


Review

A Paradigm Shift in Tissue Engineering: From a Top–Down to a Bottom–Up Strategy

Theresa Schmidt ¹, Yu Xiang ², Xujin Bao ²  and Tao Sun ^{1,*}

¹ Department of Chemical Engineering, Loughborough University, Epinal Way, Loughborough LE11 3TU, UK; T.Schmidt-20@student.lboro.ac.uk

² Department of Materials, Loughborough University, Epinal Way, Loughborough LE11 3TU, UK; Y.Xiang@lboro.ac.uk (Y.X.); X.Bao@lboro.ac.uk (X.B.)

* Correspondence: T.Sun@lboro.ac.uk; Tel.: +44-(0)-1509-223214

Abstract: Tissue engineering (TE) was initially designed to tackle clinical organ shortage problems. Although some engineered tissues have been successfully used for non-clinical applications, very few (e.g., reconstructed human skin) have been used for clinical purposes. As the current TE approach has not achieved much success regarding more broad and general clinical applications, organ shortage still remains a challenging issue. This very limited clinical application of TE can be attributed to the constraints in manufacturing fully functional tissues via the traditional top–down approach, where very limited cell types are seeded and cultured in scaffolds with equivalent sizes and morphologies as the target tissues. The newly proposed developmental engineering (DE) strategy towards the manufacture of fully functional tissues utilises a bottom–up approach to mimic developmental biology processes by implementing gradual tissue assembly alongside the growth of multiple cell types in modular scaffolds. This approach may overcome the constraints of the traditional top–down strategy as it can imitate in vivo-like tissue development processes. However, several essential issues must be considered, and more mechanistic insights of the fundamental, underpinning biological processes, such as cell–cell and cell–material interactions, are necessary. The aim of this review is to firstly introduce and compare the number of cell types, the size and morphology of the scaffolds, and the generic tissue reconstruction procedures utilised in the top–down and the bottom–up strategies; then, it will analyse their advantages, disadvantages, and challenges; and finally, it will briefly discuss the possible technologies that may overcome some of the inherent limitations of the bottom–up strategy.



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1. Introduction

Tissue engineering (TE) had its breakthrough about 35 years ago when it was introduced by two pioneers of this research field, Dr. Joseph Vacanti (Children's Hospital Boston, Boston, MS, USA) and Dr. Robert Langer (MIT, Cambridge, MS, USA) [1]. Vacanti and Langer [2] described TE as an interdisciplinary field that employs life science and engineering principles towards the development of biological substitutes to restore, maintain, and improve the functions of damaged tissues and organs. The initial motivation was to tackle the growing issue of organ shortages, which resulted in increasing deaths of patients on organ transplantation waiting lists [2,3] (pp. 3–4) [4] (p. 4). Another objective of TE was the manufacturing of assistive extracorporeal devices for defective organs, such as substitute living kidneys as alternatives to dialysis [5]. Apart from the clinical applications, the engineered biological substitutes were also intended to be used for non-clinical purposes, such as in vitro biology and physiology studies as well as drug testing [5–14].

The initiation of TE created high hopes of manufacturing and commercialising engineered tissues and organs for clinical applications, which led to the formation of several

companies [15,16]. Thus, the private sector research and development (R&D) investments in TE compared to the federal R&D investments in TE were significantly higher with, for example, more than 3.7 billion USD difference in 2001 in the US [15]. Consequently, the private sector dominated TE research, which resulted in the development of a relatively practical focus [15]. During the past 35 years, great amounts of consistent worldwide research efforts have been attracted into TE, and significant progress has been achieved. Due to the advantages of three-dimensional (3D) tissue cultures over two-dimensional (2D) monolayer cell cultures [11], a variety of engineered tissues have been manufactured successfully for non-clinical purposes. For example, engineered small intestinal organoids have been used for drug screening, the investigation of intestinal diseases, and studies of host–pathogen interactions, organ function, and physiology [17] (pp. 273–297). Some biological substitutes with one to two cell types and relatively simple anatomic structures, such as engineered human skins, are also used successfully for clinical applications [18–20]. However, there have been very few successes achieved in terms of the manufacturing of fully functional tissues with more diverse cell types and complex anatomic structures for clinical purposes [15,16].

This very limited clinical application is mainly due to the fundamental obstacles associated with the traditional top–down tissue manufacturing approach [21]. As a consequence, an alternative bottom–up strategy, also called developmental engineering (DE), has been proposed to manufacture fully functional tissues and organs. This bottom–up approach may overcome the problems associated with the traditional top–down approach and has the potential to deliver the promised successes for clinical applications [21,22].

This review focuses on the comparison and analysis of the number of cell types, the size and morphology of the scaffolds, the generic tissue reconstruction procedures utilised in the top–down and the bottom–up strategies, as well as the corresponding advantages, disadvantages, and challenges; moreover, the possible technologies that may overcome some of the inherent limitations of the bottom–up strategy will be briefly discussed.

2. Top–Down Tissue Manufacturing Approach

TE is based on a biological triad of cells, signals, and scaffolds [15,21,23,24]. As the main TE components, live cells are strategically utilised for the creation of new tissues and their integration with existing host tissues. Different signals are either provided as growth factors, cytokines, or as specific mechanical or electric stimuli to regulate various cell behaviours. Scaffolds are utilised to hold the cells together to create the tissue's physical structure; moreover, they have the same role as the extracellular matrix (ECM) of *in vivo* tissues [6,15,24]. As the functions of the ECM in natural tissues are complex and multivariate, the scaffolds are not only used to mechanically stabilise the cells by providing physical support for cell adhesion but also to regulate cell proliferation, migration, and differentiation [15,24]. Therefore, both signals and scaffolds are critical to provide the suitable environments for tissue regeneration.

In order to replicate the necessary tissue structures and functions, and to avoid the immune rejection of the engineered tissues by the hosts in clinical applications, suitable cells and scaffold materials are firstly selected [5]. The underpinning fundamental biological interactions, including the cell–cell and cell–scaffold interactions, have to be systematically investigated and then used as specific guidance for subsequent tissue reconstructions [16,25,26]. However, this has been simplified or even neglected in the top–down TE approach, which is mainly based on one-stage seeding and culturing of a few main types of cells into 3D scaffolds that replicate the sizes and morphologies of the target tissues, as opposed to the complex natural tissue development processes (Figure 1) [15,21,23,24].

