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Bioengineering liver tissue by repopulation of decellularised scaffolds

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Abstract

Liver transplantation is the only curative therapy for end stage liver disease, but is limited by the organ shortage, and is associated with the adverse consequences of immunosuppression. Repopulation of decellularised whole organ scaffolds with appropriate cells of recipient origin offers a theoretically attractive solution, allowing reliable and timely organ sourcing without the need for immunosuppression. Decellularisation methodologies vary widely but seek to address the conflicting objectives of removing the cellular component of tissues whilst keeping the 3D structure of the extra-cellular matrix intact, as well as retaining the instructive cell fate determining biochemicals contained therein. Liver scaffold recellularisation has progressed from small rodent *in vitro* studies to large animal *in vivo* perfusion models, using a wide range of cell types including primary cells, cell lines, foetal stem cells, and induced pluripotent stem cells. Within these models, a limited but measurable degree of physiologically significant hepatocyte function has been reported with demonstrable ammonia metabolism *in vivo*. Biliary repopulation and function have been restricted by challenges relating to the culture and propagations of cholangiocytes, though advances in organoid culture may help address this. Hepatic vasculature repopulation has enabled sustainable blood perfusion *in vivo*, but with cell types that would limit clinical applications, and which have not been shown to have the specific functions of liver sinusoidal endothelial cells. Minority cell groups such as Kupffer cells and stellate cells have not been repopulated. Bioengineering by repopulation of decellularised scaffolds has significantly progressed, but there remain significant experimental challenges to be addressed before therapeutic applications may be envisaged.

Key Words: Regenerative; Bioengineering; Scaffolds; Liver; Decellularisation;

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Core Tip: Given the limited resource of livers for transplantation, repopulation of decellularised scaffolds with recipient cells offers a theoretically attractive organ source without the need for immunosuppression. Bioengineered livers have progressed from small rodent to large animal blood perfusion models. Although some hepatocyte function has been achieved, challenges remain in cholangiocyte repopulation, reconstitution of the vasculature, and other minority cell groups. The cell types used in experimental models to date have yielded advances but may need to be altered if the currently distant prospect of clinical application is to be envisaged.

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INTRODUCTION

Chronic liver disease is a major health concern, with 1.5 billion individuals affected worldwide, and associated with an annual global mortality of 2 million people[1]. In the United Kingdom, liver disease is the third commonest cause of premature death[2], and is associated with societal and health care costs measured in the billions of pounds per annum[3]. In the United states, 44000 people die of chronic liver disease each year[4], with an estimated annual hospitalisation costs demonstrating an increasing trend and measured at 18 billion dollars per year in 2016[5], to which must be added similar magnitude financial costs of pre-hospital healthcare and social care burden[3].

Although vaccination programs and antiviral therapy may result in decreasing prevalence in chronic liver disease of viral aetiology, the consequences of alcohol and hepatic steatosis has resulted in a gradually increasing incidence of chronic liver disease[6-8]. Despite the enormous scope for prevention of progression to chronic liver disease through vaccination, antiviral therapy, and lifestyle interventions, the only treatment for end-stage liver disease remains liver transplantation. However, due to the shortage of available organs, 10% of patients die whilst on the waiting list for an organ[9], and many more are never considered for transplantation because of the need to optimise graft usage. Moreover, transplanted patients face the short and long-term side effects of immunosuppression.

These challenges have motivated the investigation of bioengineering liver tissue with a view to delivering bioengineered organs for transplantation. Despite progress in the generation of biogels and 3D bioprinting, reproducing the immensely complex 3D microarchitecture of liver parenchyma remains a major challenge. By decellularizing tissues with surfactant detergents, it is possible to remove the cellular component of tissues, leaving behind the 3D extracellular matrix (ECM) providing not only a scaffold but also cell fate instructions to appropriate repopulating cells. In the context of liver transplantation, many deceased organs are discarded because of inadequate cellular function[10]. As an aspirational objective, such organs could be decellularised, and repopulated with cells of recipient origin with a view to bioengineering immunologically syngeneic organs. The theoretical benefits would include timely generation of organs, transplanted in an elective manner, without the need for immunosuppression.

This review describes current progress in the field of bioengineering liver tissue from decellularised matrix and repopulating cells. To orientate the reader, the review sections will deal with the following areas: Section 2 (ECM structure and role in cell fate) provides a summary of the structure and function of the extracellular matrix, describing its paramount influence in cell fate and bioengineering, as well as an account of the evolution of synthetic and ECM substrate components to enhance tissue culture; Section 3 (General concepts in decellularisation and non-hepatic applications) provides an account of decellularization of tissues in general and non-hepatic applications, as a background context in which to consider liver decellularisation and repopulation; Section 4 (Scaffold sterilisation) discusses scaffold sterilisation; Section 5 (Liver decellularisation and recellularisation) provides an account of decellularisation and repopulation of liver tissue with subsections dealing with the variety of cellular components of liver parenchyma; Section 6 (Recellularisation of extra hepatic blood vessels) discusses the recellularisation of extra-hepatic blood vessels; Section 7 (Immunogenicity of decellularised scaffolds) provides an account of scaffold immunogenicity; and Section 8 (Conclusion) concludes the review with a discussion of the remaining challenges in the field.

ECM STRUCTURE AND ROLE IN CELL FATE

Introduction

Whilst a full account of the role of ECM in cell biology is beyond the scope of this review, its fundamental role in influencing cell behaviour requires emphasis in the context of the use of decellularised ECM scaffolds. This section describes the structure of the ECM and provides an overview of ECM cell interactions as well as the evolution in the use of ECM based substrates to enhance tissue culture.

ECM content and structure

Although the structure of ECM varies immensely between tissues in terms of proportion and layout of its constituents, common components can be identified and include Glycosaminoglycans, water, 4 major classes of extracellular proteins (the collagens, elastin, proteoglycans, and glycoproteins), and numerous growth factors as well as other bioactive cell behaviour influencing species.

Glycosaminoglycans such as chondroitin sulphate, heparan sulphate and hyaluronic acid[11] are long, negatively charged macromolecules consisting of linear repeats of uronic and amino disaccharide units. In isolation or when combined with proteins to form proteoglycans[12], Glycosaminoglycans bind water, which is critical for imparting compressive resistance to tissue.

Collagens imparts tissue tensile strength and structural integrity. They consist of 3 alpha chains, the various combinations of which make up the 28 known collagen types. In broad structure, Fibrillar collagen is assembled in triple helical structures which combine to form fibrils of varying size and thickness. Non fibrillar collagen does not form fibrils but rather a mesh like network, such as that in basement membrane by collagen type 4[13].

Elastin complements collagen's tensile strength properties to provide elasticity[14].

Glycoproteins[15,16] are peptide units covalently bound to carbohydrate groups, but not in a linear or repeating pattern, as in proteoglycans. The glycoproteins are described as connecting molecules, in that they carry binding sites to multiple other molecules including other ECM molecules, secreted growth factors, and extra-cellular membrane receptors on cells including cell adhesion molecules. The principal glycoproteins are fibronectin and laminin. Cell attachment to glycoproteins is mediated through distinct peptide domains[17] such as the Arg Gly Asp (RGD) and Arg Glu Asp Val (REDV) sequences in fibronectin[18,19], as well as Val-Al-Pro-Gly domain in elastin[20], which binds integrins on cell surface. Binding motifs may be overtly apparent or may be revealed after unfolding of ECM proteins by fibroblasts, or following the action of ECM degrading enzymes, thus introducing further complexity in the interplay between the ECM and multiple cell types in the control of cell behaviour[21]. Laminin is composed of alpha, beta and gamma heterotrimeric chains arranged in cross or Y shapes[12]. It is found in basement membrane and connects ECM components, with different forms and modifications resulting in specific controls on cell behaviour[22].

ECM cell interactions

The ECM is much more than simply a 3D scaffold which houses resident cells. It is also a source of critical biochemical and physical signalling which influences fundamental processes of cell survival, organization and differentiation[23].

ECM in development, cell migration, stem cell niche, and adult tissue fate: The importance of the ECM in cellular organisation is apparent from its synthesis and secretion in the very earliest stages of development, exemplified by the assembly of laminin and collagen 4 in mouse embryos as early as the blastocyst stage[24]. Moreover, major developmental defects are caused by ECM proteins[25], with ECM mutations resulting in wide ranging anomalies affecting body shape[26], as well as development of neural tube[27], and muscle[28].

In addition to broad control of development, the ECM acts as a regulator of the extent and direction of cell migration. Thus, laminin chain knockout results in uncontrolled and undirected neural crest cell migration[29], whilst fibronectin mutations result in impaired migration of cardiac precursor cells[30].

The ECM controls stem cell fate not only in development, but also in adult tissues where it plays a major role in the definition of the stem cell niche, keeping stem cell in a quiescent state until appropriate circumstances trigger a requirement for their proliferation[31].

The ECM also influences the behaviour of differentiated cells in adult tissues. Following cues from multitude factors (physical, chemical, oxygen partial pressure, and numerous others which together define physiological niche)[32], the ECM is altered and remodelled by resident cells in adult tissues. ECM remodelling is much more than a reconfiguration of local 3D scaffold shape: the ECM is a reservoir of multiple biologically active species which impact on cell behaviour, and which are recruited and released upon remodelling. Thus, although resident cells produce and deposit their local ECM, they are also influenced by it in a process referred to as 'dynamic reciprocity' or 'bidirectional crosstalk' between cells and their environment[33,34].

ECM – cell biochemical and biophysical signalling: The ECM interacts with cells *via* multiple receptors in the cell membrane including integrins, discoidin domain receptors, syndecans, CD44, and receptor

for hyaluronic acid. Of these, the most studied are the integrins. Distinct alpha and beta subunits combine to make 24 different known integrins, which act specifically on defined cell types in a contextual manner to determine cell growth and survival, promote invasion and migration, and direct cell differentiation and stem cell fate[35]. Mechanistically, the importance of binding motifs is emphasised by experiments demonstrating that blocking the integrin binding site of fibronectin (the RGD motif) by competitive inhibition with RGD peptides resulted in major embryonic symmetry anomalies[36].

In addition to direct communication with cells *via* cell membrane receptors, the ECM influences cell fate by acting as a reservoir of growth factors, morphogens and enzymes, which may be released as active forms in defined circumstances, in a manner that has been most studied in relation to many growth factor families including the transforming growth factor beta, platelet derived growth factor, fibroblast growth factor and insulin like growth factor superfamilies of growth factors[14]. These growth factor signals are added to and complemented by those of other bioactive species including, matrix cryptic peptides[37], matrix bound vesicles containing bioactive molecules (RNA, lipids, proteins)[38], with wide-ranging roles including impacts on cell differentiation[31] chemotaxis[39], mitogenesis[40], angiogenesis[41,42], and wound healing[43].

The ECM signals to cells and influences cell fate in biophysical ways as well as *via* biochemical mechanisms. For example, by determining cell shape with microprinted fibronectin islands, McBeath *et al*[44] showed that mesenchymal stem cells would differentiate to adipocytes if they assumed a rounded shape, and to chondrocytes if allowed to assume a spread shape.

The ECM also influences cell proliferation[45] and cell fate *via* its stiffness and elasticity. Thus, mesenchymal stem cells differentiation may be directed towards either neurological, muscle or bone phenotypes by varying the elasticity of the underlying substrate to mimic the corresponding tissue types[46], *via* mechanisms involving mechano-sensitive ion channels, and Yes-Associated Protein and Transcriptional Coactivator With PDZ-Binding Motif[14].

The evolution of complex substrates for cell culture

The powerful influence of the ECM in the control of cell fate has motivated the use of alternatives to 2D plastic cell culture with a variety of complex substrates to minimise the loss of functional specificity that is otherwise frequently observed. Thus, there has been a gradual evolution in the use of materials to mimic the ECM *in vitro*, culminating in the recent development of decellularised scaffolds, representing to date the most accurate version of native ECM.

