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The ins and outs of engineering functional tissues and organs: evaluating the in-vitro and in-situ processes

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Purpose of review
For many disorders that result in loss of organ function, the only curative treatment is organ transplantation. However, this approach is severely limited by the shortage of donor organs. Tissue engineering has emerged as an alternative solution to this issue. This review discusses the concept of tissue engineering from a technical viewpoint and summarizes the state of the art as well as the current shortcomings, with the aim of identifying the key lessons that we can learn to further advance the engineering of functional tissues and organs.

Recent findings
A plethora of tissue-engineering strategies have been recently developed. Notably, these strategies put different emphases on the in-vitro and in-situ processes (i.e. preimplantation and postimplantation) that take place during tissue formation. Biophysical and biomechanical interactions between the cells and the scaffold/biomaterial play a crucial role in all steps and have started to be exploited to steer tissue regeneration.

Summary
Recent works have demonstrated the need to better understand the in-vitro and in-situ processes during tissue formation, in order to regenerate complex, functional organs with desired cellular organization and tissue architecture. A concerted effort from both fundamental and tissue-specific research has the potential to accelerate progress in the field.

Keywords
bioreactor, cell–matrix interactions, in-situ tissue engineering, scaffold-free tissue engineering, tissue regenerative constructs

INTRODUCTION

The past two decades have seen the emergence of tissue engineering as a promising solution for alleviating the massive disparity between the demand for organs for transplantation and the available supply of organs in the clinic [1,2]. Tissue engineering proposes an alternative concept of building and regenerating tissues and organs for transplantation from their components: cells and/or (bio)materials. As these components can be made available and prepared in the laboratory, this concept can potentially yield an unlimited and even patient-specific source of tissues and organs, thereby relieving the problem of organ shortage.

Tissue engineering has been applied to engineer various organs and tissues with diverse functionalities, from kidney, tendon, and cornea to blood vessels and the heart. These applications to engineering specific organs and tissues have been separately and extensively addressed in excellent recent reviews (see, e.g. [3–6]). In this opinion article, we will instead focus on the technological perspective, take a bird's-eye view of the major tissue engineering strategies that have been recently developed, identify the common denominators as well as the unique strengths and limitations, and critically evaluate the principal challenges and opportunities that lie ahead. As will become clear, progress in the field requires a concerted effort to look more closely into the fundamental biophysical and biomechanical

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KEY POINTS

- Tissue engineering holds the potential of rebuilding new, functional tissues and organs.
- Tissue engineering involves in-vitro and in-situ steps, both of which need to be carefully designed and tailored to achieve tissue function.
- Biophysical and biomechanical interactions between cells and the scaffold or biomaterials play a crucial role in both the in-vitro and in-situ steps of tissue engineering.
- Future studies are expected to deepen our understanding of dynamic cell–matrix interactions as well as to develop innovative ways to mimic and exploit these interactions for steering tissue growth and remodeling.

processes that occur preimplantation (*in vitro*) and postimplantation (*in situ*), in order to construct an efficient and sustainable engineering methodology for achieving functional tissue and organ regeneration.

TISSUE ENGINEERING STRATEGIES

Various tissue engineering strategies have been devised and developed over the years. Here we classify and briefly summarize these strategies based on the ingredients that are used as a starting point for regenerating the tissue (Fig. 1 and Table 1).

'Conventional' tissue engineering

The 'conventional' tissue engineering methods use a combination of cells and scaffolds or matrices as a starting point. The first step is obtaining tissue-matching cells, either from primary source (i.e. the patient) or from stem cells (e.g. embryonic stem cells and stromal cells derived from adult bone marrow or umbilical cord). After in-vitro expansion, the cells are seeded onto or into the scaffold and encouraged to populate the scaffold and to produce their own extracellular matrix as a foundation of a tissue for transplantation. Finally, the engineered tissue is implanted. In this approach, the entire tissue engineering process takes place *in vitro*.

