) sequential deposition, (ii) localized etching, and (iii) module assembly.

For the sequential deposition of ECM and cells, Desai et al.52 took advantage of the shrinkage in height observed after curing ECM inside of a chamber. They filled a microchamber with a first suspension of cells and liquid ECM and allowed it to gel. Then a second suspension of cells and ECM filled the chamber and gelled. They repeated this process up to three times and created a structure consisting of a stack of three layers of various cells and ECM. Sequential deposition techniques and 3D cell patterning techniques, such as dielectrophoresis, have been combined yielding constructs with very complex architectures59. Alternatively to stacking layers one on top of another, there are techniques65-72 that produce vertical layers (or walls), which partition an initially empty microchannel. We formed walls of Matrigel® using laminar flow68 (Fig. 2). Streams of liquid Matrigel and of a solution of PEG were delivered simultaneously into a microchannel. The temperature of the microchannel was brought up to 37°C suddenly and caused the gelation in situ of Matrigel. The gel retained the shape it had as a liquid flowing down the channel under conditions of laminar flow. This is a parallel technique because several walls of Matrigel form simultaneously. Laminar flow was also the underlying mechanism in other works that created walls of synthetic gels inside microchannels65-67. Others formed walls of hydrogel in microchannels using capillarity as the driving mechanism69-72. In chambers with hydrophobic surfaces, water-based solutions – such as hydrogels in liquid phase – tend to fill the regions of the chambers as determined by surface tension, gel viscosity, and width and height of the chamber. To form walls of hydrogel, these methods69-72 use arrays of posts inside of a microchannel. In Hung et al.72, the posts, with a hydrophobic surface, confine the liquid gel because of their hexagonal shape (Fig. 3). In another technique73, different layers of gel were formed separately (by molding collagen I or fibrin54), stacked, and chemically bound to each other. The resulting 3D constructs consisted of several layers of micromolded hydrogel.

The second group of techniques for creating a multi-element construct is based on removing selectively some parts of the...
construct\textsuperscript{74–75}. In one case\textsuperscript{74}, the researchers formed a construct using collagen I and Matrigel. Then they exposed the construct to dispase, an enzyme that selectively digests Matrigel. The process generated a collagen-only construct, with empty cavities within. The researchers also showed that using dispase with a construct made of collagen, and Matrigel with cells resulted in a collagen-only scaffold, with endothelial cells cultured on the cavities within. In another example\textsuperscript{75}, constructs were built of gelatin and tissue-relevant hydrogels – such as collagen I, fibrin, and Matrigel. As the temperature was increased to 37°C, gelatin melted and left empty cavities or channels in the construct. The authors seeded mammalian cells in the empty channels and showed that this method is compatible with cells cultured within the tissue-relevant gel.

In the third group of techniques, multi-element constructs result from a two-step process: (a) production of single-element modules and (b) assembly of those modules into a higher hierarchy structure – i.e., the multi-element construct. For building modules of cells and harvested scaffolds, stamps and stencils have been used\textsuperscript{54,55,76}. In particular, for the experiment shown in the cover of this issue of Materials Today, we used a mold of PDMS to shape a mixture of collagen I and fibroblasts. After fabrication, several techniques are available in the literature for organizing the cell-laden modules. Du \textit{et al.}\textsuperscript{77} employed capillary force to allow modules of gel to self-assemble (Fig 4). Chung \textit{et al.}\textsuperscript{78} added a fin to the underside of their gel modules. The fins under the modules matched the shape of grooves patterned at the bottom of microfluidic channels and allowed guiding the modules along the channels in a similar way to how rail tracks guide trains. Although these researchers\textsuperscript{77,78} demonstrated the organization of modules of synthetic gel, the technique should be compatible with modules of harvested gel. Alternatively, sieves integrated in microfluidic devices (Fig 5) can also organize the modules by collecting them in a reduced space\textsuperscript{79}. Sieves have also been used for collecting spheroids\textsuperscript{80}.

In the comparison between techniques for creating multi-element constructs, it is important to evaluate the possibility to create an inhomogeneous soluble environment\textsuperscript{49} across the constructs. Gradients of concentration of soluble molecules arise \textit{in vitro} due to secretions of cultured cells and due to the delivery of solutions that modify the cellular microenvironment. Gradients of soluble molecules secreted by cultured cells arise from an inhomogeneous distribution of cells in the constructs. As a result, techniques that allow the fabrication of constructs with different types of cells segregated from each other will also allow the establishment of gradients of secreted soluble molecules\textsuperscript{68–70,72} (Fig 6). Of the techniques reviewed above, those that produce vertical walls of gel and partition an initially empty microchannel\textsuperscript{68–72}, offer one main advantage: the partition of a microchannel by vertical walls results in the formation of individually-addressable subchannels located adjacent to the vertical walls (Fig 2 and 3). The subchannels make it possible to deliver