Substrates for enhanced cell culture include synthetic or naturally occurring chemicals. The synthetic substrates are man-made polymers such as polycaprolactone, polyethylene glycol (PEG) and polyglycolic acid[23], or hydrogels composed of hydrophilic polymers such as polyacrylic acid, polyethylene glycol and polyvinyl alcohol[47]. These have the advantages of reliability, consistency, reproducibility, low variability, but tend to produce host inflammatory responses[48] and fundamentally lack the complexity of native ECM. The naturally occurring substrates are components of ECM, either in single form or in combinations of varying complexity.

Synthetic substrates may be bioengineered to include biological entities in a number of ways: (1) By crosslinking cell adhesion peptides (for example, the RGD domain of fibronectin or VPVG domain of elastin) to synthetic polymers like PEG to promote cell interaction[49]; (2) By incorporation of specific growth factors to favour desired cell behaviour, for example neuronal[50], bone[51], and vascular[52] differentiation, with the option of positioning of boundary forming signals[53], or temporal control by determining the mechanism of release of the bioactive species[54]; and (3) by incorporating enriched ECM components into poly-ethylene-glycol hydrogels[55].

Hydrogels are hydrated polymers or materials with $\geq 30\%$ (v/w) water content that maintain their structural integrity through crosslinks between their constituents[56], which can be synthetic polymers, or from ECM components in single form[57] or multi component form[58]. Cell derived hydrogels such as Matrigel, or hydrogels generated from specific decellularised tissues are more complex and have been used for organoid culture[59], as 2D substrates, or cell medium additives.

Whether synthetic, naturally occurring or combined, the deposition of substrate components on a given surface has evolved to high level of precision, achieving resolutions of fractions of micrometres, with micro-patterning techniques such as photolithography[60], elastomeric stamping[61], nanofiber lithography[62], electrospinning[63], and 3D bioprinting using 'bio ink' (ECM derived from specific tissue in hydrogel and colloid form)[56].

Nevertheless, despite the wide range of available substrates, be they synthetic, naturally occurring or combined, the complexity of specific tissue microarchitecture combined with the multitude of growth factors within the ECM, means reproducing ECM by the techniques mentioned above remains elusive. Hence the concept of decellularisation, whereby the cells of a specific tissue are removed, thereby leaving behind a native cell free ECM scaffold, theoretically maintaining both 3D micro-architecture and the ECM associated biological signalling.

GENERAL CONCEPTS IN DECELLULARISATION AND NON-HEPATIC APPLICATIONS

Introduction

The objective of whole organ decellularisation has 2 components: (1) To completely remove the cellular component, whilst (2) leaving the 3D microarchitecture and vital growth factor content of the ECM intact, such that repopulating cells have an environment which favours regeneration of the native tissue.

The first objective is necessary as there is evidence that residual cellular debris is not only toxic to repopulating cells, but also triggers inflammatory and destructive responses *in vivo*[64-67] (discussed in section 7 “Immunogenicity of decellularised scaffolds”) rather than the desired regenerative events. The second objective is necessary to retain the vital physical and biochemical ECM properties by which it influences cell fate.

Herein lies a fundamental difficulty in decellularisation techniques, in that whilst both necessary, these 2 objectives are in conflict, as the stringent conditions required to clear toxic debris of decellularisation will also inflict some damage to the ECM. Small molecules like growth factors will be particularly susceptible to being washed away[68], but even large macromolecules, though less vulnerable because of size and cross linking, may also be damaged[69].

The following section outlines the techniques used for decellularisation and discusses their merits and disadvantages.

Decellularisation techniques

A multitude of decellularisation techniques have been developed using physical, chemical, and enzymatic, methods either singly or in combination, and adapted to suit the differing requirements of the native tissue being treated.

Physical methods: (1) Sonication. Sonication utilises an ultrasound emitting device to transfer acoustic energy in a solvent containing tissues to be decellularised[70]. Cell membranes are disrupted by the sonication waves, and resultant debris requires removal by other methods[71-74]. Sonication process may significantly increase temperature of the solvent and tissues, risking denaturation, and therefore may need to be combined with a cooling mechanism[70]. Sonication is typically used with detergents to decellularise dense tissues such as tendons, ligaments[75], and cartilage[71], although has also been used in kidney decellularisation[76]; (2) Freeze-Thaw. Freeze-thaw achieves cell lysis through rapid thermal change, though debris requires additional methods for clearance[70]. The technique has been used in combination with detergents to reduce to the quantities of chemical reagents for decellularisation[77,78]. The formation of ice crystals may be detrimental to the ECM, leading some researchers to advocate the use of cryoprotectants to mitigate the detrimental effects without affecting cell lysis[79]; and (3) Immersion and agitation. The decellularisation effects of chemical reagents may be enhanced by agitation in instances where decellularisation is achieved by immersion in chemical reagents[70]. The length of immersion, and intensity of agitation depend on the tissue[80], and this approach is usually only appropriate for epidermal tissues and smaller organs, such as small intestine submucosa[81], trachea[82], other cartilaginous tissues[83,84], and thyroid gland[85].

Chemical methods: (1) Detergents. Detergents have been used extensively to decellularise large vascular organs by vascular perfusion[70]. Ionic detergents like sodium dodecyl sulphate (SDS) and sodium deoxycholate solubilise cell membranes and denature proteins[86-88]. Non-ionic detergents, of which Triton X-100 is the most frequently and successfully used, disrupt lipid-lipid, lipid-protein, and DNA-protein interactions[89,90]. Detergents are frequently combined in decellularisation techniques, with variations in concentration and perfusion time, and require washing steps to remove residual traces after decellularisation[91,92]; (2) Hypertonic and hypotonic solutions. Hypertonic saline causes dissociation of DNA protein interactions[93], which, combined with cell shrinkage and swelling, causes cell lysis[94]. Debris clearance further steps to achieve full decellularisation[95]; (3) Acids and bases. Bases, such as ammonium hydroxide, have been used as an adjunct to detergent based decellularisation techniques to enable clearance of DNA which, in alkali solution, denatures to low viscosity single stranded nucleic acid, facilitating its removal by perfusion[96,97]. Acids such as peracetic acid have been used predominantly for sterilisation of scaffolds (see section 4 “Scaffold sterilization”). However, both bases and acids have significant detrimental effects on the ECM, by damaging collagen and other structural proteins, as well as by denaturing key growth factors[81,98]; (4) Alcohols. Alcohols diffuse into cells and cause cell lysis by a dehydrating mechanism, and thus have been used as decellularising agents[99,100], but also as sterilising agents either alone or in combination with acids; (5) Chelating agents[101-104]. Chelating agents such ethylenediaminetetraacetic acid and ethylene glycol tetra acetic acid bind metallic ions that are essential for protein interaction[105,106], resulting in the disconnection of intercellular integral proteins and disruption of cellular adhesion in the ECM. Full decellularisation requires additional agents such as detergents[107]; and (6) Enzymatic treatments[108-111]. A variety of enzymes have been utilised for tissue decellularisation, with trypsin and nucleases being the most frequently used. Trypsin is a serine protease that hydrolyses proteins involved in cellular attachment [112], thus dissociating cells from the ECM[113]. Nucleases (DNases and RNases) cleave phosphodiester bonds between nucleotides in nucleic acids and have been used to improve the removal of remaining

nucleic acid debris in conjunction with other decellularisation agents[114,115].

Implications of decellularisation technique heterogeneity: The above summary of techniques provides an insight into the enormous heterogeneity of approaches. In addition to the variety of methods above, many protocols use varying combinations of 2 or more methodologies. Furthermore, techniques vary in other factors including temperature of decellularising process, flow rates of perfusion agents. Such considerations may be quite subtle, yet critically important: for example, one study of tracheal decellularisation involving repeated cycles of decellularising agent reported that the number of cycles critically altered the integrity of the scaffold between cycles 18 and 22[116].

This technique heterogeneity reflects the differing requirements of different tissues. Tissues with obviously different macroscopic structures require different methodology: for example, perfusing a decellularising agent *via* the circulation in vascular tissue such as liver may be effective, but unlikely to be so in tough avascular structure of tendon. In this regard, a tissue classification of laminate, amorphous, composite, whole organ suggested by Keane[117]. Moreover, the matrisome (the protein content profile of the ECM) is subtly tissue dependant[118] such that even tissues of similar consistency may behave differently. For example, similar tissues such as tendon and ligament may behave quite differently despite exposure to same decellularising agent[119,120]. Furthermore, even within a defined tissue type, individual variation with factors including age and sex may affect matrisome content[121-124].

Though not intended to be comprehensive given the scope of this review, Table 1 provides examples of the breadth of tissues in which decellularisation has been studied, and range of decellularisation protocols. This reflects the fact that the field is at an empirical stage where methodology is in an assessment phase with multiple criteria to be considered. The optimal method of decellularisation may be difficult to determine and define particularly in the context of tissues with multiple cell types, as the optimum decellularising method for one cell type may not coincide with requirements for others. Attempts at decellularisation must perhaps be seen as producing an inevitably imperfect result, which may be corrected and refashioned by repopulating cells *in vitro* and in the host.

This high level of heterogeneity in tissue samples and technique raises the question of how to assess success in decellularisation. In this regard, Crapo *et al*[125] has suggested that successful decellularisation should be determined on the basis of producing ECM which (1) Does not contain more than 50 ng of DNA per mg dry weight; (2) with residual DNA fragments no longer than 200 bp; and (3) with no visible nuclear components, based on observations of *in vivo* adverse effects of these biochemicals[64,67,126]. The area of research is rapidly changing, and thus it is anticipated that new criteria of scaffold quality are likely to arise, as suggested by other authors[127-129]. Ultimately, the success of decellularisation is surely defined by the matrix to accept repopulating cells and whether those cells survive and collectively allow physiologically significant neo-organ function. These aspects will be discussed in detail as they pertain to liver function in section 5 (Liver decellularisation and recellularisation).

SCAFFOLD STERILISATION

Introduction

In vitro culture of mammalian cells provides ideal conditions for survival of cells of interest, but also for unwanted micro-organisms. Moreover, the potential for culture infection in decellularised scaffold experiments is higher than in standard cell culture given the non-sterile tissue of origin. Thus, not only for the success of *in vitro* scaffold repopulation, but also in terms of safety in the context of scaffold *in vivo* reimplantation, there is a need to eradicate microorganisms from decellularised scaffolds.

The ideal requirements for decontaminating agents would be (1) The ability to remove all microorganisms and spores; (2) to be removeable or non-toxic to repopulating cells or potential host; and (3) to leave the scaffold ECM unaltered. Thus, the end product could be tested in terms of its sterility, toxicity, and preserved biological properties.

In addition, there is a distinction to be made between sterilisation (killing or removing all microorganisms, including bacterial spores and disinfection (killing or removing all pathogenic microorganisms but not bacterial spores). Most protocols use disinfection techniques, but these may be deemed insufficient in the clinical context, should current experimental methodology progress to that stage. The section below provides an account of techniques used to remove micro-organisms from decellularised scaffolds prior to cell repopulation, as well as a summary of the studies that have compared the efficacy of these techniques.

Sterilisation and disinfection techniques

Irradiation: Irradiation using Gamma rays or electron beam act by inflicting direct damage to DNA and proteins, and by generation of oxidative species and free radicals. The advantages of irradiation are its delivery at room temperature, with no residual chemical toxicity, but with disadvantage of matrix denaturation with increasing dose[130]. To date, the main applications have been in bone and tendon [131] decellularisation.