The first component is the cells – the producer of the new tissue. Primary autologous cells have provided substantial success as they have the advantage of being taken directly from the tissue source, hence preventing adverse immune response, are fully differentiated, and readily produce tissue-specific extracellular matrix (ECM) [7]. However, these cells require invasive cell collection, suffer from low proliferative capacity, which may be further limited with increasing donor

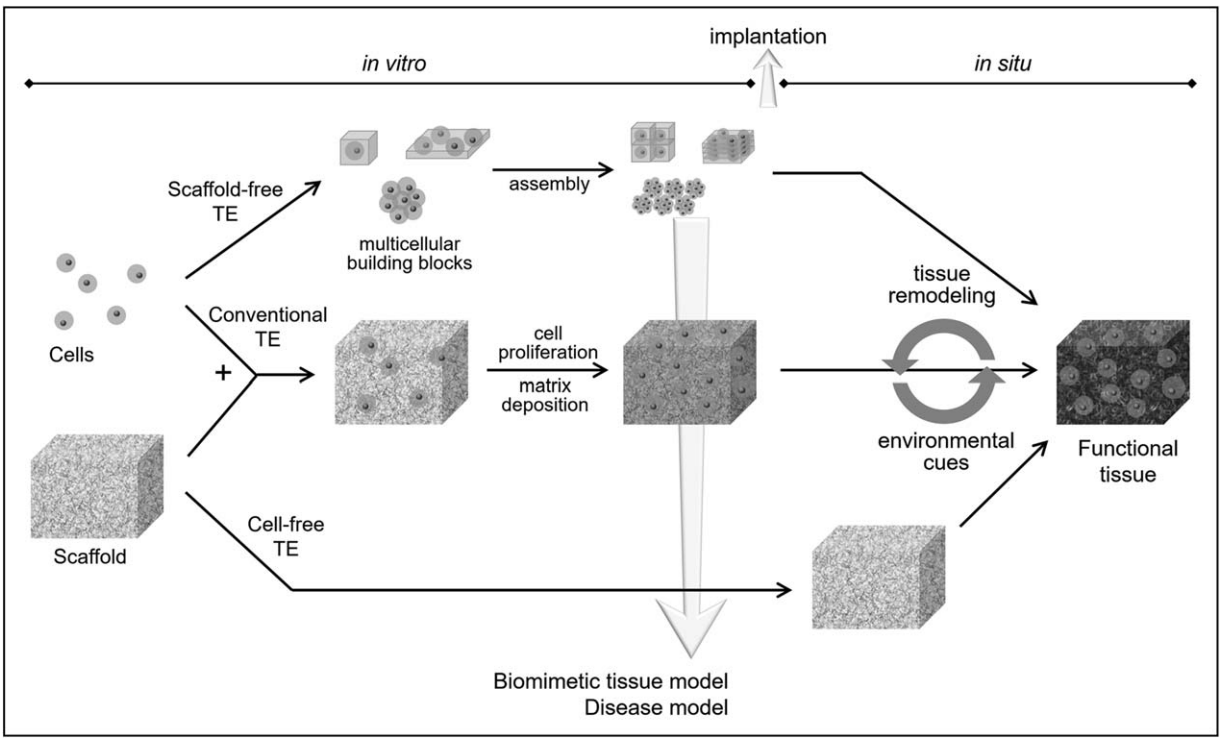

FIGURE 1. The workflow of major classes of tissue-engineering strategies. It is important to note that achieving functional tissues and organs involve processes that take place both *in vitro* and *in situ*. In both steps, cells are in continuous dynamic interactions and adapt to the cues provided by their environments.

