Survey

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The fabrication-assembly challenge in tissue engineering

Die Fabrikations-Assemblierungs-Herausforderung im *Tissue Engineering*

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Abstract: The generation of human tissue through tissue engineering has been pursued for decades and is still associated with major challenges. The comparison between natural tissue formation and engineering concepts helps to understand the fabrication-assembly challenge of tissue components and provides approaches to solutions for this multidisciplinary field.

Keywords: tissue engineering; engineering concepts; assembly; building blocks; spheroid

Zusammenfassung: Die Herstellung menschlichen Gewebes durch *Tissue Engineering* wird seit Jahrzehnten betrieben und ist immer noch mit großen Herausforderungen verbunden. Der Vergleich zwischen natürlicher Gewebebildung und Konzepten der Ingenieurwissenschaften hilft, die Herausforderung der Fabrikation und des Zusammenbaus von Gewebebestandteilen zu verstehen und liefert Lösungsansätze für dieses multidisziplinäre Feld.

Schlagwörter: *Tissue engineering*; Konzepte Ingenieurwissenschaften; Zusammenbau; Bausteine; Sphäroid

1 Introduction

Why do we speak about tissue engineering when we speak about the generation of human tissue using laboratory-based techniques? The purpose of tissue engineering is manifold: for therapeutic purposes, it is performed outside the body before application to patients or intracorporeally. Tissues are also generated in the laboratory for drug testing and research purposes. The terms "engineering of living tissues" and "tissue engineering" were coined in the 1980s by *Yuan-Cheng Bertram Fung* – an engineer with an aeronautics background whose personal interest in mechanical forces and physical phenomena of living tissues led him to enter this scientific field – and by other scientists in the context of *Fung's* U.S. National Science Foundation (NSF) proposal and at NSF meetings [1]. In a 1993 review, *Langer* and *Vacanti* later defined tissue engineering as "an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function" [2].

This article focuses on the *de novo* generation of tissues – the most complex task in this field as it is not based on any pre-existing tissue – and does not address the creation of isolated cells or cell substitutes or tissue-inducing substances per se, categories included in *Langer* and *Vacanti's* tissue engineering definition. Although there appears yet no consensus on a definition, and narrower or broader definitions can be found in the literature, one aspect frequently mentioned is the application of engineering principles to generate tissues. *Fung*, a trained engineer, was not specifically referring to engineering principles when he used the term "engineering of living tissues". "Principle" refers to basic rules and one can assume that scientists with a background in biology, medicine or chemistry who refer to "engineering principles" in the context of "tissue engineering" either have engineering processes in mind or are thinking of what I would consider engineering concepts: standardization, abstraction, function separation, function integration, modular design, design for assembly, robust design, up- and down-numbering, up- and down-scaling (Table 1). Main, superordinate engineering processes include design & development, manufacturing/production, and application/use. It is important to note that development already involves fabrication (the creation of components) and assembly, i.e. processes in which various components and subassemblies (modules) are built together to form a complete product (system). In fact, at one of the early NSF meetings in the 1980s, NASA scientist *Maurice Averner*

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Table 1: Engineering concepts[a].

Concept	Explanation	Aim
(1) Standardization	Common agreements on materials, components, processes, criteria, terms, and other categories	To facilitate reproducibility and interchangeability of products and components as well as their mass production
(2) Abstraction	Generalization, simplification by omitting details to find a superordinate context	To reduce complexity and to enable the emergence of essential features
(3) Function separation	A clear assignment of functions to different components	To simplify design, fabrication, repair processes, and functional independence
(4) Function integration	Components harbor more than one function	To achieve a higher functionality within a smaller space and/or with a lower amount of material
(5) Modular design	Subdivision of a system into smaller entities called modules which can be independently created, modified, replaced, or exchanged with other modules or between different systems	To make complexity manageable, enable parallel work, and accommodate future uncertainty
(6) Design for assembly	Consideration of the properties of components/parts in accordance with assembly processes	To minimize the number of components/parts, the assembly time and cost, and in some cases also facilitate disassembly
(7) Robust design	The system fulfils one predefined function with accepted functional quality even under uncertain resources or upon disturbances by uncertain external influences; robust design concepts include various other concepts such as (1), (3), (5)	To gain robustness, i.e. reduce unwanted variation in functional performance and accommodate uncertainty
(8) Up- and down-numbering	Operation of multiple functional entities in parallel	To immediately increase or decrease and thus adapt the productivity of a system
(9) Up- and down-scaling	Operation of an entity with increased or reduced size compared to the size of the test model; development of a model series with similar elements but different sizes	To change the productivity of a system