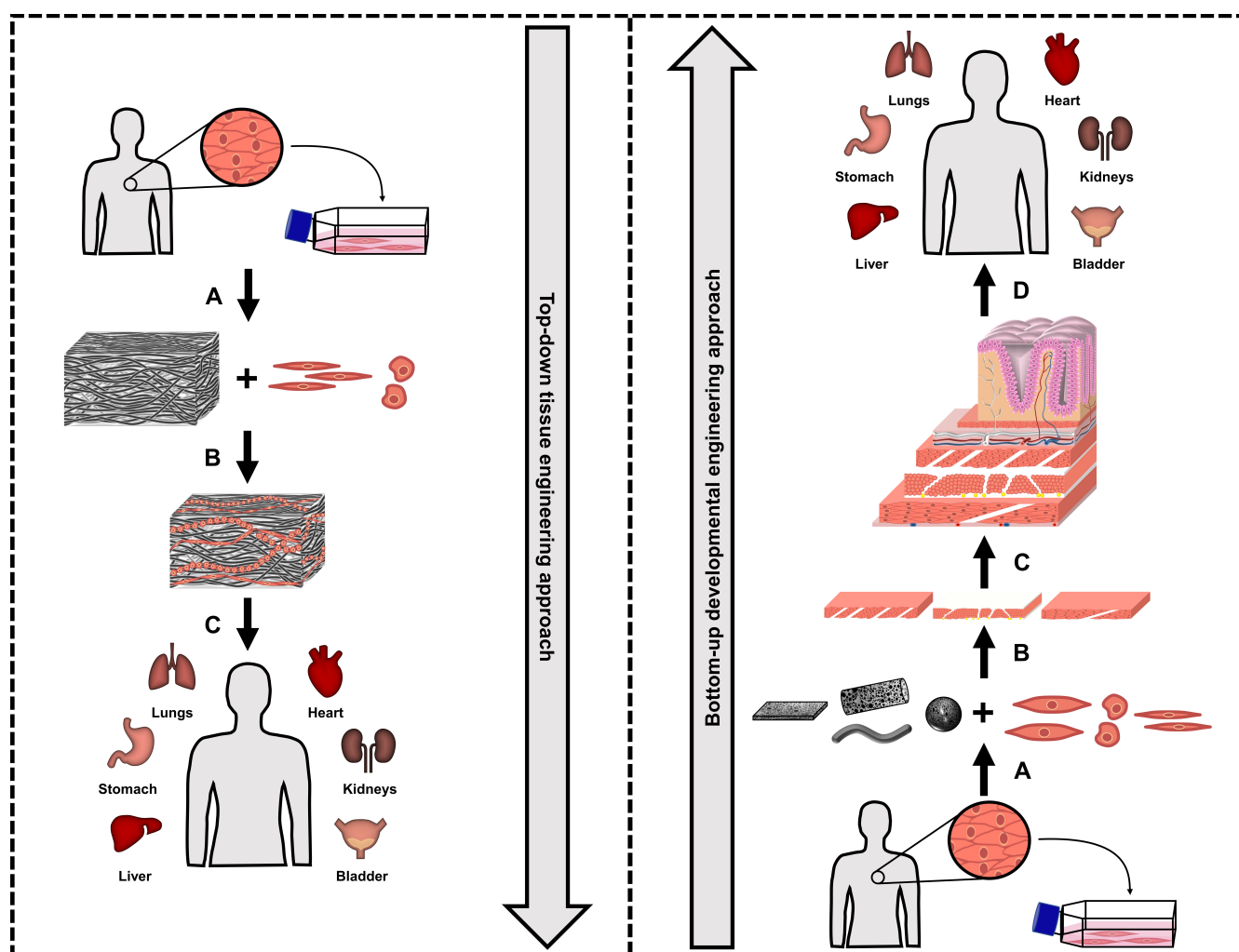


Figure 1. Comparison of the traditional top–down tissue engineering (**left**) and the bottom–up developmental engineering (**right**) approaches towards tissue reconstruction. The top–down tissue engineering approach (**left**) starts with (A) the isolation of the suitable dominant cell type(s) and the expansion of these isolated cells; the next step is (B) a one-stage process for seeding and culturing few dominant cell types of the target tissue in a three-dimensional scaffold with equivalent sizes and shapes as the target tissues/organs; (C) the reconstructed tissues can be used for clinical applications. The bottom–up developmental engineering approach (**right**) starts with (A) the isolation of multiple cell types and the expansion of these isolated cells separately; the next step is (B) the independent preparation of multiple modular tissue building blocks by culturing different cell types on corresponding modular scaffolds with specific sizes and morphologies; this is followed by (C) the gradual tissue reconstruction via layer-by-layer assembly of the modular tissue building blocks to reconstruct (D) the designated functional tissues/organs for clinical applications.

The advantages associated with this top–down TE strategy are apparent. Firstly, only the dominant cell types in the target tissues are isolated, expanded, and then utilised for tissue reconstruction, while other assistive cells, such as nerve and vascular cells, are not necessary. For example, only human dermal fibroblasts, or only human keratinocytes, or both human dermal fibroblasts and keratinocytes have been used as the dominant cell types for the reconstruction of engineered skin products, which have been used successfully for clinical purposes [16,27,28]. Chondrocytes have been employed as the single dominant cell type for the manufacture of functional cartilage products for clinical purposes, which have passed phase I or phase II of clinical trials, depending on the manufacturers [16,29,30]. Secondly, 3D scaffolds are usually fabricated to mainly replicate the overall sizes and morphologies but not the complex internal structures of the target tissues. Thirdly, a simple one-stage cell seeding and culturing in the scaffolds is adopted for the subsequent tissue reconstructions. Clearly, this top–down TE approach is suitable for the manufacturing

of tissues with relatively few cell types and simple anatomic structures, such as skin and cartilage tissues [16,22,27–30].