Table 1. Characteristics of major tissue engineering strategies

	Aspect	Conventional tissue engineering	Scaffold-free tissue engineering	Bioprinting	Cell-free tissue engineering
In-vitro and in-situ steps	Ingredients	Cells, scaffold (and other desired molecules)	Cells	Cells, biomaterial (and other desired molecules)	Scaffold
	Cell introduction	In-vitro seeding into scaffold	Cells present initially	Cells present initially	In-situ recruitment
	Extracellular matrix	Fabricated solid materials or decellularized tissues + in-vitro cell secretion	In-vitro cell secretion	Present in the bioink and in-vitro cell secretion	In-situ cell secretion
	Tissue formation	*In vitro (bioreactor)*	*In vitro*	*In vitro*	*In situ*
	Implanted product	Cell-seeded scaffold	Assembled tissue building blocks	3D-printed tissue	Cell-free scaffold
	Postimplantation	Scaffold degradation, neotissue maturation	Fusion with host tissue, neotissue maturation	Fusion with host tissue, neotissue maturation	Host response, cell recruitment, matrix deposition, scaffold degradation, tissue formation, tissue maturation
Strengths and drawbacks	Potential clinical availability	Moderate	Slow	Moderate	Fast, even off-the-shelf
	In-vitro complexity	Moderate, labor-intensive and time-consuming preparation	High, especially for the assembly of the building blocks	Moderate, especially on the optimization of bioink	Low, mostly in terms of scaffold design
	Advantages	Diverse choice of materials and scaffold fabrication, advanced control of microstructure and architecture	Possibility to recreate tissues with complex architecture	High-resolution placement of cells in tissue constructs with complex architecture	Low cost, simpler regulation for clinical translation, harnesses body's own regenerative capacity
	Common issues	Heterogeneous cell distribution	Fragile cell constructs, inadequate mechanical properties	Requires dedicated devices, high-performance bioinks, high-resolution printing	Unpredictable host response, fibrotic response
	Ideal applications	Load-bearing tissues, soft and hard tissues, disease modeling, drug screening	Tissues with defined structure, disease modeling	Tissues with defined structure, vascularized tissues, disease modeling	Vascularized tissues

age, and carry the risk of the cells being in a diseased state [8,9]. The use of stem cells circumvents many of these drawbacks, although it requires additional steps of isolating the stem cells, ensuring complete differentiation or removal of all stem cells prior to transplantation, and demonstrating the absence of teratoma formation *in vivo* because of the cells' inherent oncogenic potential [10,11].

The second component is the scaffold. The original purpose of the scaffold matrices in tissue engineering is to provide a temporary structural support for the cells. It is typically a fabricated three-dimensional construct with interconnected pores, a hydrogel, or a decellularized ECM of a tissue [12]. The scaffold consists of biocompatible and biodegradable synthetic or biological materials and will be replaced by the new ECM produced by the cells. Later, it was realized that the scaffold can be used to deliver growth factors that promote cell recruitment, proliferation, and matrix deposition, thereby aiding neotissue formation. This has led to extensive research investigating the utility of including modifying factors, such as biologically active proteins, drugs, and DNA in the scaffolds for tissue engineering [13,14▪▪,15–18]. More recently, it has also been recognized that the physical, structural, and mechanical properties of the scaffold alone (i.e. even in the absence of chemical factors) can direct a wide range of cell behaviors [19–24]. On the one hand, this opens up a wide array of possibilities to rationally design 3D scaffold structures with cell-instructive properties, exploiting the rapid advance of high-resolution scaffold fabrication techniques, to further promote directed cell infiltration and tissue formation [25▪▪]. On the other hand, this highlights the need to carefully tune the physical properties of the scaffold, not only to maintain its mechanical integrity, but also to ensure adequate cell infiltration, differentiation, and tissue formation after implantation.

Scaffold-free tissue engineering

A scaffold-free tissue engineering approach has also been widely explored, where the idea is to obtain engineered tissues directly by assembling cells without the help of scaffolds. To bridge the gap in length scale between single cells and transplantable tissues and organs, the process is typically divided into two steps: prefabrication of multicellular building blocks and assembly of these building blocks into macroscopic tissues. Because of this modular approach, scaffold-free tissue engineering is also sometimes called 'modular' tissue engineering [26]. Similar to conventional tissue engineering, here the entire tissue engineering process also takes place *in vitro*.

