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General review

Bio-scaffolds in organ-regeneration: Clinical potential and current challenges

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ABSTRACT

Cadaveric organ transplantation represents the definitive treatment option for end-stage disease but is restricted by the shortage of clinically-viable donor organs. This limitation has, in part, driven current research efforts for in vitro generation of transplantable tissue surrogates. Recent advances in organ reconstruction have been facilitated by the re-purposing of decellularized whole organs to serve as three-dimensional bio-scaffolds. Notably, studies in rodents indicate that such scaffolds retain native extracellular matrix components that provide appropriate biochemical, mechanical and physical stimuli for successful tissue/organ reconstruction. As such, they support the migration, adhesion and differentiation of reseeded primary and/or pluripotent cell populations, which mature and achieve functionality through short-term conditioning within specialized tissue bioreactors. Whilst these findings are encouraging, significant challenges remain to up-scale the present technology to accommodate human-sized organs and thereby further the translation of this approach towards clinical use. Of note, the diverse structural and cellular composition of large mammalian organ systems mean that a "one-size fits all" approach cannot be adopted either to the methods used for their decellularization or the cells required for subsequent re-population, to create fully functional entities. The present review seeks to highlight the clinical potential of decellularized organ bio-scaffolds as a route to further advance the field of tissue- and organ-regeneration, and to discuss the challenges which are yet to be addressed if such a technology is ever to become a credible rival to conventional organ allo-transplantation.

1. Background

In recent years, tissue engineering and regenerative medicine have taken center stage in the search for effective, sustainable treatments for chronic life-limiting conditions and management of end-stage organ failure. An expansion of research activity in the field has increased knowledge, advanced technology and raised the potential that transplantable tissues and organs may be created under defined laboratory conditions. In most instances, stem cells seeded onto biocompatible scaffolds composed of native or synthetic extracellular matrix (ECM) form the basis of the new tissue constructs. Once implanted to effect repair, such scaffolds are further re-populated by the recipient's own cells and connect to inputs from the host vascular system, receiving appropriate support for long-term survival. Tailor-made grafts engineered ex vivo using patient-derived stem cells have led to a small number of clinical successes [1–5], and wider use of this approach would, to some extent, address the issues of donor shortage and immune-rejection.

2. Bio-scaffolds in tissue engineering

The use of biocompatible, three-dimensional scaffolds is fundamental to the successful engineering of tissues and whole organs for use in regenerative medicine. The cells that will go on to form replacement parts depend upon a precise combination of chemical, physical, spatial and mechanical signals or cues, that regulate their differentiation and eventual function [6,7]. In the absence of this defined microenvironment cells are unable to form the cellular and vascular networks required to serve as functional constructs. Attempts to create such scaffolds from synthetic materials have enjoyed limited clinical success, most likely due to their inability to faithfully mimic the roles of naturally occurring ECM proteins, or to produce the paracrine and trophic factors that mediate effects on pluripotent stem cells and orchestrate their role in the regenerative process [6]. Large synthetic scaffolds capable of supporting the more complex tissue assemblies and to bioengineer

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surrogate, human-scale organs are also technically challenging to design [7,8].

Research focus has, therefore, turned to the use of biological scaffolds derived from native, non-transplantable organs which may be re-purposed for clinical use. The process requires the organ to first be stripped of its cellular and nuclear components; a process termed decellularization. If done efficiently the resulting structure is devoid of cells whilst retaining the original architecture within a delicate framework composed of ECM components [9–11]. As such the resulting structure not only hosts the essential cues and signaling pathways required for cell maturation, proliferation, migration and adhesion but preserves the vascular structures which are central to high density cell growth and organ survival. The ECM scaffold is also immunologically inert which confers advantages for its downstream clinical application [12,13]. Stem cells, organ-specific primary or progenitor cells of interest may be introduced to the biological scaffold, re-populating the extracellular matrix compartment before undergoing differentiation and maturation within a defined niche (microenvironment) to form tissues capable of conducting targeted functions [14].