[a]These concepts are relevant for various engineering processes.

proposed to define tissue engineering as "the production of large amounts of functional tissues for research and applications through the elucidation of basic mechanisms of tissue development combined with fundamental engineering production processes" [1]. Having addressed the possible misunderstanding of the term "engineering principles" in the field of tissue engineering, let us focus on the engineering concepts, all of which are relevant for different engineering processes, superordinate ones, mentioned above, and those integrated into them. The concepts outlined in Table 1 are considered major ones in engineering, although this list is by no means exhaustive or exclusive (personal communication with *Peter Pelz*, Mechanical Engineering, TU Darmstadt, Germany).

To understand process challenges in tissue engineering one needs to understand the components tissues are made of and how tissues are normally – naturally – formed from these components. Human tissues can be considered as systems consisting essentially of cells and their extracellular products, with extracellular matrix and fluid-filled hollow structures as intercellular spaces, representing the main components in terms of tissue architecture. Four basic human tissue types are distinguished: epithelial, connective, muscle, and nervous tissue, each consisting of specialized cell types arranged in precise patterns resulting in recognizable, defining tissue morphologies. Altogether, human cells can be classified into several hundred to thousand basic types, depending on the classification method. The extracellular matrix comprises various molecules, predominantly sugar chains and proteins, which are located outside cells. It not only serves as physical support structure, but also fulfils fundamental regulatory cell biological functions via cell-matrix interactions and as a dynamic reservoir of cell regulatory factors [3]–[5]. Epithelial cells are typically positioned very close together with direct cell-cell contacts and very little extracellular matrix in-between, whereas

connective tissue contains large amounts of extracellular matrix around the cells. Fluid-filled hollow structures include the cardiovascular system and structures that transport and store various secreted molecules, e.g. the cerebrospinal fluid-filled cerebral ventricles, the bile ducts and gallbladder that transport and store bile, and various glandular systems.

How are human tissues naturally formed from these components? I will consider two different types of natural tissue formation: tissue formation during human development and tissue regeneration in the postnatal period, more precisely, from already developed and mature human tissue.

2 Tissue formation during human development

The spatial arrangement of specific cell types within a "final", i.e. mature, human tissue as a product of development is the result of a months-long process that begins with a single cell, a fertilized egg cell (oocyte). Even after birth, several human tissues have not yet acquired their permanent basic, recognizable architecture. For example, a large part of the human brain, the cerebellum, has a prolonged maturation phase with an outer layer of immature cells – dividing progenitor cells – that does not disappear until the end of the second year after birth. The developmental steps that take place between the stage of a fertilized egg and a mature tissue include an enormous cell production through cell divisions, and the production of specific cell types – identities – from various precursor cells, often involving several intermediate cell stages. During phases of cell production by division and the formation of specific cell identities, cells change their position within the developing tissue via different types of movements, both of individual cells and of cells held together in groups. The spatial arrangement of cells in a developing tissue is thus subject to constant change, by cellular assembly and disassembly. In addition, the removal of individual cells and even tissue structures consisting of many cells is a natural step during development. During the development of the human kidney, for example, two precursor stages of this organ are formed, the tissue of which disappears completely or partially before the final kidney is formed. Finally, the composition of the extracellular matrix surrounding various cell types is different and changes during development. Although a role of the extracellular matrix in the formation of tissue structures in vertebrates is generally acknowledged, the exact composition of the extracellular matrix, its alteration and function during the formation of specific human tissues is not yet sufficiently understood.