Since this artificial top–down strategy is fundamentally different from the natural tissue developmental processes *in vivo*, there are inherent limitations [15,22]. Firstly, very limited mass transfer in these heterogeneous liquid–solid tissue culture systems [21,31] (pp. 398–400, 471–475). The mass transfer between the 3D solid scaffolds and the surrounding liquids (e.g., cell suspensions or culture media) is mainly dependent on limited diffusion rather than more effective convection. Therefore, uniform cell seeding into the 3D scaffolds is a challenge, and most of the cells are only seeded onto the exterior surfaces of the scaffolds [21,31] (p. 397). Some cells might be introduced slightly deeper underneath the exterior surfaces; however, the survival of these cells is further endangered by the limited nutrient and oxygen supply, and metabolic waste removal within the scaffolds [21,31] (pp. 398–400, 471–475). Secondly, there is a lack of essential cells and tissues. From a technical perspective, the production of functional tissues requires the capability to introduce suitable cells into specific parts of a scaffold and to control the temporal–spatial organisation of different cell types to replicate the complex, ordered architectures of natural tissues [21]. Particularly, the introduction of the essential structures, such as nerves and blood vessels, is necessary [5,21,32]. Innervation plays a critical role not only in tissue and organ development, maturation, and regeneration but also in their functional and regulatory control, and modulation [32,33]. Moreover, due to the release of specific neuropeptides by autonomic and sensory nerves, the promotion of innervation can directly facilitate angiogenesis [32]. Blood vessels, similar to nerves, are also critical structures as they supply cells with oxygen and nutrients as well as remove their metabolic wastes and carbon dioxide [5,11]. The cell viability *in vivo* depends on the diffusion of oxygen, nutrients, and growth factors from the surrounding capillaries, and the diffusion distance is limited to only 100–200 microns [11,21]. In order to manufacture vascularised thick tissues or organs, specific cell types and proper angiogenic factors are required within the scaffolds [11]. However, in the top–down tissue manufacturing processes, apart from several dominant cell types, other essential cells, such as nerve and vascular cells, are usually not included [5,32]. In addition, the control over the temporal–spatial organisation of the seeded cells is very limited, which consequently restricts the structures and functions of the fabricated tissues [21,31] (p. 397). The resulting lack of the particularly essential nerves and blood vessels is a detrimental issue that hinders the reconstruction of fully functional tissues, and the subsequent proper performance and integration of the reconstructed tissues with the host [5,32]. For example, due to the lack of blood vessels, the mass transfer in the top–down TE processes has to rely on limited diffusion, which causes poor cell survival in the necrotic central regions [21,31] (pp. 398–400, 471–475). There have been various research efforts, especially towards the incorporation of blood vessels into some tissue constructs; however, no sufficient vasculatures have been constructed yet within the currently engineered tissues [11,21]. Thirdly, there is poor controllability. Due to the fundamental differences between the top–down TE approach and the natural tissue development processes, the comparability and the viability of rational process modifications of the top–down TE processes are minimised [15,22]. Therefore, most current top–down processes, being one-stage processes, are adapted more or less according to trial-and-error, resulting in poor controllability and reproducibility [15]. As a consequence, the development of standardised protocols for these top–down TE processes is obstructed [22].

Due to the aforementioned technical obstacles, up-scaling of these traditional tissue manufacturing processes as well as the production of complex organs, such as kidneys, livers, and hearts, is not supported by the top–down TE strategy [11,21,22]. The only successful TE product for clinical applications manufactured via the top–down approach is reconstructed human skin, which is comparably simple in its anatomic structure [16,22,27]. Even though various engineered skin products have been manufactured and used for clinical applications, they are still not fully functional. This is because only human keratinocytes and/or dermal fibroblasts are commonly utilised for the reconstruction of human skin,

while other skin cells, such as melanocytes and vascular cells, are not included [28]. Thus, apart from the epidermal tissue with keratinocytes and/or the dermal tissue with fibroblasts, there is a lack of other structures, such as sweat glands, hair follicles, sensory nerve fibres, and vascular plexuses, in the currently reconstructed skins [28,34]. Other promising TE products for clinical purposes seem to be bioartificial cartilages, which have passed phase I or phase II clinical trials, depending on the producing company [16]. The attempts to apply other TE products, such as trachea, blood vessels, and bladders, for clinical purposes have not succeeded yet [16,21,35]. Therefore, several TE companies were only able to produce engineered tissues with very low profit margins while suffering from high R&D and production costs [16,22]. Hence, a number of high-profile bankruptcies occurred, such as the one of Advanced Tissue Sciences (ATS) in 2003, which resulted in the loss of about 300 million USD [16,36]. Accordingly, the scientific community raises criticism against the traditional top-down TE approach, which has not shown the initially promised potential for clinical applications, as most of the TE products just look like tissues, smell like tissues, and taste like tissues but do not fully function like the natural target tissues [22,37].

3. Bottom-Up Tissue Manufacturing Approach

Although the TE challenges for clinical applications have already been recognised by scientists for many years, they are yet to be overcome [1,21,22]. This is mainly due to the inherent, insurmountable limitations of the top-down TE approach, which is fundamentally different from natural tissue development [22]. Therefore, an alternative bottom-up TE strategy (Figures 1 and 2) has been proposed to mimic and exploit *in vivo* tissue development, structure, and function [25]. DE is closely related to systems biology and developmental biology [15,38].