Table 1 Examples of non-liver decellularisation protocols

Organ	Species	Decellularisation technique	Recellularization	Significant outcome	Ref.
Heart					
	Rat	SDS + Triton X-100	Neonatal cardio-myocytes	(1) Maintained eight constructs for up to 28 d by coronary perfusion in a bioreactor that simulated cardiac physiology; (2) Macroscopic contractions were observed by day 4; and (3) By day 8, under physiological load and electrical stimulation, constructs could generate pump function in a modified working heart preparation.	Ott <i>et al</i> [235]
	Pig	Freeze and Thaw + hypotonic solution + trypsin/EDTA/NaN ₃ + Triton X-100/EDTA/NaN ₃ + deoxycholic acid	Chicken embryonic cardio-myocytes	Cardiac extracellular matrix supported the formation of organized chicken cardiomyocyte sarcomere structure <i>in vitro</i> .	Wainwright <i>et al</i> [236]
	Rat	SDS <i>vs</i> POETE	Not performed	SDS decreased DNA and GAG and enriched the collagen content 10-fold.	Bruyneel <i>et al</i> [237]
	Pig	SDS <i>vs</i> Triton X-100 <i>vs</i> CHAPS <i>vs</i> OGP	Not performed	3% SDS as a detergent showed optimal decellularization.	Ferng <i>et al</i> [238]
	Rat	SDS + Triton X-100	Induced cardiac progenitor cells	(1) Optical mapping of recellularised scaffolds shows field-stimulated calcium transients that propagate across islands; and (2) Bipolar local stimulation demonstrated cell-cell coupling within scaffolds.	Alexanian <i>et al</i> [239]
Kidney					
	Rat	Saline + SNP + Triton X-100, DNase + SDS	Murine pluripotent embryonic stem cells	(1) Primitive precursor cells populated and proliferated within the glomerular, vascular, and tubular structures; and (2) Cells lost their embryonic appearance and expressed immunohistochemical markers for differentiation.	Ross <i>et al</i> [240]
	Monkey	1% SDS <i>vs</i> 1% Triton X-100	Not performed	SDS at 48C to be most effective in preserving the native architecture.	Nakayama <i>et al</i> [241]
	Pig	0.5% SDS <i>vs</i> 0.25% SDS <i>vs</i> 1% Triton X-100 with 0.1% ammonium hydroxide	Not performed	0.5% SDS was the most effective detergent.	Sullivan <i>et al</i> [242]
	Pig	SDS	Not performed	(1) Kidney decellularized scaffolds implanted in Yorkshire pigs easily re-perfused, sustained blood pressure; (2) Scaffolds maintained renal ultrastructure; and (3) However, presence of inflammatory cells in the pericapsular region and complete thrombosis of the vascular tree were evident.	Orlando <i>et al</i> [243]
	Rat, pig, and human	SDS	HUVECs + Rat Neonatal kidney cells	(1) The resulting grafts produced rudimentary urine <i>in vitro</i> when perfused <i>via</i> their intrinsic vascular bed; and (2) Transplanted orthotopic grafts in rats, perfused by the recipient's circulation, produced urine <i>via</i> the ureteral conduit <i>in vivo</i> .	Song <i>et al</i> [244]
	Pig	Sonication + SDS + Triton X-100	Not performed	(1) Significant decrease in decellularization time with sonication; and (2) Sonicator power proved to have significant effect on the microarchitecture integrity of the scaffold.	Manalastas <i>et al</i> [76]
Lung					
	Rat	Heparinized PBS + SDS + Triton X-100	HUVECs	Orthotopic Transplantation of grafts with 6 h of perfusion <i>in vivo</i> .	Ott <i>et al</i> [245]
	Rat	PBS + SNP + CHAPS + EDTA + Benzoylase	Rat neonatal lung epithelial + lung vascular endothelial cells	(1) <i>In vitro</i> , the mechanical characteristics of the engineered lungs were like those of native lung tissue; and (2) <i>In vivo</i> gas exchange for short time intervals (45 to 120 min).	Petersen <i>et al</i> [246]
	Mice	Triton X-100 + SDS + DNase	Embryonic stem cells	Demonstrated growth of foetal alveolar type II cells.	Price <i>et al</i> [247]
	Rat	Heparinized PBS + SDS + Triton X-100	HUVECs + rat foetal lung cells	Orthotopic transplantation of grafts with 7 d of perfusion <i>in vivo</i> .	Song <i>et al</i> [248]
Trachea					
	Rabbit	Freeze/thaw + Sonication + SDS	Not performed	(1) Orthotopic transplantation of decellularized scaffolds into segmental tracheal defects in rabbits; (2) Respiratory	Hung <i>et al</i> [249]

			epithelium regeneration on the inner surface; and (3) Cartilaginous tubular structures could not maintain structural integrity.	
Pig	Freeze and Thaw + Agitation/immersion + SDS	Not performed	Successful decellularization.	Guimaraes <i>et al</i> [82]
Rabbits	Sonication + 1 % SDS	Not performed	Orthotopic transplantation of partially decellularized trachea with no immunosuppression treatment resulted in 2 mo of survival in two rabbits and one long-term survival (2 years) in one rabbit.	Dang <i>et al</i> [71]
Nerve				
Human	Triton X-100 + SDS + EDTA + sonication	Not performed	Detergent and sonication more effective than detergent only.	Suss <i>et al</i> [74]
Small intestinal submucosa				
Pig	SDS/Triton X-100/DNase <i>vs</i> Agitation and immersion	Not performed	SDS/Triton X-100 combination for decellularization proved superior.	Syed <i>et al</i> [81]
Thyroid				
Rabbit	SDS + immersion/agitation	HTFC	The scaffolds exhibited good cytocompatibility, supported HTFCs growth, and proliferation.	Weng <i>et al</i> [85]

HTFC: Human thyroid follicular cells; SDS: Sodium dodecyl sulphate; HUVECs: Human umbilical vein endothelial cells.

Ethylene oxide: Ethylene oxide is a toxic organic compound which reacts with sulfhydryl, amino and carboxyl groups in proteins and nucleic acid molecules[132]. It is a gas at room temp and very permeable so penetrates tissues well, but is very adsorbent to decellularised ECM so difficult to clear, and may form toxic species with water such as ethylene glycol[133].

Peracetic acid: Peracetic acid is produced by the reaction of hydrogen peroxide and acetic acid, with antimicrobial activity resulting from the peroxide group (O-O) oxidation of sulfhydryl groups in proteins[134], and with activity against viral particles when combined with ethanol[135]. Although its advantages are that its decomposition molecules (acetic acid, water, and oxygen), are non-toxic and water soluble, it does result in chemical alteration of ECM[136]. There have been wide ranging applications including many examples in liver, with some favourable outcomes in comparative studies (Table 2).

Hydrogen peroxide and hydrogen peroxide low-temperature plasma: Hydrogen peroxide is a powerful oxidant which reacts with cell membranes and causes the denaturation of nucleic acids and proteins[137]. The plasma form of Hydrogen peroxide, generated by magnetic excitation of gas at low temperature, contains many charged and reactive species which also denature proteins and nucleic acids and cell membranes. Despite the advantage that the end decomposition products (water and oxygen) are non-toxic, the highly reactive original species do result in chemical alteration of proteins [138].

Alcohol: Alcohol disinfects by denaturing proteins. Although it does not eradicate spores, it has been found to be relatively sparing of ECM structure, allowing its use in a wide range of decellularised tissues tissues[131,139]. In the case of liver decellularisation, its use has been mostly in relation to processed ECM[140-142], such as ECM based hydrogels.

Ultra-violet light: Ultraviolet light in the 200–300 nm wavelength range is associated with the strongest disinfection properties, produced by direct DNA damage and generation of ozone as a reactive species. Its advantages are the relative ease of delivery, and the absence of toxic residue, but its limitations are its superficial penetration only, reflected in its use restricted to thin dimension tissues such as small intestine[136], or in case of liver, used for slices of tissue[143].

Antibiotics: Antibiotics use has been reported[131] for treatment of decellularised ECM, including liver [144,145], but their limitations are the restricted spectrum of activity and inability to eradicate spores.

Conclusions

Thus, there are numerous microorganism eradication options, and, based on the properties of sterilisation methods and suitability for specific tissue types, some authors[131] have suggested guidelines to recommend particular methods of sterilisation. In practice, whether these theoretical recommendations deliver the desired microbiological outcome is uncertain, and therefore experimental comparisons of methods seems indicated.

Table 2 Summary of studies comparing different sterilization techniques used for decellularised scaffolds

Species	Organ	Sterilization technique	Outcome	Ref.
Sheep	Liver	Compared 6 different sterilization methods: (1) Freeze drying; (2) Ethylene oxide gas; (3) Gamma irradiation; (4) Gamma irradiation + Peracetic acid; (5) Gamma irradiation + Ethylene oxide gas; and (6) Gamma irradiation + Freeze drying	(1) Peracetic acid or ethylene oxide + gamma irradiation was associated with the best outcome; and (2) Freeze drying and Gamma irradiation completely sterilized the liver, but also reduced the mechanical properties.	Kajbafzadeh <i>et al</i> [96]
Porcine	Liver	Compared 3 different sterilization methods: (1) Peracetic acid; (2) Ethanol; and (3) Slightly acidic electrolyzed water	(1) Ethanol caused a significant loss in collagen content; (2) The retained glycosaminoglycan content decreased in all treatments; and (3) Peracetic acid and slightly acidic electrolyzed water treatments achieved the highest efficiency of sterilization.	Hussein <i>et al</i> [148]
Mouse	Lung	Compared 2 different sterilization methods: (1) Gamma irradiation; and (2) Peracetic acid	(1) Irradiation produced significant structural distortion; and (2) Peracetic acid had less effect on the resulting architecture.	Bonenfant <i>et al</i> [149]
Porcine	TMJ Fibro-cartilage disc	Compared 3 different sterilization methods: (1) Peracetic acid; (2) Gamma irradiation; and (3) Ethylene oxide.	(1) Gamma irradiation and Ethylene Oxide caused structural damage leading to inferior cell adhesions; and (2) Peracetic Acid caused minimal structural damage but also induced chemical modifications leading to better cell attachments.	Matuska <i>et al</i> [146]
Porcine	Kidney	Compared 4 different sterilization methods: (1) 70% Ethanol; (2) 0.2% Peracetic acid in 1 M NaCl; (3) 0.2% Peracetic acid in 4% Ethanol; and (4) Gamma irradiation	(1) All four methods were successful in decontamination; (2) Gamma-irradiation was very damaging to collagen fibres and glycosaminoglycans, leading to less proliferation of human renal cortical tubular epithelium cells; and (3) 0.2% peracetic acid in 1 M NaCl was found to be the best method as it completely decontaminated the renal tissue and demonstrated to have preserved essential components of the ECM.	Poornejad <i>et al</i> [139]
Porcine	Liver	Compared 2 different sterilization methods: (1) Hydrochloric acid; and (2) acetic acid.	(1) ECM treated with Acetic acid showed higher initial attachment and albumin and urea production in HepG2/C3A cell cultures compared to Hydrochloric acid; and (2) Acetic acid preserved bioactive moieties compared to Hydrochloric acid.	Coronado <i>et al</i> [97]
Rabbit	Kidney	Compared 4 different sterilization methods: (1) Antibiotics (Penicillin G, Amphotericin B and Gentamicin); (2) Peracetic acid (0.5 %, 1% and 1.5 %); (3) Gamma irradiation 5 KG; and (4) 3 UV-irradiation 20-480 nm	(1) UV-irradiation is not able to sterilize; (2) Gamma irradiation resulted in reduced mechanical strength and altered microstructure; and (3) 0.5 % Peracetic acid was the most efficient method to completely decontaminate rabbit decellularized kidney while preserving the mechanical properties and main components of the matrix.	Moradi <i>et al</i> [147]

ECM: Extracellular matrix.

In this regard, only a few comparative studies have been carried out for different organ systems including liver and are summarised in Table 2. Drawing confident conclusions from these studies is difficult because of heterogeneity in the range of techniques used, range of tissues examined, in different animal species.

However, from the studies where comparisons were made, there appears to be some degree of consistency favouring the use of peracetic acid, in achieving sterility with minimal ECM damage in sheep liver[96], porcine kidney[139], porcine temporo-mandibular joint disc[146], rabbit kidney[147], porcine liver[97,148], and mouse lung[149].