3 Tissue regeneration in the postnatal period

In several mature human organs, tissue components can be newly formed. This regeneration can be a permanent, physiological process to replace cells that are continuously lost, or it occurs in the case of tissue injuries that lead to pathological cell loss. In contrast to tissue development, this type of natural tissue formation starts from an established, mature tissue consisting of specific cell types and extracellular products in a well-defined spatial arrangement. For example, the epithelial cells that form the outer section of human skin, the epidermis (Figure 1A), and the epithelial cells that line the intestine, are permanently lost by a

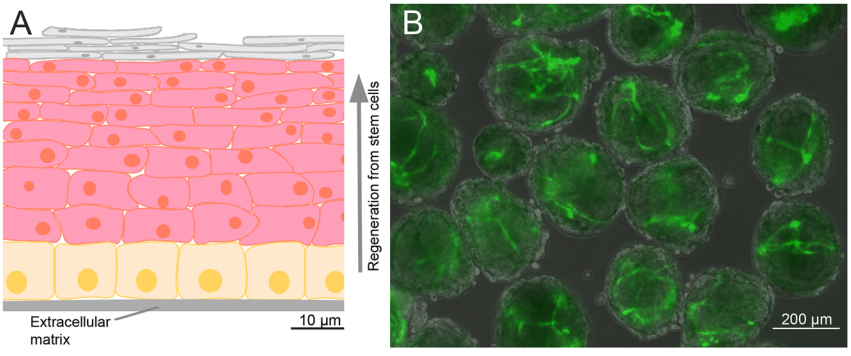

Figure 1: Schematic representation of a relatively simple natural tissue architecture and microscopic image of laboratory-generated tissue building blocks. (A) Architecture of the epidermis, the upper section of the skin, consisting of multiple layers of cell-cell contact-forming keratinocytes on an extracellular matrix that separates the epidermis from the underlying dermis. Note that the extracellular matrix layer is shown much thicker than it actually is in order to make it visible. (B) Multicellular spheroids that can be used as tissue building blocks. Endothelial cells forming a network in human liver spheroids are visible due to their production of a green fluorescent protein.

physiological process of controlled cell death of the most superficial cells or by cell death upon injury and are replaced by newly produced ones derived from stem cells located at the base of these epithelial layers [6]–[8].

4 Differences between the formation of natural tissue and the generation of engineered products

Having recapitulated – in a very simple way – the formation of tissue from components, focusing on cellular ones, the fundamental differences between the formation of natural tissue and the generation of engineered products become immediately apparent: in engineering, different components and modules can be produced separately, only begin to interact with each other during assembly, and typically do not undergo major changes in their properties/identities and position during assembly. The assembly of electronic devices in electrical engineering or machine modules in mechanical engineering immediately brings a typical engineering process to mind. Few engineering processes deviate from this, e.g. the additive manufacturing of an entire product. In biological systems, however, i. individual components (different cell types and their extracellular products as basic components) strongly influence the production and properties of other components during their own production process, ii. the production of the components and their assembly are closely linked, and iii. the properties and position of the biological components change during their assembly into tissues, and this also includes disassembly and removal steps.

5 Challenges in *de novo* tissue engineering related to differences between biology and engineering

These fundamental differences are the root of major challenges in *de novo* tissue engineering and they primarily relate to one engineering concept and two engineering processes: the design for assembly and the fabrication (creation of components) and assembly process. In addition, we lack a complete molecular and cellular understanding of all required natural tissue components and natural tissue formation processes, which is a general design problem.

At this point readers may wonder: why can't we simply follow an assembly process as applied in engineering, namely precisely position prefabricated final biological components, i.e. cells and extracellular matrix materials, according to their arrangement in natural tissues, e.g. using 3D bioprinting methods?