The potential of such an approach for clinical success is evidenced by the current use of less complex tissue matrices to affect surgical repairs and in the management of recalcitrant wounds and full-thickness burns (Table 1). Such achievements serve as proof-of-concept that new functional tissues may be derived from native material re-processed in vitro for clinical use; yet application of this emergent biotechnology to whole organs poses significant challenges. The present review highlights the recent advances in the decellularization process as applied to human and human-sized organs; the features retained by the preserved ECM and their importance to successful organ bioengineering. It will also discuss the various attempts to reconstitute the acellular scaffold, detailing the cell populations that site-specifically re-integrate with the biomimetic ECM to generate functional organs with the potential to influence future therapies for chronic disease.

3. Whole organ decellularization – reagents and methods

Decellularization protocols are organ-specific, addressing the precise physico-chemical properties of the tissue in question and adopting a combination of chemical and mechanical techniques to achieve the desired result. In general, the process is designed to efficiently remove cellular and nuclear moieties and conserve the properties of the ECM chiefly its composition, biological activity and mechanical functions. Whilst appearing straight-forward, this is not easy to achieve and often the "optimal" protocol will be a compromise, with each suggested method causing some damage to the delicate ECM ultrastructure [8].

Several different groups of reagents are routinely employed, with varying biochemical attributes which determine their use in organ decellularization. Ionic compounds such as sodium dodecyl sulphate (SDS) and sodium deoxycholate chiefly serve as solubilizing agents, with the ability to lyse cellular and nucleic membranes and denature proteins [15]. Triton-X-100 is a non-ionic detergent and acts at the level of the membrane to disrupt lipid-protein and DNA-protein binding and is effective in more dense tissues [16]. Zwitterionic detergents such as 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) show similar biochemical characteristics to ionic and non-ionic compounds, but are considered to be less disruptive to the underlying ECM components [17]. Enzymes such as dispase, lipase, trypsin and nucleases are chiefly utilized to cleave peptide bonds and thus disrupt protein-protein interactions, targeting distinct peptide sequences to detach cells from the matrix and degrade cellular and/or nucleic material [18]. Certain enzymes such as dispase also prevent cell aggregation (clumping) ensuring their complete removal from the ECM. Chelating agents notably, ethylene-diamine-tetra-acetic acid (EDTA), are often used in conjunction with enzymes due to their ability to bind Ca^{2+} and Mg^{2+} ions which impedes cell-to-cell interactions and cell adhesion to the ECM [19]. Dehydration-induced cell lysis is achieved by the incorporation of alcohols (ethanol, methanol) and acetone into the decellularization process which are also effective

Table 1
Clinical use of human and porcine ECM.

Tissue	Method for decellularization	Cell populations employed for recellularization	Clinical application	Reference
Pulmonary heart valve (human)	0.5% trypsin 0.2% EDTA	Blood-derived endothelial progenitor cells	Valve replacement in paediatric patients with congenital PV failure	Cebotari et al. [19]
Aortic heart valve (porcine)	1% (v/v) Triton-X-100 10 mM of sodium cholate	N/A	ECM graft in surgical repair of chronic heart disease	Spina et al. [90] Padalino et al. [91]
Trachea (human)	4% sodium deoxycholate 2000 kU DNA-ase	Autologous bronchial cells, bone marrow-derived chondrocytes	Airway substitute in chronic tracheitis and secondary severe bronchomalacia of the left main bronchus	Macchiarini et al. [4] Gonfiotti et al. [5]
Iliac vein (human)	1% Triton-X, 1% tri-n-butyl phosphate 4 mg/L DNA-ase	Bone marrow-derived CD133+ endothelial cells, smooth muscle cells differentiated in vitro from bone marrow stem cells	Vascular vein shunt in the treatment of extra-hepatic portal vein obstruction	Olausson et al. [3]
Dermal matrix (human)	0.07% SDS and 0.1% EDTA	N/A	Decellularized dermal skin substitute (DCD) – one-stage therapeutic strategy for recalcitrant leg ulcers	Hogg et al. [92] Greaves et al. [93]
Dermal matrix (human)	2% dispase	N/A	Treatment of full-thickness burns in co-graft model using autologous split-thickness skin	Li et al. [94]
Small intestinal submucosa (porcine)	Dilute peracetic acid; mechanical delamination	N/A	ECM patch for repair of congenital heart abnormalities	Quarti et al. [95] Woo et al. [96]
Bladder (porcine)	Cell lysis with PBS and 0.1 sodium azide NaCl and 2000 kU DNA-ase	N/A	ECM patch in esophageal reconstruction	Nieponice et al. Afaneh et al. [97,98]