LIVER DECELLULARISATION AND RECELLULARISATION

Introduction

Since the first report of successful decellularisation and repopulation of liver tissue carried out in rat liver by Uygun *et al*[86], there have been significant developments with further reports in other models, and evolution in many aspects including the challenge of sizing up technology for larger species livers, investigation of optimal decellularisation method, progress in the variety, delivery, and functional assessment of repopulating cells, culminating in recent reports providing the first evidence of physiologically significant function in large animal bioengineered organs. This section provides an account of areas of advance, highlighting studies which have contributed incremental progress in the field, and for which additional information is given in Table 3.

Liver decellularisation

Similar to the situation in the non-hepatic context, numerous protocols for liver decellularisation have been reported[150,151], varying in nature of decellularising agents, technique, and time required

Table 3 Liver decellularisation recellularisation studies

Species	Decellularisation method	Recellularisation cell type and route	Outcome	Ref.
Female Lewis rats	SDS + Triton X-100	(1) Primary rat hepatocytes <i>via</i> the Portal vein; and (2) Rat cardiac microvascular endothelial cells <i>via</i> portal vein	(1) Demonstrated Successful decellularization/Recellularization with cell viability and function; (2) Demonstrated the feasibility of transplanting these recellularised liver grafts <i>in vivo</i> with minimal ischemic damage; and (3) The recellularised graft supports liver-specific function including albumin secretion, urea synthesis and cytochrome P450 expression at comparable levels to normal liver <i>in vitro</i> .	Uygun <i>et al</i> [86]
Fisher 344 rats	Triton X-100 + SDS	Rat liver progenitor cell line WB344 through the inferior vena cava	(1) Perfusion with 0.1% SDS for 1 hour completely cleared all DNA; and (2) Supplementation of all perfusion solutions with antibiotics/antimycotics prevented microbial growth, and the IDL could be stored at 4°C for several weeks.	Shupe <i>et al</i> [156]
Male Sprague Dawley rats	Trypsin + EGTA + Triton X-100	Primary mice hepatocytes <i>via</i> : (1) Direct parenchymal injection; (2) Continuous perfusion <i>via</i> the portal vein; and (3) Multistep infusion <i>via</i> the portal vein	Systematic comparison of three different reseeded methods showed that a multistep strategy provides the greatest seeding efficiency and the presence of functional hepatocytes.	Soto-Gutierrez <i>et al</i> [164]
Male Lewis rats	SDS + Triton X-100	Primary rat hepatocytes <i>via</i> the portal vein (from spheroid culture)	(1) Layer-by-layer heparin deposition was used to avoid thrombosis, followed by repopulation of hepatocytes, and successfully implanted as a TEL into the portal system; (2) Treatment of extended hepatectomized rats with a TEL improved liver function and prolonged survival; mean lifespan was extended from 16 to 72 h; and (3) At 72 h post operation, the TEL sustained functional and viable hepatocytes.	Bao <i>et al</i> [174]
Ferret	Distilled water + Triton X-100 + ammonium hydroxide	Human foetal liver cells + human umbilical vein endothelial cells co-infusion <i>via</i> the portal vein	Demonstrated delivery of cells to different compartments of the liver tissue <i>via</i> different pathways EC delivered through the vena cava selectively seeded larger and smaller blood vessels up to the pericentral area of the liver lobule and cells seeded through the portal vein reached predominantly the periportal area of the liver lobule.	Baptista <i>et al</i> [90]
Adult male Sprague-Dawley rats	SDS or Triton X-100 + sodium hydroxide	Primary rat hepatocytes <i>via</i> the portal vein	Decellularised scaffolds constructed by perfusion of Triton X-100 were of superior quality and can provide a more effective and ideal scaffold for tissue engineering and regenerative medicine.	Ren <i>et al</i> [161]
Porcine	SDS + DNase	Porcine hepatocytes <i>via</i> the portal VEIN	Demonstrated a protocol to decellularise rapidly a full-size porcine liver with small detergent volumes within 24 h.	Bühler <i>et al</i> [153]
Human	Distilled water + SDS + Triton X-100	Human cell lines hepatic stellate cells (LX2), hepatocellular carcinoma (Sk-Hep-1) and hepatoblastoma (HepG2) <i>via</i> suspension	Decellularised human liver cubic scaffolds were repopulated for up to 21 d using human cell lines with excellent viability, motility and proliferation and remodelling of the extracellular matrix.	Mazza <i>et al</i> [154]
Piglet	Triton X-100 + ammonium hydroxide	Murine endothelial cells (MS1) with combination of static and perfusion techniques (<i>via</i> the portal vein)	(1) Developed an effective method for re-establishing the vascular network within decellularised liver scaffolds by conjugating anti-endothelial cell antibodies to maximize coverage of the vessel walls with endothelial cells; (2) This procedure resulted in uniform endothelial attachment throughout the liver vasculature extending to the capillary bed of the liver scaffold and greatly reduced platelet adhesion upon blood perfusion <i>in vitro</i> ; and (3) The reendothelialized livers, when transplanted to recipient pigs, were able to withstand physiological blood flow and maintained for up to 24 h	Ko <i>et al</i> [89]
Porcine	SDS + Triton X-100	Rat primary hepatocytes and human umbilical vein endothelial cells (cells cultured in scaffolds, but not in a perfusion circuit)	(1) The heparinized scaffolds showed improved anticoagulation and cytocompatibility compared to the control scaffold both <i>in vitro</i> and <i>in vivo</i> test; and (2) The layer-by-layer technique showed that heparinisation did not interfere with hepatocyte or endothelial cell repopulation.	Bao <i>et al</i> [176]
Porcine	SDS	Human EA.hy926 endothelial cells and HepG2 hepatic carcinoma cells <i>via</i> the portal vein	(1) The study demonstrated, exposing scaffold to heparin-gelatin mixture improved endothelial cell ability to migrate and cover vessel discs, perhaps by exploiting gelatin's multiple integrin binding sites which facilitate endothelial cell binding; and (2) Scaffolds repopulated with Hep G2 hepatocytes and endothelial cells after heparin gelatin coating showed improved <i>ex vivo</i> blood perfusion, in comparison to uncoated scaffolds.	Hussein <i>et al</i> [87]
Male Lewis rats	Trypsin + EGTA + Triton X-100	Primary rat hepatocytes <i>via</i> the bile duct and the portal vein	The study results suggest that biliary tree cell-seeding approach is promising, and that liver progenitor cells represent a good cell source candidate.	Ogiso <i>et al</i> [173]
Male Lewis rats	Trypsin + EGTA +	(1) Primary rat hepatocytes	(1) Hepatocytes co-seeded with LSECs retained their function	Kojima <i>et</i>

	Triton X-100	<i>via</i> the Bile duct; and (2) LSECs <i>via</i> the portal vein	compared with those seeded alone; (2) LSECs maintained hepatic function, and supported hepatocyte viability under blood perfusion in the engineered liver graft owing to their antithrombogenicity; and (3) Successfully achieved continuous blood flow into the vascularized liver graft by extracorporeal perfusion for at least 8 hours	<i>al</i> [172]
Female Lewis rats	SDS + Triton X-100	Human EA.hy926 endothelial cells <i>via</i> the portal vein	(1) Coupled the cell-binding domain REDV to the vasculature of decellularised rat livers; and (2) REDV coupling increased cell attachment, spreading and proliferation of endothelial cells within the scaffold resulting in uniform endothelial lining of the vasculature, and a reduction in platelet adhesion and activation	Devalliere <i>et al</i> [88]
Female Lewis rat	SDS	(1) Rat cholangiocytes <i>via</i> the common bile duct; and (2) Rat hepatocytes <i>via</i> the portal vein	(1) Demonstrated for the first time, whole liver grafts co-populated with hepatocytes and cholangiocyte; (2) Cholangiocytes formed duct-like structures, with the viable hepatocyte mass residing in the parenchymal space, in an arrangement highly comparable to the native tissue; and (3) Both albumin and urea assay results confirmed hepatocyte functionality and the gene expression analysis of cholangiocytes in recellularised liver grafts indicated viability and sustained gene expression of functional proteins.	Chen <i>et al</i> [177]
Adult Sprague-Dawley rats	Triton X-100 + NH4OH	Rat sinusoidal endothelial cells were perfused <i>via</i> the Portal vein in either RPMI media or in 5% gelatin hydrogel solution	(1) Used immortalized endothelial cells to repopulate decellularised rat liver scaffolds; (2) Gelatin hydrogels-based perfusion significantly increased the number of cells that were retained in the scaffolds; and (3) The Doppler ultrasound detected active blood flows within the re-endothelialised liver scaffolds 8 d post heterotopic transplantation.	Meng <i>et al</i> [190]
Male Lewis rats	Trypsin/EGTA solution + Triton X-100/EGTA	Human induced pluripotent stem cells derived hepatocyte-like cells <i>via</i> bile duct	(1) The first study to generate a recellularised liver model with human hepatic function using human induced pluripotent stem cells; and (2) This result suggested that the BD was an appropriate recellularization pathway regardless of the hepatocyte type.	Minami <i>et al</i> [250]
Porcine	SDS + Triton X-100	Human umbilical vein endothelial cells <i>via</i> the superior vena cava followed by <i>via</i> the portal vein	Decellularised whole porcine livers revascularized with human umbilical endothelial cells and implanted heterotopically into immunosuppressed pigs whose spleen has been removed sustained perfusion for up to 20 d.	Shaheen <i>et al</i> [191]
Porcine	Triton X-100 + SDS	(1) Human umbilical vein endothelial cells <i>via</i> the vena cava and the portal vein; and (2) Porcine hepatocytes <i>via</i> the bile duct	(1) Co-seeded primary porcine hepatocytes after human umbilical vein endothelial cell reendothelialization; and (2) Repopulated scaffolds were implanted heterotopically in a pig model and produced improved biochemical function in an acute liver failure model.	Anderson <i>et al</i> [175]
Female Sprague-Dawley rats	SDS + DNase	Human umbilical vein endothelial cells <i>via</i> the Portal vein	(1) Used aptamers (short, single-stranded DNA or RNA molecules that selectively bind to specific targets) with CD31 specificity; and (2) Aptamer coated scaffolds showed higher endothelial cell coverage, enabled perfusion with blood for 2 h with reduced platelet adhesion <i>ex vivo</i> , and restored liver function in a hepatic fibrosis rat model.	Kim <i>et al</i> [192]

TEL: Tissue-engineered liver; SDS: Sodium dodecyl sulphate; LSECs: Liver sinusoidal endothelial cells; REDV: Arg GluAsp Val.

ranging from hours[86,152] to days[153], to weeks[154] (rat, pig, human respectively) correlating with organ size. Perfusion of decellularising agents *via* the vasculature is the only means of reaching whole parenchymal space in a large organ such as the liver and has been used in all such studies.

The vessels available for infusion of decellularising agents are the portal vein, hepatic artery, and hepatic veins. Of these options, perfusion *via* the portal vein has been used most frequently although some authors report infusion *via* the hepatic veins *via* the inferior vena cava[155,156], the hepatic artery [157], and the hepatic artery and portal vein in combination[158]. Determining whether infusion route is an important factor in decellularisation quality is difficult as almost all studies report one particular technique, presumably arrived at empirically. Two studies suggest pulse flow *via* the hepatic artery provided better quality decellularisation, though whether this improved recellularisation potential with repopulating cells was not assessed[159,160].

Choice of detergent for decellularisation is equally varied though protocols using SDS and/or triton X-100 are the most frequently used, with SDS more effective at removing cellular debris, but at the expense of greater detriment to ECM structure. There are few comparative studies, with the exception of those of Ren *et al*[161], Wu *et al*[162], and Kajbafzadeh *et al*[96], showing lesser matrix degradation (with better structural protein, growth factor and glycosaminoglycan retention) and better repopulating cell function with triton X-100 in rat, porcine and sheep liver decellularisation models respectively.

In addition to biochemical content, mechanical structure of ECM important in contributing to signals which influence cell function[46]. In studies comparing protocols in sheep liver, Triton X-100 and SDS resulted in scaffolds with similar tensile strength, but Triton X-100 based protocols resulted in better retention of elasticity[96,163].