For very simple tissues or tissue segments, mainly consisting of one basic cell type and possibly different cell stages derived from it, and a simple spatial arrangement of the cells, this is feasible. For example, the outer section of human skin, the epidermis, consists mainly of one type of epithelial cells, keratinocytes, arranged in multiple cell layers, with very little extracellular matrix between the cells, but a layer of extracellular matrix underneath (Figure 1A). In this case, it is sufficient to plate cells on a suitable extracellular matrix material. Under specific culture conditions, the cells in this monolayer assemble with their neighboring cells via cell-cell adhesion and also give rise to new keratinocytes, which arrange themselves as multiple layers above the primary positioned layer [9]. Here, the cellular components (keratinocytes) perfectly self-assemble based on surface properties, including cell adhesion molecules that strongly and selectively bind to each other, and newly produced cells also possess such cell adhesion molecules, so that a stable multilayered epidermis is formed. The self-assembly of identical or similar cell types based on surface properties is a key process in tissue formation, and was demonstrated early on in elegant experiments with embryonic amphibian cells. Mixing single cells from two or three embryonic tissues (germ layers) led to the spontaneous sorting of each of these cell types into separate tissue areas, essentially recapitulating their basic spatial arrangement in the embryo [10].

However, if one wants to generate more complex tissues composed of different cell types and with a more complex spatial cell arrangement, initial positioning, e.g. by precise 3D bioprinting, may be feasible; however, to form a stable tissue that can be maintained, the cells must form permanent connections that are only disassembled in the context of a natural, i.e. desired, tissue process. Assembly involves steps that position components, but also steps that ensure they are kept together. In the latter case, the direct interface between the components must be suitable or an additional interface must be interposed to connect them. An additional interface can also be used to prevent direct interaction and mixing between the components.

In natural tissues, areas consisting mainly of extracellular matrix, also serve to stabilize cell formations. For example, a thin layer of extracellular matrix, the basement membrane, separates the superficial epithelial part of

tissues from the underlying connective tissue part and at the same time holds these two areas together. Examples include the upper section of the skin, the epidermis, and its lower section, the dermis, as well as the epithelial cells of the intestinal lining and their underlying stromal cells. Moreover, blood vessels, i.e. very complex three-dimensional arrangements of cells, are surrounded by an extracellular matrix – a thin basement membrane in case of most of the smallest blood vessels and a thicker, loose outer connective tissue layer in the case of larger vessels.

Fabrication-assembly challenges that tissue engineers face when generating complex tissues *de novo* are described in more detail below.

5.1 Tissue engineering challenges related to the fabrication of components

The number of different cell types in tissues can be very high – for example, depending on how they are classified, approximately 14 basic cell types can be distinguished in the human kidney [11] and more than 3000 in the human brain [12]. For various reasons, it is currently not possible to generate, maintain and expand all these cell types in the laboratory, e.g. from pluripotent stem cells or from human donor tissue. Many mature, i.e. differentiated cells, no longer divide and their number can therefore not be increased by cultivation in the laboratory. There is also a lack of protocols for the generation of certain, fully mature cell types from less mature precursor cells, including human pluripotent stem cells or donor-tissue derived stem or progenitor cells. This fabrication problem is related to the fact that we do not know enough about the natural processes of tissue formation and that certain cell types are not produced and maintained in isolation, but these processes take place in a multicellular tissue context, as explained above (i. – iii., Section 4), which is difficult to recapitulate in the laboratory.

Cells generated in the laboratory often do not have the functional maturity of their natural counterparts in an adult human tissue, and furthermore, the culture conditions in the laboratory can alter the state and properties of cells. Finally, the mass of cells required to generate a human tissue or even an organ is very high.

Although cells themselves produce extracellular matrix, it takes time until assembled cells have produced an amount comparable to normal tissue, and their initial functionality and assembly may require extracellular matrix to be included as a tissue component. Natural extracellular matrix can in principle be made available for tissue engineering by producing it from cells in culture or by deriving it from decellularized cadaveric tissue [13]. However, the exact composition and structure of the extracellular matrix in tissues is largely unknown, and in complex tissues, it varies at different locations in the tissue and at different time points during tissue development and regeneration. In addition, the availability of human cadaveric tissue as a source of extracellular matrix is limited, and in therapeutic applications, the use of both human and animal tissue for the isolation of natural extracellular matrix is associated with medical risks.