The type of the building blocks from which the tissue is assembled defines several scaffold-free tissue engineering strategies. A common method is to use cell spheroids or aggregates, which are usually produced by subjecting cell cultures to rotational forces or flows, whose speed and duration can be tuned to control the size of the resulting aggregates [27,28]. The aggregates can then be implanted directly or coalesced to form larger tissue structures. Another method is using cell sheets [29]. Cells are expanded until a confluent monolayer with significant amount of ECM is obtained, allowing the cell sheet to be lifted from the surface as a whole [30]. The use of stimuli-responsive materials as culture substrate allows the cell–cell junctions and the deposited ECM to be preserved during lifting [31]. The released cell sheets can then be manipulated by stacking, layering, or draping over molds to achieve thick multilayered tissues [32]. The building blocks can also be obtained using self-assembly methods [33]. Here, cells are cultured in a non-cell-adherent mold and allowed to interact with each other, coalesce, and produce their own ECM. As no external forces are introduced to the culture, the tissue formation is argued to better mimic developmental processes.

A shared advantage of these scaffold-free tissue engineering methods is that they bypass the practical issues related to the scaffolds, including the design and fabrication of the scaffold as well as cell seeding, proliferation, and migration into the scaffold. However, the lack of scaffold also carries the drawback that both the cell-based building blocks and the final tissue constructs are often mechanically fragile and prone to damage during manipulation [34]. Moreover, the scaffold-free tissue engineering methods usually require extended time to obtain sufficient number of cells and to ensure fusion of the building blocks to obtain cohesive tissue constructs. The major strength of scaffold-free tissue engineering approach is the superior control over tissue architecture, enabled by controlled assembly and placement of building blocks consisting of different cell types. In fact, there is a growing momentum to develop techniques that combine the strengths of scaffold-based and scaffold-free approaches (reviewed in [35]).

Bioprinting

A unique strategy has been developed to exploit the technological advances of additive manufacturing while at the same time retaining the spatial control of scaffold-free tissue engineering approaches: bioprinting. The concept is to deposit suspensions containing cells as well as hydrogels, biomaterials, growth factors, and any other desired bioactive

molecules ('bioink') in a spatially controlled manner ('printing') to achieve 3D tissue-like architectures [36,37]. The components and composition of the bioink can be chosen to resemble the conventional tissue engineering approach using degradable (natural or synthetic) hydrogels or polymers, or to resemble the 'scaffold-free' tissue engineering approach by directly printing cell aggregates [38]. Here, again, the complete tissue formation process takes place *in vitro*.

The main critical aspects are choosing the bioink with the desired rheological properties and gelation kinetics to ensure its printability [39,40], optimizing the printing strategies and parameters that match the properties of the bioink [41], and ensuring fusion and validating the resulting construct structure and cell viability [42]. The major strength of bioprinting is its ability to control placement of cells within a 3D tissue-like constructs.

Cell-free tissue engineering

A diametrically opposite approach of the scaffold-free tissue engineering has also recently emerged: cell-free tissue engineering. This approach focuses on endogenous regeneration of the damaged tissue, aided by acellular bioresorbable scaffolds that are implanted in the functional site [43]. The underlying hypothesis in this approach is that the host response to the implanted scaffold can be steered to induce regeneration of the functional tissue [44,45]. The complete tissue formation process occurs *in situ*, hence the approach is also known as 'in-situ TE'.

The absence of cells allows cell-free tissue engineering to bypass all issues related to cell sourcing and seeding, simplifies regulatory hurdles, allows quick and off-the-shelf availability of treatments, and eliminates the time-consuming and labor-intensive process of in-vitro tissue formation [46]. The main challenge is scaffold design. In particular, all aspects of the scaffold, from its material composition, microstructure, surface chemistry, topography, bioactivity, mechanical properties, degradation profile, to its gross macroscopic morphology, must be precisely designed and tailored *in vitro* to induce the right tissue-specific responses *in vivo*. These responses include the recruitment and infiltration of the desired (progenitor) cells, differentiation and polarization of the cells, matrix deposition, and the functional maintenance of the tissue construct. Importantly, all of these steps need to be taken into consideration in the complex in-situ context, for example, foreign-body reaction and the associated immune response, contact with blood flow, and local mechanical stretching of the tissue.