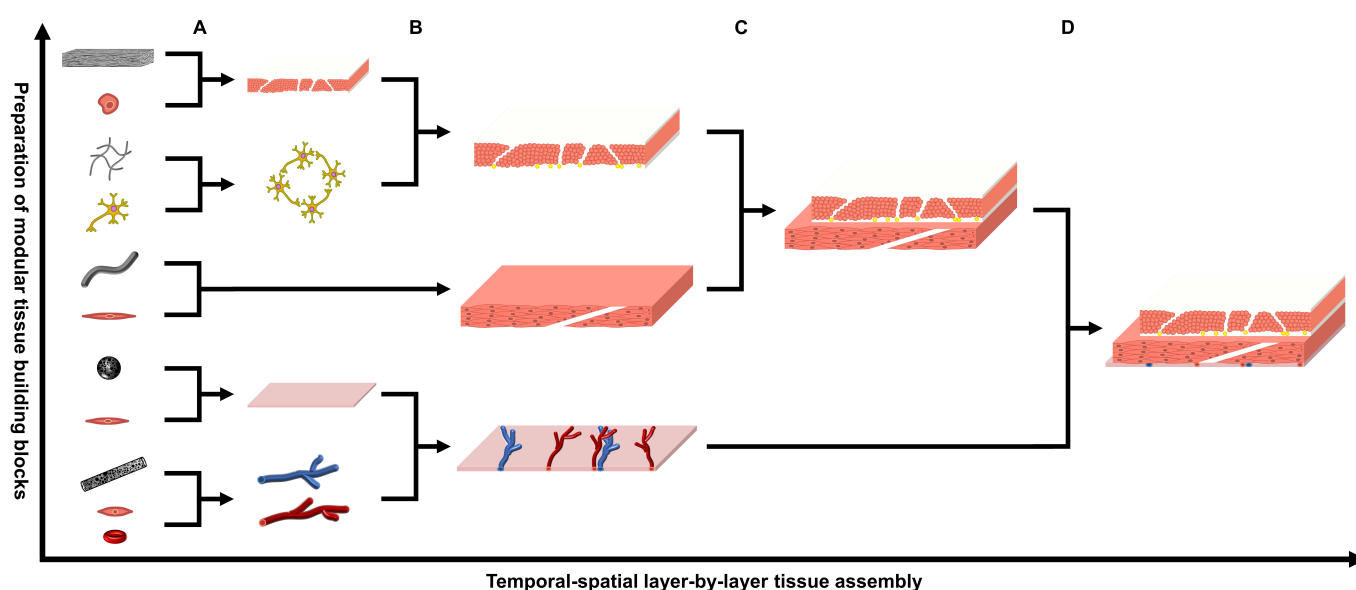


Figure 2. The gradual temporal-spatial layer-by-layer tissue assembly of functional tissues/organs via the bottom-up developmental engineering strategy. Firstly, (A) multiple modular tissue building blocks are prepared separately using different types of cells and their corresponding modular scaffolds with varying sizes and morphologies; (B–D) these modular tissue building blocks are gradually assembled layer-by-layer via specific temporal-spatial procedures to imitate the *in vivo* development processes as well as the anatomic structures of the target tissues/organs; this results in the formation of designated tissues/organs with increasing size and complexity.

Systems biology aims to investigate a whole biological system and the interactions of different components of the system at different levels [38]. Similarly, live tissues are integrated biological systems with closely related or integrated components (e.g., cells, ECM) (Figure 3) [25]. Therefore, in order to reconstruct fully functional tissues, the interac-

tions among different cells and the surrounding ECM components or scaffolds must be coordinated and regulated as in the authentic target tissues [25]. To deliberately mimic these interactions, the bottom-up DE approach utilises various suitable biomaterials or scaffolds, and multiple cell types but not just several dominant cells. Most importantly, it also imitates the in vivo-like temporal-spatial coordination of these components in vitro [15,39]. Accordingly, the tenet of this bottom-up DE strategy is to replicate the integrated functional networks of cells and ECM components. As such, it is a step towards a systems biology approach for the reconstruction of fully functional tissues and organs.

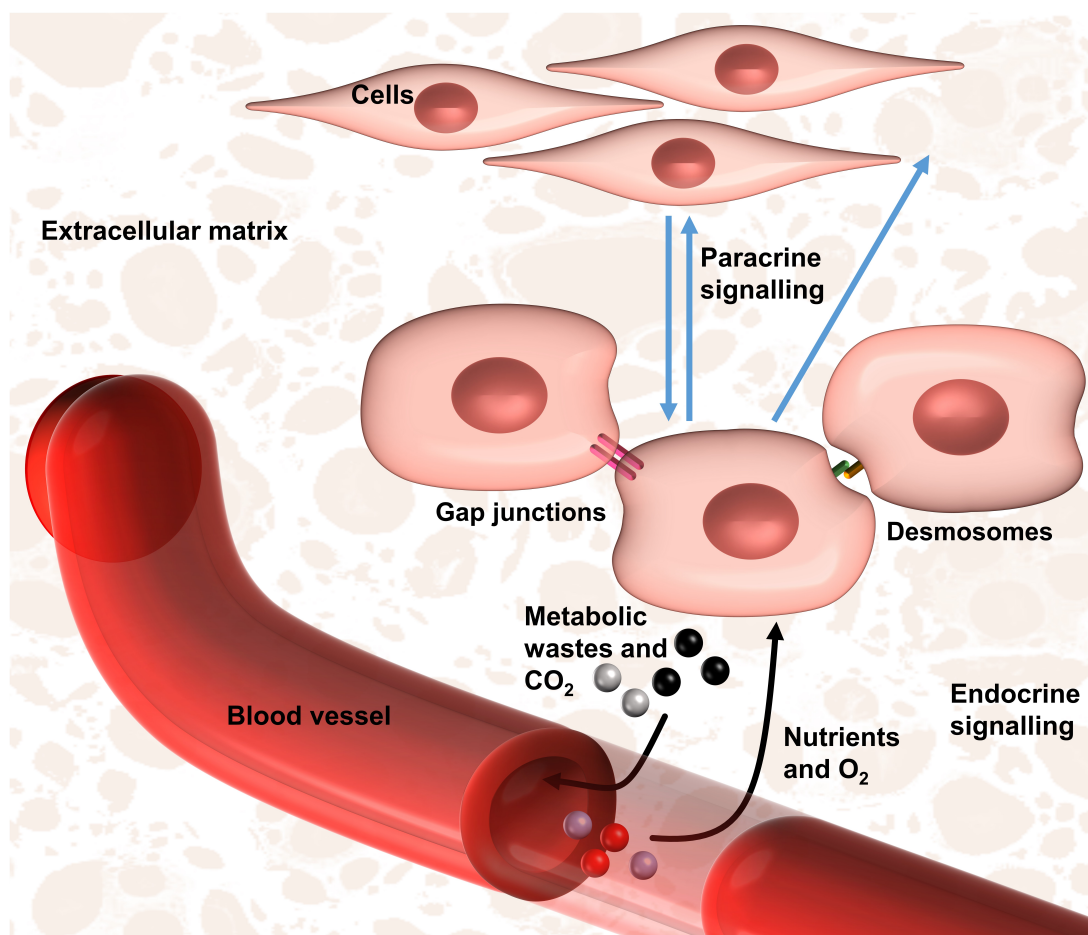


Figure 3. The native cell niche that is intended to be mimicked by developmental engineering. The cells are supplied with oxygen (O_2) and nutrients, while their metabolic wastes and carbon dioxide (CO_2) are removed via the enclosed vasculature. The cell behaviours are regulated by neighbouring cells via gap junctions or desmosomes, and by distant cells via paracrine signalling or vasculature-mediated endocrine signalling. The cells are also influenced by cell–extracellular matrix interactions.