5.2 Tissue engineering challenges related to the assembly of components

The immediate interface (surface properties) of cells generated and maintained in the laboratory may differ from those in adult tissues – particularly the state of the interfaces as they exist at the time of assembly during natural tissue formation. Furthermore, even cell types that are classified as the same, for example endothelial cells – cells that line the inner surface of blood vessels and represent the main cellular component in smallest vessels – show tissue-specific differences. Endothelial cells in different organs are not only functionally different, but can also interact differently with the surrounding cells in different tissues, leading to an assembly problem.

In tissues of the nervous system, not only local cell-cell interactions are important for functionality, but also cellular connections over long tissue distances between very specific cell types. Such cellular connections are the result of developmental or regeneration processes that cannot be formed at once in a single assembly step.

Finally, a technical challenge is the simultaneous and high-resolution printing/positioning of different material types (different cell types and extracellular matrix types or their mimics). In addition, bioprinting processes of single cells are currently too slow to produce tissue on a centimeter scale [14].

6 What are possible solutions to these fabrication-assembly challenges in tissue engineering?

Some solutions can be derived from engineering concepts mentioned in the beginning of this article: abstraction, function separation, function integration, modular design, and design for assembly (see also Table 1). An example of abstraction and modular design is the use of tissue building blocks with reduced complexity: three-dimensional formations of hundreds to thousands of cells in cell culture (spheroids and organoids, Figure 1B) that can be deposited

in a spatially controlled manner and assemble into a larger tissue formation [14], [15]. The term spheroid is mainly used for three-dimensional cell formations that result from the aggregation and division of cells. Spheroids are typically structurally and functionally less complex than organoids, which develop from stem cells through self-organizing processes and recapitulate specific structural and functional properties of an organ.

Several of the engineering concepts can be found in biohybrid systems, i.e. systems made of cells and synthetic, artificial materials. One example is an implantable biohybrid system with kidney functionalities [16]. In this case, abstraction is achieved by leaving behind the complicated architecture of the kidney and focusing on the main functions realized with simple geometries: instead of building an ultrafiltration barrier based on cells and extracellular matrix arranged as a tangled network, the filtration function is executed by flat silicon nanopore membranes. Function separation and function integration are implemented in the design and already partially realized: filtration and reabsorption functions can take place in two different compartments of the device, which are separated by a Transwell® insert. Functional integration is achieved by the silicon nanopore membrane: ultrafiltration and immunoprotection. Multiple sections and layers – a modular design – are included, and the components of the device are designed for assembly, which in turn enables a simple disassembly for analyses of the integrated cells and materials after use. The design for assembly also includes a hemocompatible U-shaped blood flow path with inlets and outlets that facilitate the connection to an artery and a vein in the human body.

Other solutions are based on the exploitation of natural cell processes, with a provision of minimal external influences to control these. For example, instead of precisely positioning single cells, one can let cellular self-organization processes of cells take place, e.g. supported by scaffolds with predefined shape and material properties [17], [18] and/or by imposing artificial gradients of biological signaling pathways to recapitulate a morphogen-driven spatial arrangement of cell types [19]. Moreover, microcarriers loaded with tissue-specific fibroblasts can be used to facilitate the production of a natural extracellular matrix and serve as connective tissue building blocks, with the carrier material subsequently removed by degradation [13].

7 Conclusions

Regardless of which solution one aims for – engineering concept-based approaches as well as biological process-based ones and certainly combinations of both – all benefit from an integration of disciplines. Combining the two worlds – natural tissue formation and engineering for the purpose of tissue engineering – requires novel elements, including components and technologies, with novel properties and functionality and thus a new world. These novel elements have their roots in natural tissues and others in synthetic materials designed to exert or to influence tissue functions. The scientists in this new world are likewise diverse – with roots in different worlds or born into this new world. This brings us back to *Yuan-Cheng Bertram Fung* who introduced the term "engineering of living tissues" in the 1980s, and about 20 years later, in an extensive oral history recorded for the Institute for Electrical and Electronics Engineers (IEEE) in 2000, said: "After many years in the field, I really think that an interdisciplinary area is not just the one area plus another. It's the new product in between, which is neither of the mother fields. The interesting part is the new in-between part" [20].

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