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Tissue engineering and surgery: from translational studies to human trials

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Abstract: Tissue engineering was introduced as an innovative and promising field in the mid-1980s. The capacity of cells to migrate and proliferate in growth-inducing medium induced great expectancies on generating custom-shaped bioconstructs for tissue regeneration. Tissue engineering represents a unique multidisciplinary translational forum where the principles of biomaterial engineering, the molecular biology of cells and genes, and the clinical sciences of reconstruction would interact intensively through the combined efforts of scientists, engineers, and clinicians. The anticipated possibilities of cell engineering, matrix development, and growth factor therapies are extensive and would largely expand our clinical reconstructive armamentarium. Application of proangiogenic proteins may stimulate wound repair, restore avascular wound beds, or reverse hypoxia in flaps. Autologous cells procured from biopsies may generate an 'autologous' dermal and epidermal laminated cover on extensive burn wounds. Three-dimensional printing may generate 'custom-made' preshaped scaffolds – shaped as a nose, an ear, or a mandible – in which these cells can be seeded. The paucity of optimal donor tissues may be solved with off-the-shelf tissues using tissue engineering strategies. However, despite the expectations, the speed of translation of *in vitro* tissue engineering sciences into clinical reality is very slow due to the intrinsic complexity of human tissues. This review focuses on the transition from translational protocols towards current clinical applications of tissue engineering strategies in surgery.

Keywords: angiogenesis; cell engineering; 3D printing; matrices and scaffolds; plastic and reconstructive surgery; tissue engineering; tissue regeneration.

Introduction

Tissue engineering attempts to exploit the cells' reproductive potential and harness the body's intrinsic capacity for healing and regeneration [1]. These cells produce growth factors and cytokines, which function as architects and coordinators of the regenerative and repair processes. Interaction and crosstalk of cells within the bioreactor or within a three-dimensional (3D) scaffold may lead to tissue and subsequently organ regeneration. As defined by Robert Langer in 1993, tissue engineering comprises (i) the isolation and manipulation of individual cells or cell substitutes, used for therapeutic infusion; (ii) the identification of tissue-inducing substances, such as growth factors, and their appropriate delivery to their target; and (iii) placing cells on or within matrices, which permit the delivery of nutrients but protect the cells from immunological destruction [2]. Non-resorbable scaffolds or matrices should be biocompatible, whereas resorbable scaffolds should not elicit a detrimental inflammatory reaction; allogenic cells will only have a temporary effect on protein release until rejected by the immunological response, whereas autologous cells may be integrated in tissues permanently and contribute to the wound microenvironment [3, 4]. Tissue engineering, therefore, is a promising and blossoming field that encompasses a wide variety of bioactive agents that are incorporated into scaffolds with the aim of restoring a region with defect [5–7]. The majority of these agents currently are analysed in a translational bench-to-bed setting. Orlando et al. listed 160 patients who received organs manufactured from autologous cells that were seeded on a supporting scaffolding material with no need for immunosuppression after implantation [8]. The majority of these (n = 106) were epithelial cell sheets for treating corneal lesions; 25 patients were treated with a vascular conduit based on autologous bone marrow mononuclear cells seeded on a

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bioresorbable scaffold; other patients were treated with 'bioengineered' tissues repairing the urethra, trachea, and bone. Corneal repair with cell- and scaffold-based treatment seems promising thus far. Many of the vascular conduits, however, developed thrombosis and the 'tissue-engineered' tracheal conduits collapsed. Ad hoc tissue engineering strategies cannot fulfil the high expectations and hopes yet, due to the intrinsic complexity of tissue regeneration [8].

Previous experiences with solid organ and composite tissue transplantation have shown the importance of a solid international registry. Registries allow strict monitoring and scrutiny of follow-ups and outcome of such technologies, and are a critical instrument for the evaluation and establishment of their risk-to-benefit ratio [9].

More simply organised animals are capable of regenerating larger parts of their bodies during adulthood, whereas animals with higher organisation and complexity, such as mammals, may show formidable regenerative capacities that are lost after birth. Humans seem radically impaired *ex utero* for reasons that remain mysterious [8].

The greatest challenge for researchers in tissue engineering and regenerative medicine probably is the understanding of reasons why the switch that determines the regenerative ability of mammals is ultimately turned off.

Thirty years have passed since tissue engineering was defined as a new and promising platform for tissue regeneration. We may need another 30 years to come significantly closer to the required needs and aims. However, on the road, novel and innovative tools may be the incentives of the learning curve.

In this review, we provide a perspective on the current strategies used in translational tissue engineering.

Mechanisms of action in tissue engineering protocols

The advent of tissue engineering and regenerative medicine strategies has fuelled another paradigm shift in our approach to biomaterials design towards complex and smart materials that interact with cells to direct their biological response and can even be responsive to cells [5]. Tissue engineering strategies are based on three pillars: (i) production and implantation of (vascularised) scaffolds or matrices, (ii) cultivation and implantation of neo-tissue derived from (stem) cells, and (iii) implantation of cells in matrices [7].

Scaffolds and matrices

Scaffolds provide a suitable 3D niche for the cells to grow, proliferate, and differentiate. Due to the varying degrees of porosities, scaffolds provide an excellent vehicle for the cells for the regular supply of nutrients and oxygen (Figure 1).

Several types of scaffolds have been reported. Natural biomaterials, such as collagen, chitosan, cellulose, etc., are polysaccharide or protein in nature and resemble the natural extracellular matrix (ECM). Synthetic biomaterials such as polycaprolactone (PCL), polylactic acid, polyethylene-glycol, and polyvinyl pyrrolidone enhance the strength of the biomaterial [10].

The optimal bioengineered tissue construct must have a morphological structure similar to the authentic tissue it must replace. The bioconstruct should be autologous to optimally integrate without rejection, 3D to bridge deep defects, porous to allow cell migration, bioinductive for cells to proliferate and to secrete ECM components and growth factors, hospitable for vascular sprouts to develop within the construct, and stimulate vasculogenesis and optimize tissue integration [3, 6]. When selective absorption of the matrix occurs, its composition should be maintained and the absorption process should not result in deposition of toxic side products that may negatively influence the mechanism of action of cells and tissues. This extensive list of required features is self-explicatory as to why an off-the-shelf preshaped vascularised scaffold has not been developed yet. It is the collaboration between material scientists, clinicians, and molecular biologists that forms the multidisciplinary environment required to design a biomimetic smart scaffold [5]. Some of the leading challenges in the field of tissue engineering consist of cell-to-scaffold interaction, accelerating cellular proliferation and differentiation, and especially vascularisation of the engineered tissues [10].

Cells and stem cells: the substrate

Donor cells can be obtained from a small tissue biopsy. Cell cultivation allows expanding the number of cells. However, adult cells may be limited in growth potential, and they may differentiate due to changing environmental factors or rapidly deteriorate by senescence [3].