\begin{figure}[h]
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\includegraphics[width=\linewidth]{figure4}
\caption{Formation of gel constructs by capillarity-driven assembly of gel modules\textsuperscript{77}. (a) Fluorescence and (b) phase-contrast images of the assembly of one cross-shaped module and two rods. (c) Fluorescence and (d) phase-contrast images of the assembly of one cross-shaped module and three rods. Fluorescence images of (e) the assembly of one cross-shaped module and one rod, and of (f) one cross-shaped module and two rods. In panels (a–d), crosses (green) and rods (red) were stained with FITC-dextran and Nile red, respectively. In panels (e–f), crosses (green) and rods (red) contained cells stained in green and in red, respectively. Scale bar = 200 μm.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\linewidth]{figure5}
\caption{Formation of constructs by collecting gel modules with a microsieve\textsuperscript{79}. (a) Schematic of a microfluidic chamber containing an array of posts that behave as a sieve for trapping gel modules as they flow through the chamber. (b) Phase-contrast image of the assembly of spheroidal modules. (c) Fluorescence image of (b) showing modules containing 3T3 fibroblasts stained with three different fluorescent dyes.}
\end{figure}
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different solutions of soluble molecules to each side of one gel wall. The presence of subchannels, therefore, provides an easy way of establishing stable inhomogeneous environments across the walls of gel by delivering solutions\cite{68,69,71}. Also, the presence of subchannels makes it possible to generate interstitial flow across the walls of gel\cite{69}, which has been found essential in the creation of new blood vessels (angiogenesis)\cite{81}.

**Histoarchitecture replicated by self-organization of cells in harvested scaffolds**

Cells are active agents in their local environment, where they interact with neighboring cells, secrete soluble cues, migrate, and modify the surrounding ECM by reorganizing the existing scaffold and even secreting new ECM molecules. The ability of cells to tailor their microenvironment can be exploited for replicating the *in vivo* histoarchitecture. In other words, instead of patterning cells in a way that resembles the cellular distribution in living tissues (such as endothelial cells adopting the shape of the patterns of adhesive molecules on the substrate\cite{82,83}), we can provide cells with cues that guide them to self-organize and to customize their microenvironment. A well-known example of cellular self-organization occurs in the endothelium of blood vessels. When cultured on a pattern-free substrate, endothelial cells exposed to flow, reshape and reorganize in response to shear stress\cite{84-86}. The elasticity of the extracellular matrix also plays a role in angiogenesis\cite{87}. In another example of cellular self-organization, the stratification of the epidermis results from microenvironmental cues, such as intercellular communication\cite{88} and undulations of the basal lamina\cite{89}. All those examples show that even in the absence of cell patterning, the inhomogeneous distribution of microenvironmental cues is able to guide the

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**Fig. 6. Cell migration induced by gradients of cell-secreted soluble molecules across collagen posts**\cite{70}. The microfluidic device consisted of three channels (left, center, and right) separated by posts of 2-mg/ml collagen (white dotted rectangles) and posts of PDMS. In the experiments, the left channel contained culture medium (control), the center channel was seeded with HMVEC cells, and the right channel with (a) MTLn3, (b) U87MG, or (c) 10T 1/2 cells. HMVEC are human dermal microvascular endothelial cells, MTLn3 and U87MG are cancer cell lines, and 10T 1/2 are smooth muscle cells. Results showed that HMVEC migrate (a) faster towards MTLn3 than (b) towards U87MG whereas (c) the presence of MTLn3 inhibits migration of HMVEC. Small white square = 150 μm x 150 μm. In (b), U87MG cells expressed GFP (green). In (c), HMVEC expressed von Willebrand factor (green). After fixation, all cells were fixed and stained for actin and DNA with rhodamin-phalloidin (yellow) and DAPI (blue).
reorganization of cells and transform the 3D architecture of the in vitro construct.

Breast epithelial cells cultured on laminin-rich extracellular matrix (resembling the basal lamina) self-organize and go from forming a monolayer to generating spheroidal acini that present lumen (Fig. 7) and produce milk in vitro. In this case, exposure to a uniform environment rich in laminin caused breast endothelial cells to reorganize and create an inhomogeneous environment for themselves:

laminin on the basal side and milk-carrying lumen on the apical side of the cells. In the context of this review, the in vitro formation of acini by cellular reorganization is paradigmatic because it shows how micrometric cellular structures can arise even from non-patterned environments.

In addition to self-organize, some cells are able to reorganize the extracellular matrix, as shown in the cover of this issue of Materials Today, where 3T3 murine fibroblasts reorganized themselves...
and contracted the matrix of collagen I in which they were seeded. Contraction of the scaffold plays an important role in wound healing. In a model of skeletal muscle, myoblasts were induced to align by means of a wavy PDMS substrate and then covered by a layer of fibrin gel (Fig. 8). Myoblasts remained aligned after transferring to the fibrin gel and caused the construct (i.e., fibrin and myoblasts) to roll up. The resulting muscle construct was cylindrical (Fig. 8e) with its longitudinal axis coinciding with the direction of alignment of myoblasts.