Developmental biology is about the upgrowth of an organism from a fertilised ovum, which is a typical bottom-up process with a gradual increase of tissue size in parallel to cell growth [4] (p. 21) [15,21]. These natural development processes are intended to be imitated in DE by firstly preparing various modular tissue building blocks using different types of cells and corresponding modular scaffolds with smaller sizes and varying shapes, and then assembling these modular tissue building blocks layer by layer to gradually reconstruct the designated tissues or organs (Figures 1 and 2) [15,21,22,31] (pp. 300–305).

The bottom-up DE strategy is different from the traditional top-down TE approach in several aspects. Firstly, more essential but not just several dominant cell types are used for tissue reconstruction. Secondly, multiple modular scaffolds with smaller sizes and varying shapes for different cell types are fabricated instead of a single scaffold with the

same size and shape as the target tissue. Thirdly, functional tissues are reconstructed via carefully coordinated temporal and spatial processes rather than a simplified one-stage cell seeding and culturing procedure. Due to the use of smaller scaffolds for the preparation of modular tissue building materials and the gradual introduction of vascular systems within the reconstructed tissues, nutrient supply and metabolic waste removal are dependent on both convection and diffusion. Consequently, mass transfer within the progressively assembled tissues is not a limiting detrimental factor. As the bottom-up DE strategy is in vivo-like, it is more comparable to the imitated natural tissue development process than the conventional top-down TE approach [15,22]. Moreover, the bottom-up strategy with sequential stages, in contrast to the top-down approach with just a one-stage cell seeding and culturing procedure (Figures 1 and 2), has improved observability and controllability [15,22]. Therefore, rational modifications to the temporal-spatial bottom-up processes are facilitated, rather than the empirically or trial-and-error-based modifications commonly adopted for the top-down procedures [15,22]. The consequentially enhanced reproducibility enables the design of standardised tissue manufacturing protocols, which is a major advantage of DE, making it significantly more efficient [15,22].

This layer-by-layer bottom-up DE approach is particularly advantageous to replicate the hierarchical architectures found in natural tissues [6,24,31] (pp. 300–305). This is mainly because the use of modular tissue building materials enables the imitation of the individual native cell niches (Figure 3) for different types of cells [5,16,26]. As previously mentioned, in order to reconstruct fully functional tissues, the whole system, as well as the complex interactions within diverse cell niches, have to be considered, including for example the cell-cell and cell-material interactions [16,25,26]. The scaffolds in TE are meant to replace the ECM in natural tissues; this has been approached by research on microgels, which mimic the fibrous structure, ECM-coated/embedded polymeric microcarriers, and ECM-derived microcarriers in cell culture and delivery applications [6,40–43]. However, the diverse ECM components fulfil more purposes than the provision of mechanical support and anchorage for the cells [15,24]. For example, they can regulate gene expression and even alter cell phenotypes via physical and/or biochemical influences, which are transduced by cell surface receptors through the cytoskeletons to the cell nuclei [5]. These cell-ECM interactions direct the tissue function and structural integrity and can even overwrite the initial cell programming [5,24]. The responses and adaptations of the cells to their niches or the surrounding matrix are exploited by DE to facilitate and regulate the designated in vivo-like tissue reconstructions [5,15,31] (pp. 227–242). Therefore, the imitation of native cell niches within the modular tissue building materials enables the bottom-up DE approach to reconstruct fully functional tissues with hierarchical architectures. Besides the layer-by-layer assembly of fully functional tissues or organs for clinical applications, the modular tissue building block materials can also be utilised as 3D tissue alternatives to 2D cell cultures or animal models for non-clinical purposes, such as drug testing.

In summary, the bottom-up DE strategy has the potential to overcome the challenges associated with the top-down TE approach and, therefore, to achieve the initially promised successes of TE, especially the manufacturing of fully functional tissues or organs to tackle the clinical organ shortage problems [21,22,31] (pp. 300–305).

4. Relevant Issues for the Bottom-Up DE Approach

There are several issues to tackle in order to successfully implement the bottom-up DE strategy [15,31,38] (pp. 300–305). In addition to the dominant cells contributing to the main characteristic function and/or structure of a particular target tissue, other cell types, especially the cells for vascularisation and innervation, need to be isolated and expanded separately under optimal conditions. Modular scaffolds with suitable physical and biochemical properties, different sizes, varying morphologies, and delicate internal structures have to be optimised for the respective modular tissue building blocks [44] (pp. 41–92). Generally, the scaffold materials are non-toxic, biocompatible, and/or biodegradable, and they also possess certain physical properties, such as suitable strength and/or stiffness, to