Stem cells have the unique ability to self-renew, proliferate indefinitely, and create offspring that differentiates into specialised, mature tissues by asymmetric replication [4]. 'Stem cells', however, represent a miscellaneous group of cells with different levels of differentiation

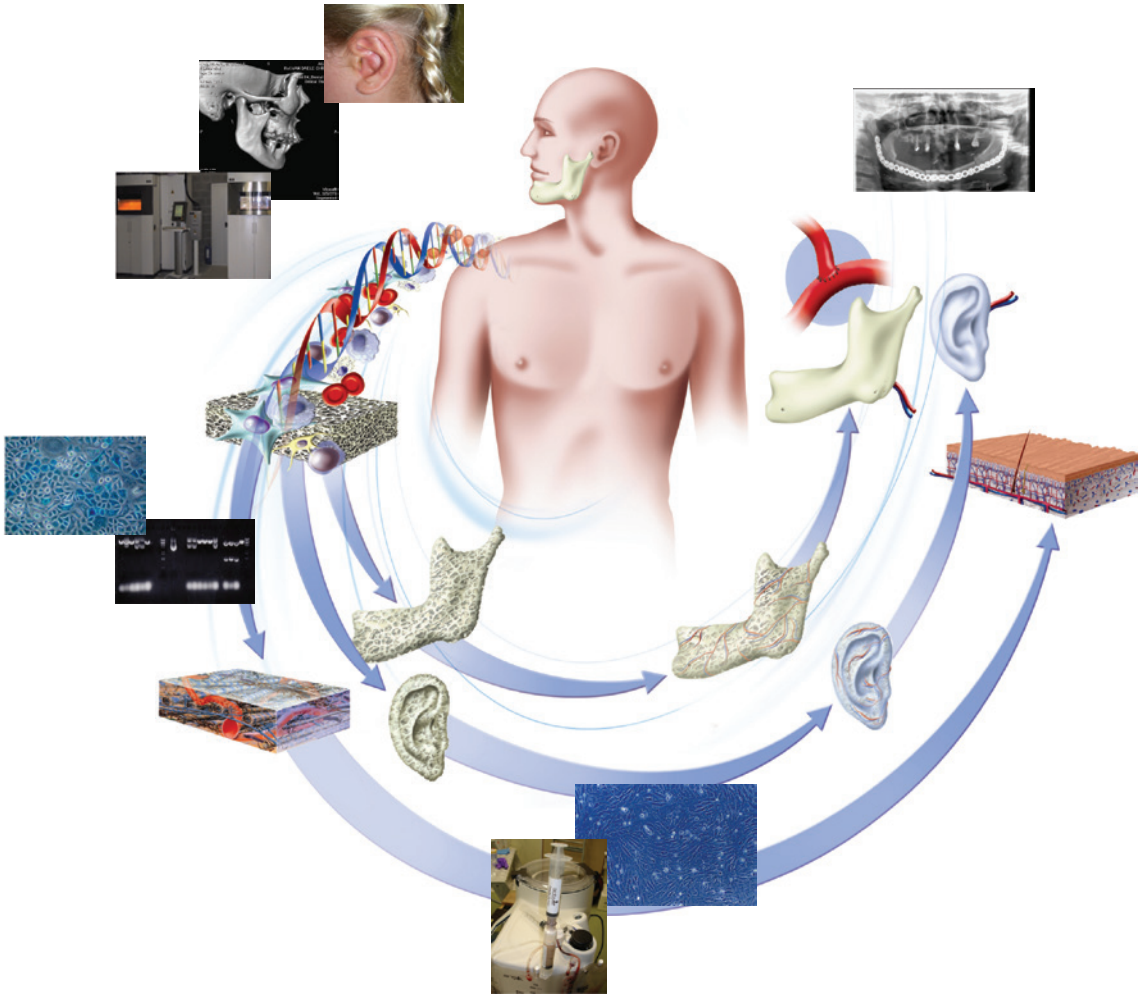

Figure 1: The tissue engineering concept.
Data from CT scans are used to create a 3D matrix by rapid prototyping technology. The porous-shaped matrices serve as scaffolds for cell seeding. Autologous cell cultures proliferate and migrate further in the matrix. A vascular matrix develops, stimulated and guided by growth factors, cytokines, and adhesion molecules. A macroscopically developed vascular supply represents the hurdle stone in this tissue engineering strategy, and is the current focus of research and translational studies. From Vranckx JJ. *Ex vivo* gene transfer to full thickness wounds. A platform for autologous tissue engineering for tissue repair. ISBN 9789082280609, 2014.

potential. Out of a clinical reconstructive perspective, it is essential that cells used in a clinical setting originate from autologous tissues if permanent integration is the aim. Embryonic stem cells per definition are allogenic. For long, adult stem cells were thought to reside mainly in bone marrow. The last 15 years' novel sources were intensively investigated for immediate use in a clinical reconstructive setting [11].

Pluripotent stem cells for use in autologous conditions

Induced pluripotent stem cells (iPSCs) In 2006, Takahashi and Yamanaka reported how adult human dermal fibroblasts or other human somatic cells could be directly reprogrammed by the introduction of four transcription factors (Oct4, Sox2, Klf4, and cMyc). iPSCs possess a differentiation potential equal to human embryonic stem cells: they are capable of differentiating into tissues of all three germ cell layers, given the correct culture conditions, growth factors, and genetic milieu [12].

As these cells are derived from adult tissues, there are no ethical issues. Moreover, unlike embryonic stem cells, they may be procured from autologous sources. Nevertheless, iPSCs also carry the risk of teratoma formation and genetic instability. Therefore, more studies are required before iPSCs could be used in a clinical reconstructive setting that is often subjected to hostile – and triggering – conditions such as radiotherapy, poor vascularisation, and severe scarring [13].

However, these data also demonstrate that adult tissues contain pluripotent stem cells that may exert their function once activated *in situ* [14]. The discovery of iPSCs is very exciting and had an enormous impact on the field, evidenced by the attribution of the 2012 Nobel Prize in Medicine 'for the discovery that mature cells can be reprogrammed to become pluripotent'.

Multipotent stem cells (MSCs) for use in autologous conditions

MSCs Adult organ-specific stem cells can be isolated from several tissue sources, including bone marrow, adipose tissue, peripheral blood, skeletal muscle, central nervous system, retina, and epithelia of skin and digestive tract. Especially MSCs from lipoaspirates are extensively investigated and used daily in a clinical reconstructive context, to soften scars and fill defects and create volume [15–17].

All organ-specific stem cells preferentially generate differentiated cells of the same lineage as their tissue of origin. However, adult stem cells from various organs may also contribute to the regeneration of dissimilar organs with stem cells crossing germ layers by 'trans-differentiation'. One of the important extracellular signals that controls stem cell fate is the secretion of growth and differentiation factors [18, 19]. In adverse clinical conditions, poor vascularisation creates a hypoxic environment, for instance after redo operations, chronic wound healing, radiotherapy, or severe scarring. This environment may trigger oncologic sleeper cells or modify growth factor profiles that influence the mechanism of action of MSCs. Therefore, vigilance is essential also when using adult MSCs, as chromosomal instability may lead to malignant transformation [20].