PUSHING THE (IN-VITRO AND IN-SITU) BOUNDARIES

The ultimate goal of tissue engineering is to regenerate tissue function at the affected site. Different elements that are needed for tissue function have started to be tackled, primarily in terms of cellular composition and tissue architecture. Looking ahead, we reflect on the in-vitro and in-situ focus points that represent the outstanding scientific challenges and exciting technological possibilities in tissue engineering for the near future.

In vitro: controlling cell organization to achieve tissue function

In-vitro tissue engineering has made considerable advances in obtaining tissue constructs with the desired cell compositions and complex tissue architectures. One of the most critical next challenges is to achieve organization at the cell level, which is especially crucial for the physiological function of mechanically active tissues like the cardiovascular and musculoskeletal tissues [47]. The challenge is two-fold: understanding the fundamental principles underlying cellular organization in native, living tissues, and figuring out ways to manipulate cellular organization in engineered tissues and organs. Importantly, cellular organization is known to be governed by the local cell–matrix physical and mechanical interactions, through processes, such as contact guidance [48–50], strain avoidance [51,52], flow-induced alignment [53,54], and curvature avoidance [55–57]. Although the molecular mechanisms underlying such processes are currently still intensely debated [58], from a tissue engineering perspective, these phenomena provide an attractive avenue for achieving tissue ordering and organization at the cell scale.

In the context of scaffold-based tissue engineering, this is possible by designing scaffolds that provide cells with specific cues like fiber size, pore size, mechanical properties, and overall geometry [59▪] – parameters that can be finely tuned using today's scaffold fabrication techniques (e.g. rapid prototyping, electrospinning/writing) [60], even in combination with additional environmental cues [61▪,62▪▪]. For scaffold-free tissue engineering, cell organization can potentially be achieved by micropatterning the multicellular building blocks, for example, aided by nanotopography/microtopography and DNA or ligand printing [63,64]. Fundamental research on cellular response to these local cues using minimal models is clearly poised to strongly accelerate efficient optimization of the experimental parameters in achieving this high-resolution cellular organization [65]. It is envisioned that this exciting