Cells can also remodel their microenvironment by generating ECM molecules as it occurs in wound healing, where fibroblasts and myofibroblasts are responsible for producing the collagen (mostly, type III) that constitutes the scar. As discussed, breast epithelial cells require the presence of laminin to polarize correctly and form the acini. However, in environments initially depleted of laminin, myoepithelial cells generate de novo a basal lamina (rich in laminin) to support the formation of acini by self-organization of the epithelial cells. In another example, synthetic (polyglycolic acid coated with poly-4-hydroxybutyrate) trileaflet heart valves seeded with myofibroblasts and endothelial cells showed evidence of de novo produced collagen, glycosaminoglycans (GAGs), and elastin. In addition to secreting new ECM proteins, cells can modify their environment by digesting the extracellular matrix with specialized enzymes, such as matrix metalloproteinases (MMPs). Proteolytic digestion of the extra-cellular matrix is a key mechanism for in vivo cell migration and metastasis. Localized enzymatic expression of MMP-14 is also relevant in the morphogenesis of the mammary tissue.

**Histoarchitecture replicated by seeding cells on decellularized scaffolds**

Typically, harvested scaffolds are prepared as an aqueous solution that can easily coat a Petri dish or a membrane. Alternatively, ECM can be harvested from tissues and decellularized, retaining some or all of its in vivo structure. Recent works have demonstrated that tissue engineering could benefit of methods for harvesting scaffolds that preserve the original shape of the scaffold. Taylor and colleagues decellularized the cadaveric whole heart of a rat by coronary perfusion with detergents. This technique preserved the scaffold (collagens I and III, laminin, and fibronectin) after depleting it from cells. The authors then re-seeded the scaffold with cardiac or endothelial cells (recellularization) and maintained the cardiac construct under perfusion with a fluidic system that replicated the characteristics of cardiac physiological load (Fig. 9a). The construct presented macroscopic contractions after four days, and was able to perform pump function (at about 2% of the performance of an adult heart) after eight days (Fig. 9b). In another paradigmatic example of
the use of decellularized cadaveric scaffolds for tissue engineering. Macchiarini and colleagues demonstrated the decellularization of a cadaveric human tracheal scaffold and its recellularization with epithelial cells and mesenchymal stem-cell-derived chondrocytes from the patient—a 30-year-old woman with end-stage bronchomalacia. The graft was implanted to replace the patient’s left bronchus and presented normal appearance and mechanical properties four months after implantation. The graft, consisting of autologous cells and decellularized scaffolds, did not cause antigenicity in the patient, who did not receive anti-donor antibodies or immunosuppressants.

Conclusions
The complexity of living tissues is overwhelming and its study in vivo is oftentimes limited by the researcher’s inability to control all the experimental parameters, which depend on the systemic response of the living organism. In vivo studies using animal models might be limited in their application to human studies as a result of the intrinsic differences that exist between animal and human beings. In this context, realistic in vitro models of human biological tissues would find application in the study of cell and tissue biology as well as in the development of cell-based assays for drug development and test. The formation of realistic tissue models depends on two main principles. First, the number of elements in a living tissue replicated in a tissue model determines how realistic that model is. (As an illustrative example, monolayers of endothelial cells under static conditions or under shear stress are both models of the vascular endothelium. The latter, though, incorporates the stimulus of shear stress on the cells and therefore is more realistic than the former.) Second, the techniques chosen to form a tissue model determine the elements that can be integrated to that tissue model. (For instance, laminar flow develops in microfluidic channels. Hence, models of the vascular endothelium formed in microfluidic channels provide a more precise control of the shear stress on the cells than similar models on Petri dishes.) As a result, the selection of the methods to form a tissue model will ultimately determine how closely that model replicates a living tissue. In this review we have discussed a variety of methods presently available for replicating in vitro the architecture and components of living tissues. Among those methods, researchers select the combination of techniques that yields a tissue model most similar to the tissue under study.

At this time, when induced pluripotent stem (iPS) cells start making it possible to develop disease-specific (and even patient-specific) cellular models, improvements in cell culture techniques are required to replicate more closely the in vivo disease-specific (or maybe patient-specific) cellular microenvironment. We expect those improvements in cell-culture techniques to arise from an increase in the number of components (different types of cells, chemical and mechanical cues, etc.) integrated to a tissue model, from the appearance of new methods to replicate in vitro the living tissues and their components, and from the development of harvesting protocols and commercialization of tissue- and disease-specific extracellular matrices.