Unipotent progenitor cells

Blood outgrowth endothelial cells (BOECs) BOECs are 'late outgrowing endothelial-like colonies' of collagen-adherent cells that show much higher proliferation capacity than early outgrowth endothelial progenitor cells (EPCs) [21, 22]. BOECs stimulate angiogenesis actively by incorporation into the host vessel, and passively by secretion of proangiogenic growth factors. BOECs can be isolated from umbilical cord blood but also interestingly from a simple adult intravenous blood donation, with the former showing higher proliferation potential but also susceptibility to karyotypic aberrations [22–24]. As a result, BOECs can be obtained in an autologous fashion, which may show its benefit in a clinical setting, in tissue engineering strategies, and in reconstructive surgery to promote perfusion of hypoxic tissues or isolated flaps [24, 25].

Implantation of cells in matrices to generate full-thickness constructs

A controlled induction of vascular networks by *ex vivo* or *in situ* strategies is the determinant of the timing in which tissue engineering will be incorporated successfully in our clinical practice.

These phenomena could be induced by an *in situ* or *ex vivo* approach [3, 4]. With *in situ* cultivation of cells and tissues, the patients' own body is used as an incubator. Such a strategy sounds logical, as *in situ* cell populations that respond to local – autologous – biomolecular cues produce the proangiogenic and matrix-forming growth factors. However, infiltration of undesired tissues such as fibroblasts may generate undesired granulation tissue, which modifies the shape and content of the tissue-engineered construct, and the obligatory use of shaped biocompatible implants as a guiding scaffold make this approach not evident at all.

An *ex vivo* approach to overcome the invasion of undesired tissues would require an immediate reperfusion once the regenerated tissue would be 'transplanted' *in situ*. This would be clinically feasible if macroscopic blood vessels could be cultivated within the construct *ex vivo* and subsequently connected to a vascular pedicle – artery and vein – of the recipient site by microsurgical techniques. Thus far, this is science fiction, as so far blood vessels cannot be generated *ex vivo*.

Fundamental role of vascularisation

Full-thickness tissue constructs consisting of cell-seeded matrices can only be implanted successfully in a clinical reconstructive setting when supplied by vascular networks. The key limiting factor in the evolution of tissue engineering is the absence of vascular networks that are capable of distributing oxygen and nutrients within the matrix. Nutrient supply and waste removal in limited-thickness engineered tissues, such as skin, can initially be overcome by diffusion, until neovascularisation takes over. Already in 1973, Folkman demonstrated that cells only survive within a 3-mm distance from a nutrient source [26]. A viable capillary network within the tissue construct serves as the required link between the host and

the engineered implant [3, 4]. The strongest stimulus for capillary sprouting is hypoxia. Moreover, the ECM functions as a reservoir of growth factors to induce incoming blood vessels (angio-induction), and it serves as a scaffold for migrating cells that participate in angiogenesis [27].

Growth factors such as VEGF, bFGF, PDGF, and IGF-1 function as architects of the regenerative wound healing process. They convert the temporary scaffold of the early wound healing phase into a vascularised scaffold by attracting vascular progenitor cells to interact with local cells to grow a vascular network *in situ* [4, 28]. In a hostile wound environment, absent cell populations and missing growth factors could be added to induce healing and integration [27, 29]. However, the half-lifetime of externally supplied recombinant proteins to the healing wound is very short, especially at body temperature of 37 °C. *In vivo* and *ex vivo* gene transfer protocols that bring in the gene that will translate into proangiogenic growth factors to the construct or the healing wound may overcome the short-acting impact of proteins, by turning local cells into production units of those proangiogenic growth factors. However, it will be essential to control this proangiogenic stimulus to avoid tumour growth [28–31].

Other strategies to generate vascular networks focus on a combination of engineering methods combined with biomolecular approaches. Miller et al. described 3D printed networks of carbohydrate glass as a mould that was coated with poly-lactic-co-glycolic acid (PLGA) before being encapsulated within a suspension of cells in a range of hydrogels. These were cross-linked before the glass particles dissolved away to reveal patent fluidic PLGA channels [32]. An alternative approach is the use of prelamination of layers of photo-cross-linkable gelatin metacrylate by projection stereolithography and computer-aided design (CAD) and computer-aided manufacturing (CAM) [33].

Cell sheet engineering and stacking strategies seem promising: a temperature-responsive surface is used to culture cells. Upon reaching confluence, the surface can be cooled to 20 °C to reduce its hydrophobicity, and the intact cell sheet can be removed easily, preserving cell-cell junctions, ECM, and cell surface proteins. These cell sheets can then be stacked to generate cell-dense tissues. Stacked layers of cardiomyocytes that beat simultaneously without the use of a formal scaffold are a powerful example of the promise of this method [34].

Decellularisation and recellularisation of tissues

Decellularisation strategies aim to remove the resident cells and a large proportion of the major histocompatibility complex of a tissue or an organ using protocols that rely considerably on perfusion with detergents. This strategy sounds clinically very useful and realistic, as the purpose is to preserve the ECM along with the native structure including the perfusable vasculature. The decellularised matrices subsequently serve as an 'authentically designed' scaffold that can be repopulated with 'autologous' cells to restore the intrinsic morphology. The strength of this strategy is the maximal preservation of biomolecular environmental cues that are presumed to direct the cellular phenotype and structure [35]. Song et al. decellularised cadaveric rat kidneys by detergent perfusion through the renal artery, and recellularised with human umbilical venous endothelial cells and rat neonatal kidney cells (Figure 2). After seeding, the organs were transferred into a perfusion bioreactor to provide whole-organ culture conditions. Filtrate 'urine' was produced *in vivo* from the recellularised kidney [36]. Similar whole-organ decellularisation methodologies are also tailored for the lung, liver, and heart, and to create an acellular tracheal tube.

Rapid prototyping and 3D bioprinting

Advances in computer technology in recent years have offered to surgeons innovative tools for conducting preoperative surgical simulations and use intraoperatively surgical-precision cutting guides that add significant accuracy, reduce the operative time, and improve the quality of outcome. Preshaped biomatrices can also be manufactured by CAD and CAM technologies. Unlike 'conventional' 3D printing that has been used to print temporary cell-free scaffolds for use in surgery, 'bioprinting' requires a different approach that is compatible with depositing living cells. Bioprinting is a 3D fabrication technology used to precisely dispense cell-laden biomaterials for the construction of complex 3D functional living tissues or artificial organs [37].