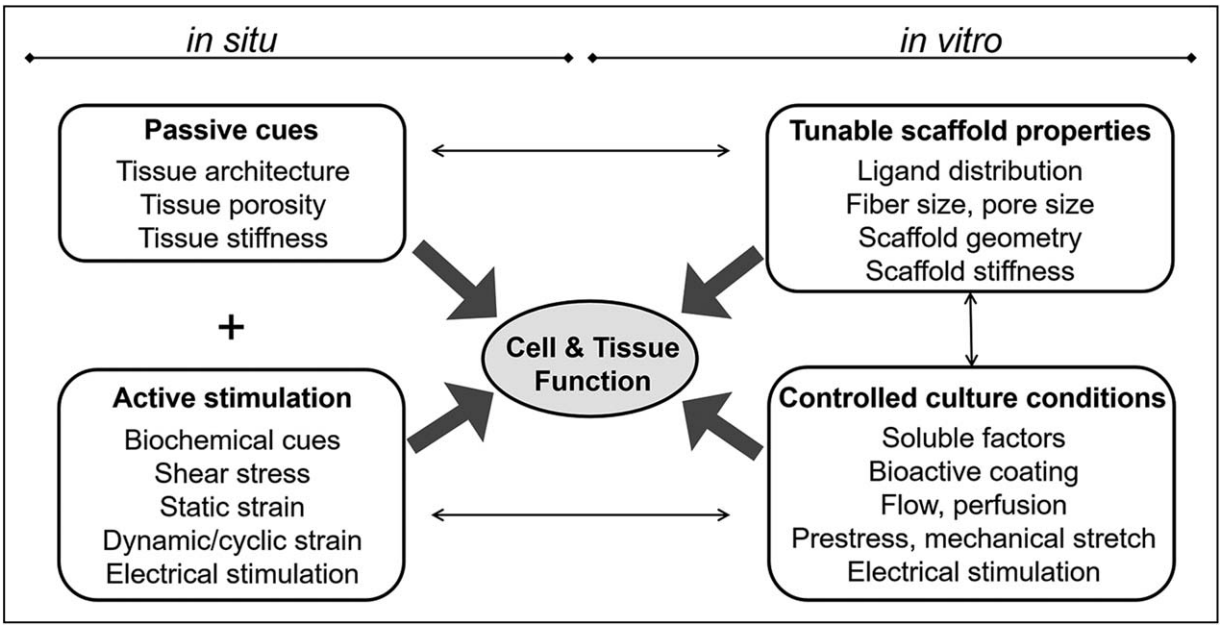

FIGURE 2. Cell and tissue response is sensitively dependent on cues and stimulation present in their environment. For tissue engineering purposes, these in-situ cues and stimulations can be mimicked and exploited *in vitro* to steer functional tissue formation and regeneration.

development will pave the way to engineered autologous tissues and organs that are as function-ready as possible for transplantation.

In situ: understanding, recreating, and steering tissue remodeling

Urged by the obvious role of the biophysical and biomechanical interactions between cells and the cellular environment in tissue formation and function, there has been a growing need for experimental platforms that allow one to subject cells and tissue constructs to a variety of biomimetic physical and mechanical cues in a controllable manner – 'bioreactors' [66,67▪]. By providing culture conditions that closely recapitulate the environmental conditions of the native/desired tissues (Fig. 2), the hypotheses are that bioreactors can promote the formation and growth of viable tissues and organs for the in-vitro steps of tissue engineering [68] and that bioreactors can help us predict and steer tissue formation and evolution for the in-situ steps of tissue engineering in the presence of passive and active cues from the cellular microenvironment [69].

In addition to improving the existing bioreactors to further fine tune the in-vitro culture conditions of the tissue constructs, two promising research directions have started to be explored. First, in most in-vivo environments, multiple cues are simultaneously at work, but the combinatorial effects of multiple environmental cues on cells and tissues are still poorly understood. Smart designs of bioreactors will allow decoupling of such cue combinations and help bridge our fundamental understanding of cell response (typically to single cues) and physiological tissue functions. For example, recent development of bioreactors that allow decoupling of mechanical stretch and shear flow demonstrates that these cues act nonsynergistically in regulating cell–cell signaling [70], cell proliferation [71], and neovessel formation [72], highlighting the need to better understand the underlying mechanisms of tissue growth. Second, bioreactors endow us with the possibility of quantitatively controlling the cues in a spatiotemporally resolved manner. When combined with physiologically relevant scaffold and tissue geometries, this has an enormous potential for disease modeling and drug screening. For example, by simulating different in-vivo conditions *in vitro*, one can mechanistically identify the possible causes of the disease and test different therapeutic strategies systematically [73].

CONCLUSION

Research over the past few years has not only demonstrated significant advances in each of the TE approaches, but has also resulted in new, innovative strategies and methodologies that are designed to overcome the limitations of the existing methods. This strong technological progress has the potential to elevate the physiological functionality of the

engineered tissues, to broaden the application areas (i.e. the possibility to engineer more diverse tissues and organs), and to increase the clinical accessibility and robustness of the engineered constructs (e.g. by simplifying the constructs in view of the ethical, social, and regulatory concerns). At the same time, fundamental research on cell–matrix and cell–material interactions has proven to be vital in deepening our understanding of the in-vitro and in-situ processes during tissue formation and remodeling. A concerted effort in these basic and technologically oriented research lines will enable a more directed, hypothesis-based tissue engineering and away from inefficient trial-based and error-based approach.

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Conflicts of interest

There are no conflicts of interest.

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