Disinfection and sterilisation of scaffold

Diverse methods have been used to eradicate micro-organisms from decellularised liver scaffolds. Once again there are few studies directly comparing the available methods, but those that exist provide some consensus in favour of perfusion with peracetic acid with reports in sheep[96] and porcine[97,148] liver models, suggesting that peracetic acid was optimal in the dual objective of achieving sterility and maintenance of matrix structure, albeit with protocols varying in concentration and time of exposure.

Characterisation of decellularised scaffold

As a result of the many decellularisation and sterilisation techniques, arises a need for some means of assessing the resultant scaffold to enable comparisons of scaffold quality not only for comparative research but also in view of future clinical applications. Ultimately, although the most meaningful quality criterion is how successfully a scaffold accommodates repopulating cells to generate a neo-organ with useful function, this high-level objective has proved difficult to achieve, resulting in the use of intermediary scaffold assessment methods. It is likely that as research advances, new criteria will emerge, with those which best predict end function becoming dominant.

DNA content: Some of the earliest scaffold quality criteria were put forward by Crapo *et al*[125], who suggested that successful decellularisation should be determined on the basis of producing ECM which (1) Does not contain more than 50 ng of DNA per mg dry weight; (2) with residual DNA fragments no longer than 200 bp; and (3) with no visible nuclear components[125], based on observations of *in vivo* adverse effects of these biochemicals[64,67,126]. In addition to gel electrophoretic methods to determine DNA fragment length, light microscopy with hematoxylin and eosin stain and DAPI stain have been used to demonstrate absence of residual DNA and supplemented by electron microscopy to visualise cell free matrix microarchitecture[86].

Protein and complex polysaccharide content: In contrast to nucleic acids which must be removed, there is a need to preserve structural proteins, growth factors and other complex molecules in the matrix. Many studies report qualitative and quantitative measures of the structural proteins collagen, laminin, elastin, fibronectin as well as glycosaminoglycans[86,158,161], whilst others quantify pre and post decellularisation content for known ECM associated growth factors including hepatocyte growth factor [161], basic fibroblast growth factor[164], vascular endothelial cell growth factor (VEGF) and insulin-like growth factor 1[165], and many others described by Park *et al*[166].

Non-destructive scaffold assessment: The above methods of scaffold assessment require physical sampling and destruction of the decellularised scaffold, preventing its subsequent use for recellularisation. Thus, pursuing the need to establish methods of scaffold assessment that leave the scaffold intact for further experimentation, Geerts *et al*[167] describe non-destructive methods of scaffold assessment by computerised tomography and biochemical analysis of decellularisation effluent perfusate.

Vascular tree structural integrity: The vasculature has a particular importance in the intended aim of recellularisation as parenchymal cell populations are critically dependant on a reliable blood supply. Thus many authors report preservation of ECM scaffold which define vessels as demonstrated by injection of coloured Dextran[90], radio-opaque dye[165], and corrosion casts[168].

Liver scaffold recellularisation

Repopulating cell heterogeneity: With recellularisation of scaffolds comes the choice of repopulating cells. Many different cell types have been investigated including cell lines, induced pluripotent stem cells (iPSCs), mesenchymal stem cells, foetal stem cells, primary adult cells, and their propagated form after culture in organoids -all with associated advantages and shortcomings.

Immortalised cell lines are useful experimental work tools in that they offer a homogeneous population with a stable phenotype, which can be easily propagated in large numbers. However, there is little or no scope for a role beyond experimentation and into clinical applications given the risk of unchecked proliferation and malignant transformation. iPSCs[166] are also very powerful experimental tools with all the advantages of cell lines, and the added benefits of phenotypic versatility, but are similarly limited in clinical applications because of malignant transformation concerns. Mesenchymal stem cells offer a potentially clinically relevant cell type in terms of sourcing, propagation and safety, with possible beneficial immune modulation effects[169], but are probably limited in their range of differentiation end points[170]. Hepatic foetal cells[90] offer advantages of propagation and differentiation plasticity, but have little clinical application potential because of ethical, availability, and immuno-allogeneicity issues. Primary cells[164] offer the advantages of stable, mature phenotype without concerns for malignant transformation, but present difficulties in terms of sourcing, and propagation to clinically relevant cell numbers during which loss of function is often observed. Organoid cultured primary cells (discussed in more detail in the section on cholangiocyte recellularisation below) may offer a realistic solution to expanding primary cells *in vitro* without loss of desirable phenotype.

Thus, there are a multitude of studies reporting hepatic scaffold repopulation using a variety of cell types, introduced into scaffolds *via* different routes, and using various cell combinations, and reporting different means of assessing the repopulated scaffold. The sections below deal with this heterogeneity by describing progress in recellularisation by considering each main hepatic cell type. It is entirely acknowledged however, that optimal function will be achieved by simultaneous co-recellularisation of a variety of cell types, as cell interactions are critical for optimal cell function[171]. Key examples of this concept in the liver recellularisation literature include the reports of (1) Baptista *et al*[90] showing that human foetal liver cells and human umbilical vein endothelial cells (HUVECs) exhibited better function when infused together in scaffold than individually; (2) Barakat *et al*[168], showing that human foetal stellate cells and human foetal hepatocytes together resulted in the generation of mature hepatocyte phenotype; and (3) Kojima *et al*[172], showing that co-seeding of hepatocytes with liver sinusoidal endothelial cells (LSECs), but not HUVECs, improved hepatocyte function.

Hepatocyte recellularisation: The first report of liver tissue decellularisation and repopulation by Uygun *et al*[86] in a rat model was followed by others in rodent models[172-174], and thereafter on a larger scale in pig[153] and human livers[154].

These and other models have used a variety of hepatocyte sources for recellularisation including mostly primary hepatocytes[175,176], but also primary hepatocytes after spheroid propagation[174], foetal hepatocytes[90] and hepatocyte carcinoma cell lines[87].

The mechanism of re-introduction of hepatocytes has been by means of infusion *via* the portal vein in the vast majority of studies, though infusion *via* multiple vascular routes (Hepatic artery, Portal vein, supra and infra hepatic vena cava)[89] and *via* the *via* bile duct[175] have also been reported. There are few comparative studies to determine whether one or other route is optimal, though one study reports significantly higher parenchymal engraftment of hepatocytes after infusion *via* the biliary tree in comparison to the portal vein[173]. For portal vein infusion of hepatocytes, multiple sequential infusions result in better cell engraftment efficiency, cell proliferation, and cell function than infusion of the same number of hepatocytes in one single infusion[86,164].

Many indicators of function have been used to assess the function of hepatocytes reintroduced into decellularised scaffolds, including: (1) Albumin and urea production[86,90]; (2) elimination of ammonia, consumption of glucose and expression of cytochrome p450 metabolic enzymes[161,164,175]; (3) Immunofluorescence demonstration of expression of hepatocyte-specific marker fumarylacetoacetate [175]; (4) Immunostaining demonstration of hepatocyte viability enzymes such as UDP glucuronosyl-transferase 1, glucose6phosphatase[86]; (5) Expression of dipeptidyl peptidase- 4, a bile canaliculus marker, demonstrating hepatocyte polarity[172]; and (6) Immunofluorescence demonstration of hepatocytic lineage markers α -fetoprotein, CYP2A and CYP3A[90].

Ultimately, however, the most meaningful measure of hepatocyte function is whether a repopulated scaffold can exhibit significant function in the harsh test of *in vivo* physiological environment. Two studies have reported the early stages of such function: Bao *et al*[174] repopulated decellularised and heparin treated rat liver scaffolds with primary rat hepatocytes from spheroid culture. Repopulated scaffolds were implanted heterotopically in rats having undergone 90% hepatectomy, with control animals undergoing 90% hepatectomy without scaffold implantation. At 72 h post-operation, hepatocytes in the implanted scaffolds expressed liver specific genes, including coagulation factor X, albumin, and cytochrome P450. In contrast to control rats whose ammonia levels rose substantially, scaffold implanted rats had significantly slower ammonia increases, and mean survival in this acute liver failure model was increased from 16 h to 72 h.

Anderson *et al*[175] repopulated a decellularised porcine liver using HUVECs infused *via* the vena cava and portal vein and porcine hepatocytes *via* bile duct infusion.

In vitro assessment of the repopulated scaffolds showed increasing production of Von Willebrand factor over time, albumin production, ammonia detoxification and urea production. The presence of HUVEC repopulated vasculature was essential to sustain blood flow in an *ex-vivo* blood circuit. The authors also investigated a porcine heterotopic liver transplant model of acute liver failure. Thus, scaffold portal vein and vena cava were anastomosed to native portal vein and inferior vena cava respectively, and native liver blood flow was entirely abolished by ligation of native portal vein branches and arteries to native liver. The scaffolds sustained flow for 48 h during which intracranial pressure (ICP) and ammonia levels (indicators of acute liver failure) were monitored. Control animals underwent portocaval shunt and liver devascularisation without scaffold implantation. Although no definite differences were seen in ICP measurements, the scaffold transplanted animals showed clear evidence of ammonia level stabilisation in contrast to inexorable increase in control animals. The authors suggested that scaffold functionality was limited by the small size of the grafts (required by the heterotopic implantation) which restricted the number of implanted hepatocytes and resulted in significant small for size syndrome.

Cholangiocyte repopulation: In comparison to hepatocyte repopulation, there are to date few if any reports of repopulation of the biliary tree using primary cholangiocytes. In a rare report in this category, Chen *et al*[177] repopulated a decellularised rat liver scaffold with primary rat cholangiocytes *via* the bile duct and hepatocytes *via* the portal vein, and perfused the repopulated scaffold for 48 h *in vitro*,

with assessments showing expression of a number of cholangiocyte genes including cytokeratin 7, Cystic Fibrosis transmembrane conductance regulator (CFTR), hepatocyte nuclear factor-1 alpha (HNF-1 α), gamma glutamyl transferase (GGT).

The reason for the relative absence of studies reporting repopulation with primary cholangiocytes is the longstanding challenge of propagating and maintaining cholangiocytes in conventional cell culture with loss of essential phenotype[178]. As an alternative, driving pluripotent stem cells towards cholangiocyte differentiation requires extensive manipulation[179] and the clinical applicability of such cells remains in doubt in terms of the risk of malignant change[180]. The evolution of organoid culture, however, offers possible opportunities.

Organoid cultures are 3D cell culture systems whereby cells of choice, when placed in the 3D environment of a supporting substrate (typically Matrigel) undergo cell differentiation, self-organization, whilst retaining the ability to propagate[181]. Thus, organoid culture has provided a potential solution to the supply of biliary epithelial cells, allowing expansion of cholangiocytes from small adult tissue samples whilst retaining cholangiocyte phenotype[182] such expression of such as cytokeratins 7 and 19, and epithelial cell adhesion molecule. The technique, first achieved with intra-hepatic human cholangiocytes from a liver biopsy by Huch *et al*[183], was then confirmed subsequently using extrahepatic bile duct cholangiocytes[184], and bile derived cholangiocytes[185,186], with demonstrable transcriptomic and phenotypic differences between cholangiocytes of different origin within the biliary tree[187].

The availability of cholangiocytes provided by organoids has allowed their use in repopulation of decellularised biliary tissue in several models. Thus, Willemse *et al*[188] repopulated decellularised human bile duct tissue with intra hepatic, extra hepatic, and bile derived cholangiocytes from organoid culture and analysed expression of cholangiocyte markers and biliary function of the tissue engineered constructs. In contrast to intra hepatic counterparts, extra hepatic and bile derived cholangiocytes repopulated decellularised bile duct efficiently, exhibited tight junctions and polarity with apical cilia, showed a gene expression profile suggesting maturation of cholangiocytes, as well as appropriate expression cholangiocyte-specific transporter genes such as CFTR, which was active in a functional assay. Similarly, Roos *et al*[189] isolated cholangiocytes from human bile collected from gall bladders after cholecystectomy, percutaneous trans-hepatic cholangiography, and endoscopic retrograde cholangio-pancreatography (ERCP), and demonstrated efficient and long-term organoid culture (passage > 15 over > 5 mo). The cholangiocytes in organoids showed transcriptomic patterns consistent with native cholangiocytes, expressed functional ion channel protein MDR1, and efficiently repopulated decellularised human bile duct scaffolds.