The advantages of bioprinting include accurate control of cell distribution, high-resolution cell deposition, scalability, and cost-effectiveness. To date, no single bioprinting technique enables the production of all scales and complexities of synthetic tissues. All current bioprinting techniques – using inkjet, laser-assisted, or extrusion printers – are based on layer-by-layer printing. This method generally has difficulty printing hollow structures. In addition, the preparation of bioink is time consuming due to cell culturing and biomaterial synthesis [38]. Today, researchers print small-scale tissues that can survive through diffusion. However, full-scale organs – such as full-thickness skin – and complex tissue

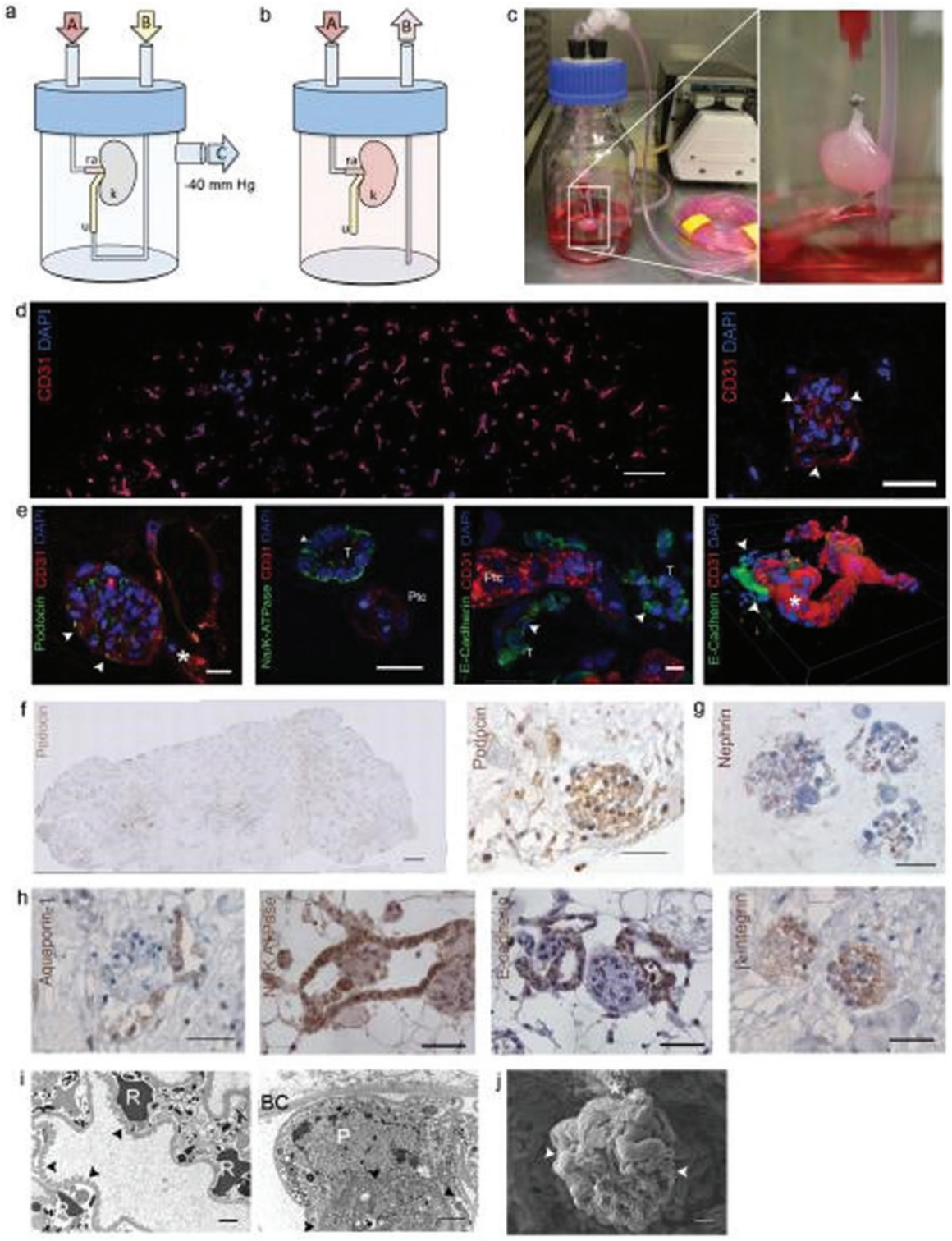

Figure 2: Perfusion decellularisation of whole rat kidneys.
(A) Time-lap pictures of a cadaveric rat kidney undergoing antegrade arterial perfusion decellularisation. Ra refers to renal artery, Rv to renal vein, and U to ureter. (B) Movat's pentachrome-stained sections. Black arrowheads indicate Bowman's capsule. (C) Cell seeding and (D and E) whole-organ culture setup for decellularised kidneys. (F–H) Immunohistochemical images of (F) an entire graft cross section confirming engraftment of podocin-expressing epithelial cells (left) and of a reseeded glomerulus showing podocin expression (right). (G) Nephrin expression in regenerated glomeruli, and (H) aquaporin-1 expression in regenerated proximal tubular structures (left); Na/K-ATPase expression in regenerated proximal tubular epithelium (middle left); E-cadherin expression in regenerated distal tubular epithelium (middle right); and b-1 integrin expression in a regenerated glomerulus (right). From: Song et al. [36].

constructs require an embedded vascular network to connect to host arteries and veins. Moreover, small bioprinted tissues may take only minutes or hours to print, but what would be the cell viability both within a prepolymer bioink and within the polymerised early printed layers of large multiday prints [39]?

Sieira et al. showed in a pilot study a prelamination procedure on microvascular fibula flaps either with oral mucosa constructs before inset into the defect or in a second stage after inset. They demonstrated good results with dental implants [40].

Tissue engineering-based clinical applications

In 1997, Cao et al. published a report of a 'nude' immunosuppressed laboratory mouse that had what looked like a human ear grown on its back. This ear consisted of an ear-shaped cartilagenous structure generated by seeding bovine chondrocytes onto a synthetic polyglycolic acid (PGA) biodegradable ear-shaped mould [41]. The related pictures spread worldwide and ignited the fascination for tissue engineering and generated enormous – unrealistic – expectations. Walles performed a Medline search in 2011 using the term 'tissue engineering', which provided 37,000 hits [42]. A similar search today results in 10-fold hits, indicating the growing fascination and research interest and the belief in its inherent potential. The majority of reports are proof-of-principle studies and only few thus far have resulted in a clinical protocol. For instance, in the context of auricular cartilagenous reconstruction, >50 studies have been published in the past decade using cell- and matrix-based techniques for ear reconstruction. The major shortcomings of these protocols are long-term resorption of the biological scaffold and collapse of the construct in an *in vivo* clinical setting [43].