imitate the target tissues [44,45] (pp. 41–92). Polymers are the most commonly used scaffold materials alongside metals, ceramics, and their composites [46]. Polymeric scaffolds can be prepared using natural polymers (e.g., chitosan, hyaluronic acid (HA), collagen, silk, and gelatin) and/or synthetic polymers (e.g., poly (lactide) (PLA), poly (ϵ -caprolactone) (PCL), poly (glycolic acid) (PGA), and poly(lactic-co-glycolic acid) (PLGA)). In comparison with synthetic polymers, the advantages of natural polymers are very obvious. They are usually the most suitable materials with delicate structures for the cultured cells. However, due to limited human sources, animal-derived natural polymers are commonly produced at large scales. Consequently, the use of natural polymers for clinical purposes can be limited by the potential pathological agents and/or immune reactions [47,48]. Depending on the cultured cells and the imitated target tissues, the scaffolds need to be fabricated into different shapes, such as microspheres, fibres, membranes/films, tubes, sponges, non-woven fabrics, hydrogels, and porous 3D scaffolds. Depending on the designated shapes, various manufacturing techniques can be utilised, such as electrospinning to produce nanofibres, membrane/film formation, emulsion to create micron-sized or nanoparticles, lipid templating to generate microporous scaffolds, 3D printing to print non-woven fabrics, and gas foaming to manufacture sponges [49–55]. For example, porous PLA nanocomposite sponges or chitosan-gelatin blend nanofibres are prepared for skin cell cultures [55,56]; hollow PCL fibres and PGA sutured mesh tubes seeded with smooth muscle cells are prepared for blood vessel cultures [57–59]; conductive polyaniline (PANI) and polypyrrole (PPy), freeze-dried PANI composite sponges, electrospun biodegradable PLA nanofibres, and natural silk hydrogels are prepared for nerve cell cultures [48,60–63]; silk hydrogels are prepared for tendon and cartilage cell cultures; and natural polymer composite nanofibres as gelatin/hydroxyapatite and collagen/hydroxyapatite are prepared for bone tissue cultures [54,64–68]. Table 1 summarises the commonly used polymeric scaffolds with varying shapes for different human cells and target tissues. Apart from these self-made scaffolds, some of the commercially available, biodegradable microcarriers can be used as the modular scaffolds [44] (pp. 41–92) [69,70]. According to the bulk material and surface property, morphology, size, and degradation rate, suitable microcarriers not only provide the necessary physical support for cell expansion but also act as the tissue building materials for the layer-by-layer tissue assembly [11]. Therefore, the use of microcarriers as the modular scaffolds for both cell expansion and tissue reconstruction will facilitate process intensification in DE.

As exemplified in Table 2, porous and non-porous spherical particles and microgel microcarriers are commonly used for cell cultures beside other modular scaffolds such as nanoparticles, hydrogels, and cryogels [40,41,52,53,96–100]. In contrast to non-porous microcarriers, porous microcarriers are usually fabricated with interconnections, enlarged surfaces, and even incorporated with growth factors to facilitate cell adhesion and growth [101] (pp. 149–178). The internal structure, particularly the porosity, is a critical characteristic of porous microcarriers [11]. The pore size not only affects the mass transfer but also the respective cell colonisation within the microcarriers [102]. Regarding the particle size, micron-sized microcarriers are commonly used for cell cultures [11,101,103–106] (pp. 149–178). This is because particles at nano-scale have been found to be toxic to the cultured cells, while macro-sized ones can cause physical damage to the cultured cells, as higher energy input is needed to suspend these large solid particles for cell cultures [11,101,103–106] (pp. 149–178).

Table 1. Summary of the commonly used polymeric scaffolds with varying morphologies and respective cell types for the engineering of different human target tissues.

Engineered Tissue	Cell Types	Scaffold Morphologies	Common Polymers	References
Vascular tissue (e.g., blood vessel)	Human mesenchymal stem cells (hMSCs)	Tubes	PCL ¹ , PLLA ²	[71,72]
	Human turbinate mesenchymal stromal cells	Tubes	PEG ³	[71]
	Human umbilical vein endothelial cells (HUVECs)	Tubes	PPO-PEO ⁴	[71]
	Human smooth muscle cells	Mesh tubes	PGA ⁵	[59,73]
	Human smooth muscle cells	Tubes	PGA	[58]
	Vascular smooth muscle cells from human induced pluripotent stem cells (hiPSCs-VSMCs)	Tubes	pNIPAm-grafted PDMS ⁶	[74]
Bone tissue (e.g., tendon, cartilage)	hMSCs	Three-dimensional (3D) porous scaffolds	PCL/HA ⁷	[75]
	Human bone marrow stromal cells (hBMSCs)	Membrane/3D porous scaffolds	PHA ⁸	[50]
	hBMSCs	3D porous scaffolds	PLA ⁹	[76]
	Human placenta-derived mesenchymal stem cells (hPMSCs)	Fibres	PLA, PLGA ¹⁰	[77]
	MG-63 human osteoblast-like cells	Porous microspheres	PLA	[78]
	Human nasal chondrocytes	Porous microspheres	PEGT/PBT ¹¹	[79]
	Human skeletal stems cells (hSSCs)	Sponges	PLA/HA ¹² , PLGA/HA ¹³	[80]
	Human fetal osteoblasts (hFOBs)	Nanofibres	Chitosan/HA ¹⁴	[81]
	hFOBs	Nanofibres	Gelatin/HA ¹⁵	[82]
	hFOBs	Nanofibres	Collagen/HA ¹⁶	[68]
	Adipose-derived mesenchymal stem cells (hADMSCs)	3D fibrous scaffolds	Keratin	[54]
	Human bone marrow derived mesenchymal stem cells	Hydrogels	Silk fibroin	[64]
	Human bone marrow stem cells	Sponges	Silk	[83]
	hADMSCs, Human tenocytes (HT), HUVECs	Non-woven meshes, nanofibrous woven fabrics	PCL	[84]
Urinary tissue (e.g., bladder, urethra, ureter)	Human bladder smooth muscle cells (hBSMCs), urothelial cells (UCs)	Porous microspheres, meshes, nanofibres	HA ¹⁷ , PGA, PLGA, PLLA	[85,86]
	hBSMCs, UCs	Meshes	PLAC ¹⁸ copolymer	[87]
Dermal tissue	HUVECs	Non-porous microspheres	PLGA	[88]
	Human Skin Fibroblast cells (HSFs)	3D porous scaffolds	CPCP ¹⁹ composite	[89]
	Human dermal fibroblasts (HDFs)	Porous microspheres	PCL	[52]
	Human keratinocytes (HaCaTs)	Hydrogels	Gelatin	[90]
	HSFs, HaCaTs	Sponges	Gelatin–chitosan	[91]
Nerve tissue	Nerve stem cells (NSCs)	Nanofibres	PANI/PG(PCL&Gelatin) ²⁰	[92]
	Human glioma cells (A-172 cells)	Nanofibres	PLGA	[49]
	Human glioma cells (A-172 cells)	Nanofibres	PLGA/PCL/PANI ²¹	[93]
	Human embryonic stem cell-derived neural crest stem cells (hESC-NCSCs)	Porous scaffold 3D printed from fibres	PPy-b-PCL ²²	[94]
	hMSCs	Macroporous hydrogels	PANI/PEGDA ²³	[95]