The potential of organoid cultured cholangiocytes was further emphasised by Sampaziotis *et al*[184] who cultured biliary organoids using human cholangiocytes from deceased donors as well as ERCP brush samples. Transcriptomic analysis showed maintained genetic stability over passages and expression of key biliary markers, including cytokeratins 7 and 19, HNF-1 β , GGT, secretin receptor, sodium-dependent bile acid transporter (SLC10A2), CFTR and SRY-box 9. Electron microscopy revealed the presence of ultrastructural features characteristic of cholangiocytes, including cilia, microvilli, and tight junctions. Finally, several assays demonstrated key functionalities: (1) Rhodamine 123 accumulated in the ECO lumen only in the absence of the MDR1 antagonist verapamil; (2) fluorescent bile acid cholyl-l-lysyl-fluorescein was actively exported from cholangiocyte organoids; and (3) Secretin promoted water secretion, resulting in distension of the bile duct lumen, whereas somatostatin negated the effects of secretin. Moreover, *in vivo*, the cholangiocytes self-organized into bile duct-like tubes after transplantation into nude mouse kidney capsule. Finally, the cholangiocytes maintained their phenotype in biodegradable polyglycolic acid scaffolds discs and densified collagen cylinders. Respectively, the repopulated structures were used in mouse *in vivo* models to successfully repair gall bladder wall and reconstitute a functional extra-hepatic biliary tree.

In a further analysis of the potential of organoid cultured cholangiocytes, Sampaziotis *et al*[186] isolated human cholangiocytes for intrahepatic, extrahepatic and gall bladder bile. Transcriptomic analysis showed that cholangiocytes from different sites expressed a core of similar genes but differed in others. The cholangiocytes displayed a gradual shift in their transcriptional profile along the biliary tree, suggesting a response to region-specific microenvironments. Thus, when grown in organoid culture, cholangiocytes of different regions of the biliary tree reverted to a single common expression profile but, when exposed to gall bladder bile adopted the expression profile corresponding to the site of origin of bile. Using a mouse model of cholangiopathy induced by 4,4'-methylenedianiline, intraductal delivery of human gallbladder organoids resulted in engraftment of cholangiocytes, correction of cholangiopathy and phenotype rescue, in comparison to 100% fatality amongst the control group. In a human liver model using discarded deceased donor livers with ischaemic biliary injury, injected organoids engrafted in areas of denuded biliary epithelium, and corrected cholangiopathy.

Thus, in conclusion, whilst there has been a deficit in reports of biliary tree repopulation for decellularised liver scaffolds since the first report of this approach in 2010 from Uygun *et al*[86], the advent of organoid culture appears to have provided a novel means of propagating stable, functional cholangiocytes in sufficient numbers. This would appear to be the best current way of progressing with biliary repopulation of decellularised liver scaffolds.

Hepatic vascular recellularisation: Reconstitution of a viable vasculature in a decellularised liver scaffold is of paramount importance, to allow not only function but survival of the other liver cell populations. The objective is complicated in the case of the liver because of its dual blood inflow supply *via* hepatic artery and portal vein, the immensely complex architecture of liver sinusoids, and the uniquely specialist functions of the sinusoidal endothelial cells. This area of research has progressed in terms of the range of cells used, attempts to optimise the quality of endothelial cover to minimise thrombosis, and advancement in large animal blood perfusion models.

In the first report relating to liver scaffold repopulation, Uygun *et al*[86] used commercially sourced rat cardiac microvascular cells to create an endothelial lining, allowing the repopulated scaffold to be perfused in an *ex-vivo* rat blood circuit for 24 h, and in an *in vivo* heterotopic implantation model to renal vessels for an 8 h perfusion period. Subsequent reports have used a variety of cell types to create vascular cover including Ms1 cells[90], HUVECs[90,172,175,176], human EA.hy926 endothelial cell line [87,88], immortalised endothelial cells[190], and primary liver sinusoidal endothelial cells[172].

Functionality of these repopulated vascular cells has been assessed by various criteria including (1) light microscopy to show vascular cover[90], and supplemented with electron microscopy to demonstrate the presence of sinusoidal cell fenestrae[172]; (2) demonstrating the expression of endothelial cell gene product such as of Von Willebrand factor[90,175], endothelial nitric oxide synthase (eNOS)[90], Lymphatic vessel endothelial hyaluronan receptor 1 and stabilin 2 expression[191], Platelet endothelial cell adhesion molecule 1 (PECAM-1), CD34, VE-cadherin (vascular endothelial cadherin), eNOS, VEGF expression[87], sinusoidal endothelial marker (SE- 1) and stabilin-2[172]; (3) platelet adhesion studies[90]; (4) Transcriptomic analysis of infused HUVECs assuming an LSEC phenotype [191]; and (5) Glucose consumption rate[175,191] of infused endothelial cells.

Given the prime importance of preventing thrombosis in the scaffold, several approaches have explored treating the scaffold with anticoagulants and enhancing endothelial cell cover of the decellularised vascular network. Thus, Bao *et al*[174] investigated layer by layer deposition of heparin in decellularised scaffolds, with hepatocyte repopulation, and reported sustained blood perfusion up to 72 h in a heterotopic rat implantation model, in comparison to rapid thrombosis in un-heparinised scaffolds. In a later study, the same group[176] optimised the layer-by-layer technique and showed that heparinisation did not interfere with hepatocyte or endothelial cell repopulation.

Whilst interesting as a possible method of improving initial thrombogenicity, maintaining heparin deposition is not achievable in the longer term, and could present undesirable consequences. Thus, some authors have investigated the use of heparin to maximise endothelial cell cover, rather than chemically bonding it to scaffold. Studies reporting better endothelial cell repopulation in the presence of heparin preparations include that of Hussain *et al*[87], who reported that exposing scaffold to heparin-gelatin mixture improved endothelial cell ability to migrate and cover vessel discs, perhaps by exploiting gelatin's multiple integrin binding sites which facilitate endothelial cell binding. Scaffolds repopulated with Hep G2 hepatocytes and endothelial cells after heparin gelatin coating showed improved *ex vivo* blood perfusion, in comparison to uncoated scaffolds. Similarly, Meng *et al*[190] 2019 used immortalized endothelial cells to repopulate decellularised rat liver scaffolds. Gelatin hydrogels-based perfusion significantly increased the number of cells that were retained in the scaffolds, and Doppler ultrasound detected active blood flows within the re-endothelialised liver scaffolds 8 d post-transplantation.

Adopting a different approach, some groups have investigated the manipulation of endothelial cell attachment to scaffold to improve vascular cover. Devalliere *et al*[88] covalently coupled the cell-binding domain REDV to the vasculature of decellularised rat livers before seeding endothelial cells *via* the portal vein. REDV coupling increased cell attachment, spreading and proliferation of endothelial cells within the scaffold resulting in uniform endothelial lining of the vasculature, and a reduction in platelet adhesion and activation. Ko *et al*[89] conjugated anti-endothelial cell antibodies to liver scaffolds resulting in uniform endothelial attachment and reduced platelet adhesion upon blood perfusion *in vitro*. The re-endothelialised livers, withstood physiological blood flow *in vivo* for up to 24 h in a porcine implant model. Kim *et al*[192] used aptamers (short, single-stranded DNA or RNA molecules that selectively bind to specific targets) with CD31 specificity. Aptamer coated scaffolds showed higher endothelial cell coverage, enabled perfusion with blood for 2 h with reduced platelet adhesion *ex vivo*, and restored liver function in a hepatic fibrosis rat model.

In the most significant advances to date in the area of successful hepatic vascular perfusion of repopulated scaffolds, at least in terms of length of *in vivo* perfusion, Shaheen *et al*[191] seeded decellularised whole porcine livers with HUVECs and showed successful perfusion of the heterotopically implanted scaffolds into for up to 20 d. The same group[175] later co-seeded primary porcine hepatocytes after HUVEC reendothelialisation. Repopulated scaffolds were implanted heterotopically in a pig model and produced improved biochemical function in an acute liver failure model.

In conclusion, the difficult problem of repopulating the vasculature of decellularised scaffolds has seen significant progress, with reports of *in vivo* blood perfusion lasting many days. Whilst encouraging, there remain advances to be made in the development of clinically relevant cell populations for this purpose, and the repopulation of the highly specific liver sinusoidal endothelial cells.

Conclusions

The field of hepatic scaffold recellularisation has advanced from *in vitro* rodent liver scaffold models to large animal *in vivo* blood perfusion. Whilst this represents much progress, significant areas of development remain to be investigated. Of the different liver cell types, even in the case of hepatocytes where repopulation results are the most advanced, the degree of hepatocyte function observed to date is still limited. Cholangiocyte recellularisation is far behind, though organoid sourced cells may help with this challenge. Intra-hepatic vascular recellularisation has allowed impressive *in vivo* perfusion but using cells which have limited application beyond experimental models. Minority cell groups such as Kupffer cells and stellate cells, though important in their influence on other cell types, have not been repopulated decellularised scaffolds.

RECELLULARISATION OF EXTRA HEPATIC BLOOD VESSELS

Introduction

If the objective of whole liver recellularisation is the bioengineering of neo-organs is implantation to provide useful function, neo-livers will need to be fully reconnected to the recipient circulation, with both hepatic arterial and portal venous inflow, and hepatic vein outflow. To date, because the focus of investigation has understandably been to achieve viable blood circulation through the sinusoidal network, extra-hepatic vascular inflow has relied exclusively on portal reperfusion of recellularised grafts. Whilst much progress has been made with sustained portal perfusion of up to 20 d in large animal models[191], recellularisation and perfusion of the hepatic artery has not been reported. This gap in the field will need to be addressed, as, unlike hepatocytes which may survive on portal flow alone, the biliary tree is critically dependant on hepatic arterial supply.

Arterial scaffold recellularisation precedents

In addition to thrombogenicity, the hepatic artery presents considerable other difficulties stemming from the biophysical demands of withstanding arterial pressure in the short and long term. In the short term, a recellularised artery and its arterial anastomosis needs to be able to tolerate pressures of 3000 mmHg[193], and then do so in the long term without accelerated atherosclerosis.

The challenges of bioengineering viable arterial conduits[193] is an entire field in itself, with much research motivated by the clinical need represented by the immense burden of cardiac, cerebrovascular and peripheral vascular disease. The research trajectory of vascular biologists and clinicians in vessel bioengineering has followed much the same path as those studying the liver. As a result of the drawbacks of synthetic[194] and allogeneic and xenogeneic grafts[195] (long-term patency issues due to thrombosis, inflammation, and stenosis), there has been an evolution towards cellular repopulation of scaffolds of various types. Thus, following pioneering reports by Weinberg *et al*[196] of early bioengineered vessels containing collagen, Dacron and a combination of smooth muscle and endothelial cells, L'Heureux *et al.* reported the use of extracellular matrix with vascular cells to bioengineer a blood vessel[197], with subsequent reports of successful bioengineered grafts in clinical practice[198,199].

Arterial vessel anatomy is complex and consists of three concentric layers (1) the intima layer, composed of endothelial cells resting on an internal elastic lamina layer of type 4 collagen and elastin, which separates it from media; (2) the media, composed of smooth muscle cells (SMC), type I and type III collagen; and (3) the adventitia, containing fibroblasts embedded in a loose collagen matrix of type I and type II collagen. The ability of arteries to withstand arterial pressure waves stems from the complex tri-layer of cells and ECM above, which therefore likely requires recapitulation to achieve similar function in recellularised grafts. In the context of tubular grafts, this challenge has been investigated using a variety of biofabrication techniques including biomaterial moulding[200], cell sheet engineering [201], bio-ink applications, with tissue maturation[202] under fluid flow[203] in purpose designed bioreactors[193].