The progression from bench to bed in tissue engineering protocols has been hindered by many difficulties, mostly related to the lack of vascularisation and the loss of tissue strength and coherence. In the human body, not only the regular regenerative responses exert a more complex role but accompanying clinical morbidity – such as cardiovascular disease, diabetes, and radiotherapy, to name some – or exposition to medication – cytostatica, corticosteroids, and immunosuppressive agents – may strongly influence any of the cascades previously investigated in more controlled laboratory settings. After all, tissue-engineered constructs should exactly be applicable in those 'hostile' tissue conditions where regular wound healing and tissue regeneration processes fail.

The majority of clinical – translational – tissue engineering protocols were performed in five domains: skin, urethra and bladder, the cardiovascular system, bone, and trachea. Many domains will follow.

Skin

Skin substitutes were developed for clinical use especially to treat extensive wounds such as third-degree burns [44–46] (Table 1). Reconstitution of skin wounds deeper than the lamina basalis requires a restoration of all basement membrane components to restore thickness, texture, and elasticity, while providing biomechanical resistance to aggressors. To date, this has been far from realistic to mimic. Skin is not only the largest organ, but also a very specialised and ultimately complex tissue with intrinsic biomechanical, neurosensible, proprioceptive, and thermo- and immunoregulatory features.

Distinct features categorize skin substitutes or skin equivalents

Natural polymers used in skin tissue engineering include chitosan, fibrin, gelatin, and hyaluronic acid. The disadvantages of natural polymers include low mechanical strength, shrinkage and contraction, rapid biodegradability, and risk of immunological rejection.

Synthetic materials include nylon, PGA, and polylactic acid. Due to the ease of fabrication, synthetic polymers such as nylon and PGA/polylactic acid are less expensive. Synthetic materials possess limited tissue compatibility, limited cellular recognition, and incorporation. These substitutes show their importance mainly in combination with natural polymers [47–50].

Skin substitutes can also be described according to cell content: cell-free (acellular) versus cell-containing (cellular) substitutes

Acellular products only contain matrix elements on natural or synthetic materials. These products serve as a template for dermal reconstitution, allowing migration of host cells during the wound healing process. Cellular products contain living cells such as keratinocytes and/or fibroblasts, and may be seeded in a matrix.

Table 1: List of biological and composite skin substitutes.

Materials	Composition	Thickness	Brand	Indication
Biological				
Alloderm	Acellular human dermis	0.79–3.3 mm	Lifecell Corporation, NJ, USA	Burns, soft tissue defects
Allomax	Acellular human dermis	0.8–1.8 mm	Bard Davol, RI, USA	Soft tissue defects
DermaMatrix	Acellular human dermis	0.2–1.7 mm	Synthes, PA, USA	Soft tissue defects
Glyaderm	Acellular human dermis	0.2–0.6 mm	Beverwijck, Netherlands	Full-thickness wounds
Graftjacket	Acellular human dermis	0.5–2 mm	Wright Medical Technology, TN, USA	Soft tissue defects
Oasis	Porcine small intestine Submucosa acellular collagen	0.15–0.3 mm	Healthpoint Ltd., TX, USA	Burns, chronic wounds
Permacol	Acellular porcine dermis	0.4 or 1.5 mm	Covedien, OH, USA	Full thickness wounds
Strattice	Acellular porcine dermis	1.5–2 mm	LifeCell, NJ, USA	Soft tissue reconstruction
SurgiMend	Acellular bovine dermis	0.4–1.54 mm	TEI Biosciences, MA, USA	Soft tissue reconstruction
Tiscover	Acellular human dermis Autologous FB	1–2 mm	A-SKIN, BV, Netherlands	Chronic wounds
Xenoderm	Acellular porcine dermis	0.3 mm	MBP Neustadt, Germany	Full-thickness wounds
Composite				
Apligraf	Allogenic neonatal FB Allogenic neonatal KC	0.4 mm 0.75 mm	Organogenesis, MA, USA	Donor sites, EB
Dermagraft	Mesh + allogenic FB	0.19 mm	BioHealing, CA, USA	Wounds, diabetic ulcers
Hyalomatrix	Hyaluron-based scaffolds With autologous FB	1.2 mm	Fidia	Burns, chronic wounds
Integra	Human collagen I With GAG and silicone top	1.3 mm	Integra Life Sciences, NJ, USA	Burns, chronic wounds
Matriderm	Bovine collagen I, elastin	1 and 2 mm	Care AG, Germany	Burns, chronic wounds
OrCel, previously CCS	Collagen I sponge Gel allogenic FB and KC	1 mm	Ortec International, NY, USA	Chronic wounds, donor sites
Renoskin	Bovine collagen I and GAG	1.5–2.5 mm	Perouse Plastie, France	Burns, defects
Terudermis	Calf collagen Polyester mesh ± silicone top	Four types	Olympus Terumo Biomaterials, Japan	Burns, mucosal defects
Transcyte	Collagen with neonatal FB Nylon mesh + silicone top	1.2 mm	Sciences Inc., CA, USA	Burns

The composition of the substitute is represented in column 2. The thickness of the layer of the substitute is represented in column 3. Note that not all these substitutes are available worldwide. Also, they may not be approved for the same indication in different countries: none of the engineered cell-containing skin substitutes have been approved for the European market. FB, fibroblasts; KC, keratinocytes; GAG, glucosaminoglycans; EB, epidermolysis bullosa.

Acellular scaffolds could be applied for short periods to stimulate autologous healing. Cell-free biodegradable scaffolds may stimulate colonisation by autologous cells in the wound environment as a biological dressing. Cell-containing skin substitutes may provide immediate functional skin replacement. However, in a clinical context, allogenic cells will get rejected and thus rather serve as a temporary supply of proteins, but without 'autologous' biomolecular cues.

Skin substitutes can also be characterised according to the skin layer they represent: epidermal, dermal, or combined (Table 1)

Epidermal substitutes The concept of 'cell engineering' emerged from the discovery that human keratinocytes could be cultivated on a carpet of irradiated murine 3T3 fibroblasts in a culture dish in serum-enriched growth medium [51, 52]. This breakthrough led to the application of cultured autologous keratinocytes on burns in 1981, and later proved to be lifesaving in extensive burn wounds with limited donor site for skin grafts. However, nowadays, we hardly will use keratinocyte layers in clinical burn treatment, as the graft take has proven to be <70% in >50% of cases. Even after healing, the reconstituted epidermis remained excessively fragile mainly due to loss of the rete-peg pattern of the epidermis [53, 54]. In order to restore function and structure of the skin, an epidermal layer will not suffice.

Dermal substitutes Dermal substitutes were processed in a partnership with skin cells. In analogy to the ECM, the scaffold provides support for dermal fibroblasts and epidermal keratinocytes. The rationale of using an absorbable

artificial dermis on deep wounds is the temporary scaffold it provides for cells that should be able to migrate into the construct from the wound environment [45]. Therefore, the pore size should be around 100–250 μm. The collagen-glycosaminoglycan dermal architecture of most dermal scaffolds approaches that of normal dermis and completely biodegrades after 30 days. Faster biodegradation might lead to toxic by-products that impair healing [55]. When the basement membrane is preserved in the construct, keratinocyte engraftment is superior [55]. This finding may be of great importance in further skin engineering protocols.