¹ PCL: Poly(ϵ -caprolactone); ² PLLA: Poly(L-lactide); ³ PEG: Polyethylene glycol; ⁴ PPO-PEO: Poly(propylene oxide-co-ethylene oxide); ⁵ PGA: Poly(glycolic acid); ⁶ pNIPAm-grafted PDMS: N-isopropylacrylamide-grafted polydimethylsiloxane; ⁷ PCL/HA: Poly(ϵ -caprolactone) and hydroxyapatite composites; ⁸ PHA: Polyhydroxyalkanoates; ⁹ PLA: Poly(lactide); ¹⁰ PLGA: Poly(lactic-co-glycolic acid); ¹¹ PEGT/PBT: Polyethylene glycol terephthalate/polybutylene terephthalate; ¹² PLA/HA: Poly(lactide) and hydroxyapatite composites; ¹³ PLGA/HA: Poly(lactic-co-glycolic acid) and hydroxyapatite composites; ¹⁴ Chitosan/HA: Chitosan and hydroxyapatite composites; ¹⁵ Gelatin/HA: Gelatin and hydroxyapatite composites; ¹⁶ Collagen/HA: Collagen and hydroxyapatite composites; ¹⁷ HA: Hyaluronic acid; ¹⁸ PLAC: Poly(lactic acid-co- ϵ -caprolactone); ¹⁹ CPCP: Collagen/PEG/Chi/PCL; ²⁰ PANI/PG(PCL&Gelatin): 10 and 15 wt % doped polyaniline (PANI) with poly(ϵ -caprolactone)/gelatin (PG) (70:30); ²¹ PLGA/PCL/PANI: Doped polyaniline (PANI) with poly(lactic-co-glycolic acid)/poly(ϵ -caprolactone); ²² PPy-b-PCL: Copolymer of polypyrrole and poly(ϵ -caprolactone); ²³ PANI/PEGDA: Polyaniline in polyethylene glycol diacrylate.

Table 2. Summary of commonly used polymeric materials as porous and non-porous spherical and microgel microcarriers of particular sizes together with respective cell types.

Microcarriers	Polymers	Sizes (μm)	Cell Types	References
Non-Porous Spheres	PCL ¹	261 \pm 71	Rat bone-marrow-derived stromal cells (rBMSCs)	[107]
	PLA ²	180–280	Rabbit chondrocytes	[108]
	PLGA ³	47–210	Sheep articular cartilage chondrocytes	[109]
	PLLA ⁴	100–200	Human OUMS-27 chondrosarcoma cells	[110]
	PLGA	30–80	White rabbit chondrocytes	[111]
	PLG ⁵	52–68	Calves chondrocytes	[112]
	PLG	52–199	Calves chondrocytes	[113]
	PLGA	80–90	Bovine chondrocytes	[114]
	PLLA	80–120	Rabbit ear chondrocytes	[115]
	PCL-b-PEO ⁶ or PCL	100–150	MG-63 human osteosarcoma cells	[116]
	PLGA	165 \pm 40.4	Human umbilical vein endothelial cells (HUVECs)	[99]
Porous Spheres	Collagen	\approx 250	Human and rat bone marrow-derived mesenchymal stem cells (hMSCs/rMSCs)	[117]
	Gelatin	260 \pm 50	Human mesenchymal stem cells (hMSCs) and nasal chondrocytes (hNCs)	[118]
	PCL	168–220	rBMSCs	[107]
	Blend of PCL and PLA	50–100	rBMSCs	[51]
	PLA	160–320	Rat bladder smooth muscle cells	[96]
	PEGT/PBT ⁷	130–180	Human nasal chondrocytes	[90]
	PLA	150–250	MG-63 human osteoblast-like cells	[89]
	PLGA	343 \pm 60	NIH 3T3 mouse embryo fibroblasts	[119]
	PLAGA ⁵	500–860	Human SaOS-2 line HTB-85	[120]
	PLGA	\approx 50	3T3 L1 mouse preadipocyte cells	[121]
	PCL	100–600	Human dermal fibroblasts (HDFs)	[52]
Non-Porous Microgels	PLGA	500–800	L929 fibroblasts and rat adipose-derived stromal cells (ADSC)	[53]
	pNIPAM ⁸	120 \pm 15	Rat hippocampal neuronal cells	[74]
	Collagen coated PS ⁹	125–212	Human bone marrow-derived mesenchymal stem cells (hBM-MSCs)	[122]
	Collagen-gelatin	80	3T3 fibroblast cells	[123]
Porous Microgels	Alginate	<200	Rat adipose-derived stem cells (rASCs)	[124]
	GelMA ¹⁰	\approx 90	NIH 3T3 mouse embryo fibroblasts/HUVECs	[73,75]
	PLGA-g-HEMA and MCS ¹¹	200–300	Human adipose stem cells (hASCs)	[125]
	Silk fibroin	503	MG-63 human osteoblast-like cells	[126]
	PEG ¹²	\approx 200	hMSCs	[127]

¹ PCL: Poly(ϵ -caprolactone); ² PLA: Poly(lactide); ³ PLGA: Poly(lactic-co-glycolic acid); ⁴ PLLA: Poly(L-lactide); ⁵ PLG/PLAGA: Poly(lactide-co-glycolide); ⁶ PCL-b-PEO: Poly(ϵ -caprolactone-b-ethylene oxide); ⁷ PEGT/PBT: Polyethylene glycol terephthalate/polybutylene terephthalate; ⁸ pNIPAM: Poly(N-isopropylacrylamide); ⁹ PS: Polystyrene; ¹⁰ GelMA: Gelatin methacryloyl; ¹¹ PLGA-g-HEMA and MCS: Poly(L-glutamic acid)-g-2-hydroxyethyl methacrylate and maleic anhydride-modified chitosan; ¹² PEG: Polyethylene glycol.