In the specific case of arterial scaffolds obtained by decellularisation techniques, followed by repopulation with appropriate cells, there are many examples of successful long term outcomes in a variety of experimental models, reviewed by Krawiec *et al*[204], and including (1) Cho *et al*[205] who used canine bone marrow mononuclear cells differentiated under different culture conditions to generate smooth muscle and endothelial phenotypes. These were reintroduced into decellularised dog carotid arteries sequentially to create media and intimal layers in neo-vessels, which were reimplanted in a canine carotid model. Seeded grafts were patent at 8 wk compared to thrombosis at 2 wk in unseeded controls; (2) Similarly, Zhao *et al*[206] used ovine bone marrow stem cells and differentiated them into endothelial and smooth muscle phenotypes, before seeding them onto decellularised carotid artery scaffolds. Seeded scaffolds were mechanically stable and patent at 5 months, in comparison to unseeded controls, which all occluded at 2 wk or less; (3) Kaushal *et al*[207] isolated endothelial precursor cells from peripheral blood of sheep, expanded them *ex vivo* and then seeded them on decellularised porcine iliac vessels. Seeded grafts remained patent for 130 d as a carotid interposition graft in sheep, whereas non-seeded grafts occluded within 15 d; (4) Borschel *et al*[208] repopulated decellularised rat femoral arteries with primary endothelial cells, which were implanted as interposition grafts. Patency rates at 4 wk were

89% and 29% recellularised grafts and control grafts respectively; (5) Ma *et al*[209] repopulated decellularised foetal pig aortas with canine endothelial cells and demonstrated 6-mo patency after reimplantation in a canine carotid model; and (6) Dahan *et al*[210] repopulated decellularised pig carotid artery with autologous endothelial and smooth muscle cells and demonstrated 6-week patency in a carotid interposition graft model.

Conclusion

Thus, the problem of arterial recellularisation brings very significant and specific challenges, but with some promising possible solutions suggested by long term successful perfusion bioengineered repopulated decellularised arterial neo-vessels in several animal models.

IMMUNOGENICITY OF DECELLULARISED SCAFFOLDS

Introduction

In its most ambitious objective, bioengineering neo-organs by decellularisation and recellularisation would involve the use of allogeneic or even xenogeneic scaffolds repopulated with appropriate cells originating from the intended recipient. The resultant neo-organ would thus in theory be immunologically syngeneic, at least from the perspective of the repopulating cells. The question remains, however, whether non-self scaffold, even if covered by syngeneic cells may elicit an adverse immune or inflammatory reaction.

Scaffold immunogenicity studies

Overwhelmingly, *in vivo* animal studies and human clinical studies examining implantation of decellularised scaffold show non pathological and constructive, functional tissue remodelling with the partial restoration of tissue appropriate to the site of implantation[211]. Examples of such animal studies include that of Mirmalek-Sani *et al*[157], who observed no local or systemic adverse host response to decellularised porcine liver scaffold introduced into rats, and similar report of studies involving further xenogeneic introduction of decellularised scaffolds of goat into mouse[212], rat into rabbit[213] and mouse into rat[214]. These results are matched by successful use of decellularised scaffolds in the clinical setting without adverse effect, such as that used by Lawson *et al*[199] who constructed bioengineered vascular grafts for dialysis in patients with chronic renal failure, and other clinical reports describing favourable results with the use of decellularised scaffolds in oesophageal tissue[215], tendon [216], major cardiac vessel[217], and chronic wound management[218]. However, there have also been reports, albeit in a small minority, of scaffold related inflammatory reactions[219,220], thus raising questions relating to the immunogenicity of decellularised ECM.

Depending on the nature of an implanted material into a host, the host response may broadly be characterised as either (1) Pro-inflammatory, eventually leading to the deposition of non-functional dense scar tissue, or, in contrast; and (2) 'constructive remodelling', leading to the controlled incorporation/degradation of the implanted material and its replacement with functional tissue consistent with the site of implantation[221,222].

The factors that determine which of these responses prevails are incompletely understood, but involve the interaction of the implanted material with innate[223] and adaptive immune system cells [224] such as the natural killer cells, macrophages, and lymphocytes, which can be directed to assume very different phenotypes, resulting in either a reconstructive or inflammatory reaction. The constructive remodelling response is characterised by the directing of macrophages towards the M2 (reconstructive) rather than M1 (inflammatory) phenotype, and the presence of T helper cells of Th2 phenotype, with cellular upregulation and downregulation of anti-inflammatory and proinflammatory genes respectively[225].

In relation to the reaction elicited by the implantation of decellularised ECM, investigation suggests that decellularised ECM per se does not elicit an inflammatory reaction, but does stimulate a strong pro-healing phenotype of the innate and adaptive immune systems[66,225,226]. Adverse reactions do result, however, as a consequence of retained cellular products from incomplete decellularisation[67,227], post decellularisation processing of scaffolds such as cross linking[100,228], or remnants of decellularising cells[229], or sterilising agents methods in the implanted scaffold[222].

The mechanism whereby decellularised ECM elicits a reconstructive response is incompletely understood but likely relates to molecular homology, the effect of bioactive molecules within the ECM, and the influence these biomolecules have on host immune and regenerative cells.

Thus, the constituent biochemicals of ECM, including laminin, collagens, fibronectin, and glycosaminoglycans are amongst the most highly conserved molecules in mammalian species[230]. As a result of this high degree of conservation, allogeneic and even xenogeneic ECM implants elicit similar 'self' recognition and constructive cell responses[225,231]. The infiltration of implanted decellularised scaffold by host cells results in the exposure and release of bioactive molecules including cryptic peptides, which modulate the immune response and direct innate and adaptive immune cells towards a reconstructive phenotype[232]. These, and other bioactive molecules within the ECM also act as

chemotactic agents for stem and progenitor cells *in vitro* and *in vivo*[233]. Indeed, cryptic peptides from collagen III can reproduce progenitor cell chemotaxis[40,234].

Conclusion

In conclusion, although there are some reports of adverse reactions to implantation of decellularised ECM, these examples are due to retained cellular products or decellularising agents, rather than the ECM itself, which elicits a favourable remodelling response, even if xenogeneic. This allows some optimism for the prospect of recellularising appropriate animal ECM scaffolds for clinical use in humans.

CONCLUSION

In the 12 years since the first report of liver decellularisation[86] and repopulation to the present, there has been much progress in the field, which has moved from predominantly *in vitro* small animal models to *in vivo* large animal models sustaining bioengineered liver perfusion for up to 20 d *in vivo*[191]. Despite this, many challenges and areas of investigation remain.

Firstly, even in the restricted domain of a single organ such as the liver, decellularisation protocols remain varied, and more often arrived at in empirical rather than comparative ways. Assessment of the quality of the decellularised scaffold is described according to numerous criteria with only some having been validated in terms of recellularisation efficacy. Standardisation of technique and quality assessment will need to progress significantly not only to facilitate experimental investigation, but also in future to meet clinical application standards. In the anticipation of sourcing human scaffolds from decellularised deceased donor livers, such considerations would apply particularly given the likely variability of scaffold quality, in contrast to the relative reproducibility of scaffolds originating from experimental animals. Should xenogeneic scaffolds ever be considered and repopulated with human cells, zoonotic as well as immunological concerns would have to be addressed.

In the area of recellularisation, the first hurdle remains the establishment of a viable vasculature, as no parenchymal function or survival is possible without it. In the liver, this is a particularly difficult problem because of the dual blood supply, and the uniquely specific functions of the sinusoidal endothelial cells. Thus, hepatic arterial recellularisation, and the fashioning of a neo-hepatic artery capable of withstanding arterial pressure has not been attained, but will be essential, as survival of the biliary tree will not be achieved without it.

Although recellularisation of portal sinusoidal and hepatic venous compartments has much progressed, with the achievement of *in vivo* perfusion albeit with portal hypertension[175], these results have been achieved with cells (often HUVECS) which, whilst providing excellent experimental tools, raise barriers to progress to the ultimate aim of recellularising scaffolds with cells from the intended recipient, and generating a syngeneic organ obviating the need for immunosuppression.

Immune considerations aside, the diversity of cell function in the vasculature of the liver is another area requiring investigation. Whilst HUVECs seem to assume some characteristics of liver sinusoidal cells when introduced into decellularised scaffolds, it remains to be shown that they can carry out the numerous, unique, and vital functions of LSECs. If they do not, a more refined recellularisation population will be required.

Assuming that a viable and fully functional vasculature is achieved, recellularisation of the main parenchymal elements, the hepatocytes and cholangiocytes, is also far from attained. In terms of the former, repopulation of decellularised scaffolds with primary hepatocytes has proved reproducible, but has only provided the beginnings of significant function, with temporary stabilisation of serum ammonia in the most successful *in vivo* models[175]. Amongst many others, endocrine, synthetic, detoxifying, and bile metabolic functions have not yet been demonstrated. Repopulation of the biliary tree is also unattained, till now largely due to the difficulty of propagating cholangiocytes in sufficient numbers, though this challenge may be alleviated by the advent of organoid culture. Other cell types, such as Kupffer cells and stellate cells, present as minorities in terms of numbers but significant in their influential interaction with hepatocytes and cholangiocytes, have not been investigated at all in recellularisation.

Although currently very distant, matters relating to clinical applications will also need much consideration. Thus, the entire process, decellularisation agents and methods, the resultant scaffold, and repopulating cells would need to meet stringent clinical grade standards. Concerns regarding scaffold immune response on the part of the host, though thus far not an observation in the context of experimental models, would have to be addressed more rigorously, as would zoonosis in the scenario of xenogeneic scaffolds.

Finally, it seems difficult to envisage that a clinical grade neo-organ could be generated entirely *in vitro*. More likely a partially recellularised scaffold may be produced, and require completion of repopulation *in vivo*, implying, at least temporarily, an auxiliary role for such neo-organs, rather than the prospect of transplantation in the manner that is practised with retrieved donated organs.

In summary, bioengineering of organs by decellularisation and repopulation remains a fascinating area still in an early phase of investigation, where the last decade has produced major advances but also left vast opportunity for research and development.