Compound substitutes with epidermal and dermal elements Recently, more compound scaffolds were presented, featuring dermal matrix elements in combination with allogenic foreskin keratinocytes and/or fibroblasts. Thus far, however, none of the cell-containing artificial skin equivalents have provided a significant clinical advantage to full-thickness healing despite their more corresponding structure and biocontent. This explains why none of the engineered cell-containing skin substitutes have thus far been approved for the European market. The cells added usually are allogenic and therefore will get rejected within a 2-week timespan, similar as an allograft (homograft) in a clinical setting.

Using autologous cell populations will enhance the pool of biomolecular cues in the matrix and may generate better outcome. However, seeding autologous cells into the substitute largely complicates the processing protocols, as these cells must be obtained from the later recipient and must undergo a time-consuming cell culture expansion protocol before being integrated into the matrix; these elaborate steps explain the steep price for these compound substitutes. Further research is required to evaluate their added value in a clinical setting.

De novo assembly of matrices In order to mimic the functional and structural properties of live human ECM, new techniques are being developed. Recently, electrospinning of polymeric nanoscale fibres such as collagen has shown a promising outcome; moreover, this technique is relatively easy and inexpensive [56]. Collagen nanofibrous scaffolds possess a structure mimicking native ECM. They possess excellent biocompatibility, with similar cellular organisation, proliferation, and maturation compared to current techniques such as freeze-dried collagen [57].

As these skin equivalents should, before all, thrive in hostile healing conditions when vascularisation is poor, chronic inflammation exists, proteinases are abundantly present, and, in accompanying morbidity such as diabetes or cardiovascular disease, the future in ready-to-use custom-made sheets of skin requires enormous advances in the fields of ECM biology [58–61].

Urethra and bladder

Approximately 400 million people worldwide suffer from urinary bladder cancer. When radical cystectomy is performed, usually the intestine is used for urinary bladder reconstruction; however, the complication rate is rated up to 35%.

Atala et al. treated seven patients in need for a cystoplasty of the urinary bladder using adult autologous urothelial and muscle cells grown in culture [62]. These cells were seeded on a biodegradable bladder-shaped scaffold made of collagen, or a composite of collagen and PGA. About 7 weeks after the biopsy, the engineered bladder was used for reconstruction and implanted either with or without an omental wrap. They reported excellent results in a follow-up period of 22–61 months [45]. Postoperatively, the mean bladder leak point pressure decreased at capacity, and the volume and compliance increases were greatest in the composite engineered bladders with an omental wrap.

Nevertheless, the construction of a neo-bladder for patients with muscle-invasive bladder cancer is much more challenging because of the inability to use autologous stem cells derived from urinary tracts [63].

Raya-Rivera et al. cultivated smooth muscle cells and epithelial cells obtained from bladder biopsies. These cells were cultured for 1 week to ensure migration, proliferation, and matrix production, and were then seeded onto tubular PGA and poly-L-lactic-acid (PLLA-PGA) scaffolds. The authors analysed the biopsies of five patients and confirmed a tissue organisation similar to native tissue. They reported excellent functional flow rates at 36–72 months [64].

Cardiovascular system

In 1919, Guthrie stated that for repairing a blood vessel, 'an implanted segment needs only to temporarily restore mechanical continuity, serving as a scaffolding bridge for the ingrowth of tissue from the host' [65]. The defining characteristics of regenerative cardiovascular implants have not strayed from that statement, but have taken it one step further: the tissue-engineered implant does not only restore continuity and serve as a scaffold for tissue migration; it also has the ability to degrade, leaving remaining cells to integrate in the host [1].

Vascular grafts

In a *New England Journal of Medicine* report in 2001, Shinoka et al. described a tissue engineering approach to

treat atresia of the right intermediate pulmonary artery in a 4-year-old patient [66]. They used autologous adult cells that were seeded on a tube composed of PCL-PGA. They reported an excellent outcome with a 9-year follow-up.

In a multicentre study, McAllister et al. reported the use of a tissue-engineered autologous vascular graft to create a vascular access for haemodialysis in nine patients in need for an arteriovenous (AV) fistula but without having appropriate veins [67]. They cultivated confluent sheets of autologous adult fibroblasts and their deposed ECM, and seeded them around a stainless-steel mandrel. The patency rate was 60% at 6 months, equal to the patency rate of natural AV fistula.

Hibino et al. used autologous bone marrow mononuclear cells seeded on a biodegradable scaffold of PGA and PLLA to create a cavopulmonary conduit; 5 out of 25 patients developed a thrombosis or stenosis of the graft. There was no reported aneurysm or graft rupture [68].

Olausson et al. used a decellularisation procedure on a donor iliac vein and reseeded it with endothelial and smooth muscle cells derived from bone marrow of the recipient. This procedure was used in a 10-year-old patient with portal vein thrombosis. This first construct failed after 1 year; however, a similar second procedure led to normal portal blood flow [69].

Cell therapy for myocardium

Tee et al. [70] and Morrison et al. [71] previously used an AV chamber to create a large adipose tissue construct to restore the breast in five female postmastectomy patients. In one patient, substantial volume was generated. The other three failed to develop significant enlargement of the original fat flap, which, at the time of chamber explantation, was encased in a thick vascular fibrous capsule [71]. The same authors subsequently reported the use of neonatal cardiomyocytes implanted into a similar AV loop chamber and assembled into a contractile flap [70].

Bolli et al. reported in the *Lancet* in 2011 the early functional results of a phase 1 trial using bone marrow-derived progenitor cells in patients with ischaemic cardiomyopathy. Their approach led to enhanced function following myocardial infarction and failure of coronary stents [72].

Heart valves

Mechanical valves offer exceptional durability coupled with a considerable risk of thrombogenesis. Biological valves do not need anticoagulation, but have a limited lifespan. Cebotari et al. treated 38 patients with decellularised pulmonary homografts, and showed that in contrast to conventional homograft and xenografts, decellularised fresh allograft valves exhibited adaptive growth [73]. Enzymatic treatment with trypsin/EDTA converts pulmonary valves in a cell-free scaffold with 98% reduction of DNA content. Histology revealed a well-preserved 3D network of collagen fibres in the ECM.

The same authors reported the clinical implantation of a decellularised human pulmonary allogenic heart valve that was reseeded with autologous EPCs. They reported a 3.5-year follow-up in two patients of age 13 and 11 years. Postoperatively, a mild pulmonary regurgitation was documented in both children. No signs of valve degeneration were observed. These tissue-engineered valves have the potential to remodel and grow accordingly to the somatic growth of the child [73].

Bone and articular cartilage

Warnke et al. used a cell-based strategy to treat mandibular defects after oncologic resection. The authors used a titanium mesh cage filled with bone mineral segments. They added bone morphogenic proteins and bone marrow [74]. The construct was matured into the latissimus dorsi muscle for 7 weeks. However, despite a reported excellent outcome, the procedure was performed in only one patient when it was reported in the *Lancet*, and the follow-up was limited to 4 weeks.