In order to manufacture the necessary modular tissue building block materials using different cells and the corresponding modular scaffolds, systematic investigations of the underpinning tissue development processes are crucially important. Particularly relevant are the mechanistic insights and biomimetics of the complex, reciprocal interactions among different cells, ECM components, and physical and biochemical signals within the native cell niches [31] (pp. 227–242). For example, cells are able to interpret and then react to their surrounding environment according to the material properties [31] (pp. 227–242, 261). However, cells do not interact directly with bulk materials but through surface materials such as layers of deposited proteins from the culture media or supplemented serum respectively [31] (p. 227). Research indicated that it only takes milliseconds for the proteins to be deposited on the material surfaces and that this process is also affected by the material topography, surface chemistry, and surface charge [31] (pp. 227–242, 261). The influences of the modular scaffolds and the surface materials on the cultured cells have to be accounted for in the preparation of each type of modular tissue building block. During the manufacture of the necessary modular tissue building block materials, it is also possible to co-culture two or three types of cells on suitable 3D scaffold(s), which has already been successfully demonstrated in the top-down tissue engineering approach [128,129]. For

example, human mesenchymal stem cells (hMSCs) and human umbilical vein endothelial cells (HUVECs) have been successfully co-cultured in 3D-printed PCL-based nanocomposite scaffolds and poly (d,l-lactic acid) (PDLLA) sponges within silk fibres [130,131]. It was demonstrated that the *in vitro* vascularisation was promoted via the co-culture. The proliferation rates of keratinocytes and fibroblasts were increased at the air–liquid interface when both cell types were co-cultured on a porous scaffold [132]. Wu and co-workers [84] found that the tri-culture of adipose-derived mesenchymal stem cells, human tenocytes, and HUVECs on PCL non-woven fabrics could enhance collagen formation in comparison with monocultures. The sequence and the required cultivation time for each modular tissue building block, as well as the temporal–spatial layer-by-layer assembly of these modular building materials, have to be investigated as they are the key factors for the bottom–up tissue manufacturing approach [15,22]. Therefore, growth factors, cytokines, extracellular vesicles, or specific mechanical or electric stimuli could be considered to orchestrate the complex temporal–spatial processes and maintain a high degree of differentiation in the tissue blocks [21,23,101,133–135] (pp. 149–178). Moreover, certain indicators or benchmarks, such as specific cell/tissue morphologies or biochemical compounds, have to be established to evaluate the appropriate timing for the gradual tissue reconstruction processes [15,22]. Due to the gradual increase in tissue size and complexity during the assembly, the supply of the cells with sufficient nutrients and oxygen, and the removal of metabolic wastes and carbon dioxide have to be considered [11,21,31] (pp. 398–400, 471–475). However, the internal mass transfer within the cultured assembled tissues can be achieved via convection, by media perfusion through the gradually reconstructed vasculature, and then diffusion within 100 to 200 μm [11,21].

In addition to the aforementioned issues, there are also several inherent limitations to overcome for the successful implementation of the bottom–up DE approach. Firstly, there are high contamination risks. Due to the comparably long time periods for both the preparation of the necessary modular building block materials and the temporal–spatial assembly of the target tissues, the bottom–up DE approach usually bears relatively high contamination risks. Therefore, apart from scaffold and culture media sterilisation, strict aseptic techniques are needed for the preparation of the modular building blocks and the tissue assembly processes. Additional strategies, such as the use of a bacteriophage to prevent specific bacterial infection or contamination, might be considered [136–138].

Secondly, there is the low mechanical strength and coalescence of the assembled tissues. Due to the use of modular tissue building blocks, the assembled tissues might be mechanically fragile and, thus, less stable than the tissues prepared via the top–down TE approach using large scaffolds. Therefore, it might be necessary to exploit suitable mechanisms to integrate the modular building blocks with sufficient tensile strength. Despite the potential of methods such as sheet stacking and 3D tissue structure bioprinting using cell ink to assemble dual building layers, the suggested method aims to employ cell–cell interactions and cell migration between the tissue building blocks for the tissue assembly [139,140]. The integration of the modular tissue building blocks could potentially follow a similar mechanism as the aggregation of microcarriers during cell cultures conducted in bioreactors. Research indicated that one of the bead-to-bead cell transfer mechanisms is cell bridging between two microcarriers [141]. As similar cell migration or cell bridging could occur among the assembled modular building blocks, further study of the cell transfer, cell bridging between the microcarriers or different modular scaffolds, and the ECM components (e.g., collagen) produced by these bridging cells might be useful to increase the tensile strength or the mechanical stability of the gradually assembled tissues.

5. Conclusions

The top–down TE approach has so far been obstructed from achieving the promised successes in terms of clinical applications, which can be attributed to one-stage seeding and culturing of few dominant cell types in scaffolds with equivalent sizes and morphologies as the target tissues. Due to the lack of vascular systems, mass transfer within the cultured

tissues is heavily dependent on diffusion. Consequently, the top-down TE approach is suitable for the reconstruction of tissues for non-clinical applications and a few engineered tissues with relatively simple anatomic structures, such as skin and cartilage, for clinical applications. In contrast, the bottom-up DE strategy aims to mimic natural tissue development processes by culturing multiple cell types in corresponding smaller modular scaffolds, and then assembling these modular tissue building blocks layer-by-layer into fully functional tissues. Due to the use of micro-sized modular scaffolds and the reconstruction of vascular systems, mass transfer within the gradually expanded tissues is not a limiting factor. However, in order to successfully implement the bottom-up DE approach, it is crucially important to develop standardised procedures for the isolation and expansion of multiple cell types, the manufacture of modular scaffolds with the required sizes and morphologies, and the temporal-spatial assembly of different functional tissues or organs. Moreover, research efforts and suitable technologies are also needed to investigate and overcome the inherent challenges associated with this bottom-up DE strategy. Firstly, mechanistic insights into the underpinning biological processes, such as the cell-cell and cell-material interactions, are crucially important for the preparation of the diverse modular tissue building blocks. Secondly, specific sterilisation and aseptic techniques are required to tackle the high contamination risks induced by the elongated temporal-spatial tissue reconstruction processes. Thirdly, as multiple modular scaffolds are assembled layer-by-layer to provide the physical support for the cultured cells, the mechanic stability of the reconstructed tissues might be another problematic issue to be considered.

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