Mumme et al. used a nasal chondrocyte-based engineered autologous cartilage construct to treat 10 patients with symptomatic posttraumatic full-thickness cartilage defects in a safety-and-efficacy human trial with a 24-month clinical follow-up. Radiological assessments indicated variable degrees of defect filling and development of repair tissue approaching the composition of native hyaline-like cartilage. The next step is analysing efficacy in large controlled human trials [75].

Trachea

It may be relatively easy to generate a hollow tube that mimics the windpipe; however, it is very complex to create a long circular flexible vascularised tube that has a mucosal lining with ciliated epithelium and that withstands the rigors of negative pressures during respiration.

Delaere et al. used a trachea allotransplantation strategy to restore long-segment defects of the trachea. A donor trachea is prefabricated in the forearm and native allogenic mucosa is replaced with recipient mucosa

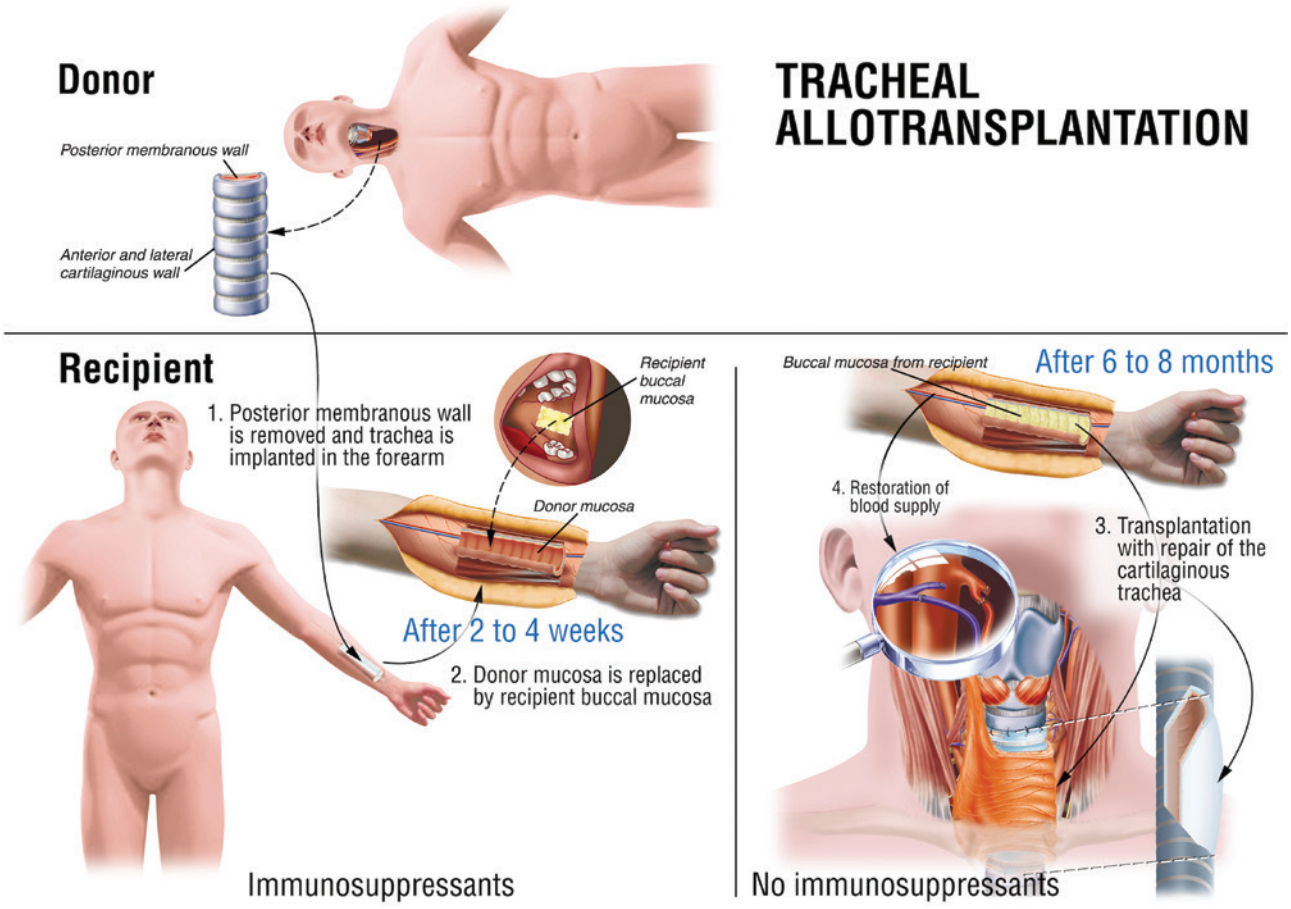

Figure 3: Trachea allotransplantation to restore a long-segment defect of the trachea.
A donor trachea is prefabricated in the forearm and allogenic mucosa was replaced with recipient buccal mucosa creating tolerance. Microvascular orthotopic transfer to the defect occurs in a second stage. Immunosuppression is tapered and stopped after control bronchoscopy and CT scan. Currently, the authors are investigating in a preclinical setup the cultivation of autologous respiratory cells and decellularisation techniques on the allotrachea to diminish immunogenicity further, and to induce faster reepithelialisation and angiogenesis to the inner mucosal lining. From Vranckx [3] and Delaere et al. [76].

creating tolerance (Figure 3). This allows stopping immunosuppression after the orthotopic transfer to the defect in a second stage. Using their prefabrication protocol as vascular platform, they investigated respiratory cell cultivation to induce faster reepithelialisation and decellularisation of the allotrachea to further diminish immunogenicity [76].

Kojima and Vacanti reported in 2002 a strategy forming a tube with sheets of bioresorbable PGA, both seeded with fibroblasts and chondrocytes from the nasal septa of sheep. This construct was then implanted under the sternocleidomastoid muscle to allow for extrinsic vascularisation [77]. The concept was innovative but unfortunately failed: the graft degenerated and collapsed.

Omori et al. reported in 2005 the first human case applying regenerative medicine principles to restore partially the cricoid cartilage and cervical trachea. They used an acellular biosynthesised polypropylene-reinforced mesh coated with collagen and preclotted with autologous blood in eight human cases with a 4-year follow-up [78]. They added fibroblasts and bone marrow-based cells, and coated the lumen of the construct with poly-L-lactic-acid-co-caprolactone to delay the degradation of the collagen. The authors were sceptical about the durability of regenerating cartilage from implanted chondrocytes. At the luminal surface, reepithelialisation occurred very slowly without decent vascularisation. However, in a follow-up editorial, it appeared that the bronchus had collapsed and that a stent was placed. A decellularised tracheal tube was used as a scaffold to generate a tracheal tube. Short-term incubation was performed with autologous bone marrow-derived cells. Eight months after implantation, a collapse of the construct occurred and a stent had to be placed [79].

Conclusion

The concept of tissue engineering is intriguing and promising. There is a logarithmic growth of reports describing tissue engineering principles using (stem) cells seeded in matrices. The translation to clinical practice does not occur abruptly, but by creeping substitution. Research in each of the pillars of tissue engineering – bioengineering principles, biomolecular sciences, and clinical strategies – resulted along the road to innovative approaches and materials that are used in daily practice. The key feature – and major hurdle – in further development of tissue engineering for clinical practice is the integration of vasculature within the construct. The first human trials are conducted currently.

Author Statement

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Author Contributions

Jan Jeroen Vranckx: Formal analysis; funding acquisition; investigation; methodology; writing – original draft; writing – review and editing. Margot Den Hondt: Data curation; formal analysis; investigation; methodology; writing – review and editing.

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Supplemental Material: The article (DOI: 10.1515/iss-2017-0011) offers reviewer assessments as supplementary material.

Reviewer Assessment **Open Access**

Jan Jeroen Vranckx* and Margot Den Hondt

Tissue engineering and surgery: from translational studies to human trials

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Reviewers' Comments to Original Submission

Reviewer 1: anonymous

Mar 19, 2017

Reviewer Recommendation Term:	Accept with Minor Revision
Overall Reviewer Manuscript Rating:	N/A

Custom Review Questions	**Response**
Is the subject area appropriate for you?	4
Does the title clearly reflect the paper's content?	4
Does the abstract clearly reflect the paper's content?	4
Do the keywords clearly reflect the paper's content?	4
Does the introduction present the problem clearly?	4
Are the results/conclusions justified?	4
How comprehensive and up-to-date is the subject matter presented?	4
How adequate is the data presentation?	4
Are units and terminology used correctly?	4
Is the number of cases adequate?	N/A
Are the experimental methods/clinical studies adequate?	N/A
Is the length appropriate in relation to the content?	3
Does the reader get new insights from the article?	4
Please rate the practical significance.	3
Please rate the accuracy of methods.	N/A
Please rate the statistical evaluation and quality control.	N/A
Please rate the appropriateness of the figures and tables.	3
Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	4
Please judge the overall scientific quality of the manuscript.	5 - High/Yes
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:
Some small corrections in diction or grammar.

Reviewer 2: anonymous

Apr 10, 2017

Reviewer Recommendation Term:	Revise with Major Modification
Overall Reviewer Manuscript Rating:	50

Custom Review Questions	**Response**
Is the subject area appropriate for you?	3
Does the title clearly reflect the paper's content?	3
Does the abstract clearly reflect the paper's content?	4
Do the keywords clearly reflect the paper's content?	5 - High/Yes
Does the introduction present the problem clearly?	3
Are the results/conclusions justified?	3
How comprehensive and up-to-date is the subject matter presented?	4
How adequate is the data presentation?	3
Are units and terminology used correctly?	4
Is the number of cases adequate?	N/A
Are the experimental methods/clinical studies adequate?	N/A
Is the length appropriate in relation to the content?	2
Does the reader get new insights from the article?	3
Please rate the practical significance.	4
Please rate the accuracy of methods.	N/A
Please rate the statistical evaluation and quality control.	N/A
Please rate the appropriateness of the figures and tables.	3
Please rate the appropriateness of the references.	1 - Low/No
Please evaluate the writing style and use of language.	3
Please judge the overall scientific quality of the manuscript.	3
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:
Tissue Engineering strategies for surgery; by creeping substitution

The authors review the recent developments in the field of Tissue engineering. The review was unfortunately provided to the reviewer without a list of references so that an acceptance is not possible at this stage.
The review is quite lengthy and in parts repetitive as parts of the specialized section describing the approaches to different tissue types are already discussed in the first part of the manuscript.
Some crucial contributions to the field seem to be missing, e.g. the developments in cardiac tissue engineering pioneered by Wayne Morrison or the first-in-human trials regarding the clinical application of tissue engineered cartilage by Ivan Martin and coworkers.
The figure quality is relatively low and needs to be improved.
Page 11, last sentence of the first paragraph requires citations. What does (CFR infra) stand for?

Authors' Response to Reviewer Comments

May 03, 2017

Reviewer 1:

Required changes were made in the tekst.

A copy of the manuscript with all changes still visible was added for further convenience.

Reviewer 2:

The manuscript has been substantially shortened.

The reference list is once more added as well as the refs of Wayne Morisson et al. and Martin et al. The work of both authors was briefly explained into the manuscript.

Cfr infra: “conferatur infra”: “see below/further”

The resolution of the pictures was enhanced.

Sincerely Yours

Reviewers’ Comments to Revision

Reviewer 1: anonymous

May 15, 2017

Reviewer Recommendation Term:	Accept
Overall Reviewer Manuscript Rating:	70

Custom Review Questions	**Response**
Is the subject area appropriate for you?	3
Does the title clearly reflect the paper’s content?	4
Does the abstract clearly reflect the paper’s content?	4
Do the keywords clearly reflect the paper’s content?	4
Does the introduction present the problem clearly?	4
Are the results/conclusions justified?	3
How comprehensive and up-to-date is the subject matter presented?	3
How adequate is the data presentation?	4
Are units and terminology used correctly?	4
Is the number of cases adequate?	N/A
Are the experimental methods/clinical studies adequate?	3
Is the length appropriate in relation to the content?	2
Does the reader get new insights from the article?	3
Please rate the practical significance.	3
Please rate the accuracy of methods.	N/A
Please rate the statistical evaluation and quality control.	N/A
Please rate the appropriateness of the figures and tables.	4
Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	4
Please judge the overall scientific quality of the manuscript.	3
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:

-

Reviewer 2: anonymous

May 08, 2017

Reviewer Recommendation Term:	Accept
Overall Reviewer Manuscript Rating:	75

Custom Review Questions	**Response**
Is the subject area appropriate for you?	3
Does the title clearly reflect the paper's content?	3
Does the abstract clearly reflect the paper's content?	3
Do the keywords clearly reflect the paper's content?	3
Does the introduction present the problem clearly?	3
Are the results/conclusions justified?	1 - Low/No
How comprehensive and up-to-date is the subject matter presented?	3
How adequate is the data presentation?	N/A
Are units and terminology used correctly?	3
Is the number of cases adequate?	N/A
Are the experimental methods/clinical studies adequate?	N/A
Is the length appropriate in relation to the content?	3
Does the reader get new insights from the article?	3
Please rate the practical significance.	N/A
Please rate the accuracy of methods.	N/A
Please rate the statistical evaluation and quality control.	N/A
Please rate the appropriateness of the figures and tables.	N/A
Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	3
Please judge the overall scientific quality of the manuscript.	3
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:
All questions have been answered sufficiently.