FOOTNOTES

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REFERENCES

- 1 **Moon AM**, Singal AG, Tapper EB. Contemporary Epidemiology of Chronic Liver Disease and Cirrhosis. *Clin Gastroenterol Hepatol* 2020; **18**: 2650-2666 [PMID: [31401364](https://pubmed.ncbi.nlm.nih.gov/31401364/) DOI: [10.1016/j.cgh.2019.07.060](https://doi.org/10.1016/j.cgh.2019.07.060)]
- 2 **Ratib S**, West J, Fleming KM. Liver cirrhosis in England-an observational study: are we measuring its burden occurrence correctly? *BMJ Open* 2017; **7**: e013752 [PMID: [28710203](https://pubmed.ncbi.nlm.nih.gov/28710203/) DOI: [10.1136/bmjopen-2016-013752](https://doi.org/10.1136/bmjopen-2016-013752)]
- 3 **Williams R**, Alexander G, Armstrong I, Baker A, Bhala N, Camps-Walsh G, Cramp ME, de Lusignan S, Day N, Dhawan A, Dillon J, Drummond C, Dyson J, Foster G, Gilmore I, Hudson M, Kelly D, Langford A, McDougall N, Meier P, Moriarty K, Newsome P, O'Grady J, Pryke R, Rolfe L, Rice P, Rutter H, Sheron N, Taylor A, Thompson J, Thorburn D, Verne J, Wass J, Yeoman A. Disease burden and costs from excess alcohol consumption, obesity, and viral hepatitis: fourth report of the Lancet Standing Commission on Liver Disease in the UK. *Lancet* 2018; **391**: 1097-1107 [PMID: [29198562](https://pubmed.ncbi.nlm.nih.gov/29198562/) DOI: [10.1016/S0140-6736\(17\)32866-0](https://doi.org/10.1016/S0140-6736(17)32866-0)]
- 4 **Tapper EB**, Parikh ND. Mortality due to cirrhosis and liver cancer in the United States, 1999-2016: observational study. *BMJ* 2018; **362**: k2817 [PMID: [30021785](https://pubmed.ncbi.nlm.nih.gov/30021785/) DOI: [10.1136/bmj.k2817](https://doi.org/10.1136/bmj.k2817)]
- 5 **Hirode G**, Saab S, Wong RJ. Trends in the Burden of Chronic Liver Disease Among Hospitalized US Adults. *JAMA Netw Open* 2020; **3**: e201997 [PMID: [32239220](https://pubmed.ncbi.nlm.nih.gov/32239220/) DOI: [10.1001/jamanetworkopen.2020.1997](https://doi.org/10.1001/jamanetworkopen.2020.1997)]
- 6 **Wong MCS**, Huang JLW, George J, Huang J, Leung C, Eslam M, Chan HLY, Ng SC. The changing epidemiology of liver diseases in the Asia-Pacific region. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 57-73 [PMID: [30158570](https://pubmed.ncbi.nlm.nih.gov/30158570/) DOI: [10.1038/s41575-018-0055-0](https://doi.org/10.1038/s41575-018-0055-0)]
- 7 **Younossi ZM**. Non-alcoholic fatty liver disease - A global public health perspective. *J Hepatol* 2019; **70**: 531-544 [PMID: [30414863](https://pubmed.ncbi.nlm.nih.gov/30414863/) DOI: [10.1016/j.jhep.2018.10.033](https://doi.org/10.1016/j.jhep.2018.10.033)]
- 8 **Liangpunsakul S**, Haber P, McCaughan GW. Alcoholic Liver Disease in Asia, Europe, and North America. *Gastroenterology* 2016; **150**: 1786-1797 [PMID: [26924091](https://pubmed.ncbi.nlm.nih.gov/26924091/) DOI: [10.1053/j.gastro.2016.02.043](https://doi.org/10.1053/j.gastro.2016.02.043)]
- 9 **Hart A**, Schladt DP, Zeglin J, Pyke J, Kim WR, Lake JR, Roberts JP, Hirose R, Mulligan DC, Kasiske BL, Snyder JJ, Israni AK. Predicting Outcomes on the Liver Transplant Waiting List in the United States: Accounting for Large Regional Variation in Organ Availability and Priority Allocation Points. *Transplantation* 2016; **100**: 2153-2159 [PMID: [27490411](https://pubmed.ncbi.nlm.nih.gov/27490411/) DOI: [10.1097/TP.0000000000001384](https://doi.org/10.1097/TP.0000000000001384)]
- 10 **Watson CJE**, Kosmoliaptis V, Randle LV, Gimson AE, Brais R, Klinck JR, Hamed M, Tsyben A, Butler AJ. Normothermic Perfusion in the Assessment and Preservation of Declined Livers Before Transplantation: Hyperoxia and Vasoplegia-Important Lessons From the First 12 Cases. *Transplantation* 2017; **101**: 1084-1098 [PMID: [28437389](https://pubmed.ncbi.nlm.nih.gov/28437389/) DOI: [10.1097/TP.0000000000001661](https://doi.org/10.1097/TP.0000000000001661)]
- 11 **Fraser JR**, Laurent TC, Laurent UB. Hyaluronan: its nature, distribution, functions and turnover. *J Intern Med* 1997; **242**: 27-33 [PMID: [9260563](https://pubmed.ncbi.nlm.nih.gov/9260563/) DOI: [10.1046/j.1365-2796.1997.00170.x](https://doi.org/10.1046/j.1365-2796.1997.00170.x)]
- 12 **Mouw JK**, Ou G, Weaver VM. Extracellular matrix assembly: a multiscale deconstruction. *Nat Rev Mol Cell Biol* 2014; **15**: 771-785 [PMID: [25370693](https://pubmed.ncbi.nlm.nih.gov/25370693/) DOI: [10.1038/nrm3902](https://doi.org/10.1038/nrm3902)]
- 13 **Gordon MK**, Hahn RA. Collagens. *Cell Tissue Res* 2010; **339**: 247-257 [PMID: [19693541](https://pubmed.ncbi.nlm.nih.gov/19693541/) DOI: [10.1007/s00441-009-0844-4](https://doi.org/10.1007/s00441-009-0844-4)]
- 14 **Muncie JM**, Weaver VM. The Physical and Biochemical Properties of the Extracellular Matrix Regulate Cell Fate. *Curr Top Dev Biol* 2018; **130**: 1-37 [PMID: [29853174](https://pubmed.ncbi.nlm.nih.gov/29853174/) DOI: [10.1016/bs.ctdb.2018.02.002](https://doi.org/10.1016/bs.ctdb.2018.02.002)]
- 15 **Singh P**, Carraher C, Schwarzbauer JE. Assembly of fibronectin extracellular matrix. *Annu Rev Cell Dev Biol* 2010; **26**:

- 397-419 [PMID: 20690820 DOI: 10.1146/annurev-cellbio-100109-104020]
- 16 **Kadler KE**, Hill A, Canty-Laird EG. Collagen fibrillogenesis: fibronectin, integrins, and minor collagens as organizers and nucleators. *Curr Opin Cell Biol* 2008; **20**: 495-501 [PMID: 18640274 DOI: 10.1016/j.ceb.2008.06.008]
 - 17 **Kanie K**, Kondo Y, Owaki J, Ikeda Y, Narita Y, Kato R, Honda H. Focused Screening of ECM-Selective Adhesion Peptides on Cellulose-Bound Peptide Microarrays. *Bioengineering (Basel)* 2016; **3** [PMID: 28952593 DOI: 10.3390/bioengineering3040031]
 - 18 **Bellis SL**. Advantages of RGD peptides for directing cell association with biomaterials. *Biomaterials* 2011; **32**: 4205-4210 [PMID: 21515168 DOI: 10.1016/j.biomaterials.2011.02.029]
 - 19 **Hubbell JA**, Massia SP, Desai NP, Drumheller PD. Endothelial cell-selective materials for tissue engineering in the vascular graft via a new receptor. *Biotechnology (N Y)* 1991; **9**: 568-572 [PMID: 1369319 DOI: 10.1038/nbt0691-568]
 - 20 **Gobin AS**, West JL. Val-ala-pro-gly, an elastin-derived non-integrin ligand: smooth muscle cell adhesion and specificity. *J Biomed Mater Res A* 2003; **67**: 255-259 [PMID: 14517884 DOI: 10.1002/jbm.a.10110]
 - 21 **Friedland JC**, Lee MH, Boettiger D. Mechanically activated integrin switch controls alpha5beta1 function. *Science* 2009; **323**: 642-644 [PMID: 19179533 DOI: 10.1126/science.1168441]
 - 22 **Sugawara K**, Tsuruta D, Ishii M, Jones JC, Kobayashi H. Laminin-332 and -511 in skin. *Exp Dermatol* 2008; **17**: 473-480 [PMID: 18474082 DOI: 10.1111/j.1600-0625.2008.00721.x]
 - 23 **Hussey GS**, Dziki JS, Badylak SF. Extracellular matrix- based materials for regenerative medicine. *Nat Rev Mater* 2018; **3**: 159-173 [DOI: 10.1038/s41578-018-0023-x]
 - 24 **Leivo I**, Vaheri A, Timpl R, Wartiovaara J. Appearance and distribution of collagens and laminin in the early mouse embryo. *Dev Biol* 1980; **76**: 100-114 [PMID: 6991310 DOI: 10.1016/0012-1606(80)90365-6]
 - 25 **Adams JC**, Watt FM. Regulation of development and differentiation by the extracellular matrix. *Development* 1993; **117**: 1183-1198 [PMID: 8404525 DOI: 10.1242/dev.117.4.1183]
 - 26 **Kramer JM**, Johnson JJ, Edgar RS, Basch C, Roberts S. The sqt-1 gene of *C. elegans* encodes a collagen critical for organismal morphogenesis. *Cell* 1988; **55**: 555-565 [PMID: 3180220 DOI: 10.1016/0092-8674(88)90214-0]
 - 27 **Ishii N**, Wadsworth WG, Stern BD, Culotti JG, Hedgecock EM. UNC-6, a laminin-related protein, guides cell and pioneer axon migrations in *C. elegans*. *Neuron* 1992; **9**: 873-881 [PMID: 1329863 DOI: 10.1016/0896-6273(92)90240-e]
 - 28 **Volk T**, Fessler LI, Fessler JH. A role for integrin in the formation of sarcomeric cytoarchitecture. *Cell* 1990; **63**: 525-536 [PMID: 2225065 DOI: 10.1016/0092-8674(90)90449-o]
 - 29 **Coles EG**, Gammill LS, Miner JH, Bronner-Fraser M. Abnormalities in neural crest cell migration in laminin alpha5 mutant mice. *Dev Biol* 2006; **289**: 218-228 [PMID: 16316641 DOI: 10.1016/j.ydbio.2005.10.031]
 - 30 **Trinh LA**, Stainier DY. Fibronectin regulates epithelial organization during myocardial migration in zebrafish. *Dev Cell* 2004; **6**: 371-382 [PMID: 15030760 DOI: 10.1016/s1534-5807(04)00063-2]
 - 31 **Gattazzo F**, Urciuolo A, Bonaldo P. Extracellular matrix: a dynamic microenvironment for stem cell niche. *Biochim Biophys Acta* 2014; **1840**: 2506-2519 [PMID: 24418517 DOI: 10.1016/j.bbagen.2014.01.010]
 - 32 **Yue B**. Biology of the extracellular matrix: an overview. *J Glaucoma* 2014; **23**: S20-S23 [PMID: 25275899 DOI: 10.1097/IJG.000000000000108]
 - 33 **Bonnans C**, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol* 2014; **15**: 786-801 [PMID: 25415508 DOI: 10.1038/nrm3904]
 - 34 **Bissell MJ**, Hall HG, Parry G. How does the extracellular matrix direct gene expression? *J Theor Biol* 1982; **99**: 31-68 [PMID: 6892044 DOI: 10.1016/0022-5193(82)90388-5]
 - 35 **Humphries JD**, Chastney MR, Askari JA, Humphries MJ. Signal transduction via integrin adhesion complexes. *Curr Opin Cell Biol* 2019; **56**: 14-21 [PMID: 30195153 DOI: 10.1016/j.ceb.2018.08.004]
 - 36 **Yost HJ**. Regulation of vertebrate left-right asymmetries by extracellular matrix. *Nature* 1992; **357**: 158-161 [PMID: 1579165 DOI: 10.1038/357158a0]
 - 37 **Banerjee P**, Shanthi C. Cryptic Peptides from Collagen: A Critical Review. *Protein Pept Lett* 2016; **23**: 664-672 [PMID: 27173646 DOI: 10.2174/0929866522666160512151313]
 - 38 **Huleihel L**, Hussey GS, Naranjo JD, Zhang L, Dziki JL, Turner NJ, Stolz DB, Badylak SF. Matrix-bound nanovesicles within ECM bioscaffolds. *Sci Adv* 2016; **2**: e1600502 [PMID: 27386584 DOI: 10.1126/sciadv.1600502]
 - 39 **Sicari BM**, Zhang L, Londono R, Badylak SF. An assay to quantify chemotactic properties of degradation products from extracellular matrix. *Methods Mol Biol* 2014; **1202**: 103-110 [PMID: 24155230 DOI: 10.1007/7651_2013_37]
 - 40 **Agrawal V**, Tottey S, Johnson SA, Freund JM, Siu BF, Badylak SF. Recruitment of progenitor cells by an extracellular matrix cryptic peptide in a mouse model of digit amputation. *Tissue Eng Part A* 2011; **17**: 2435-2443 [PMID: 21563860 DOI: 10.1089/ten.TEA.2011.0036]
 - 41 **Sottile J**. Regulation of angiogenesis by extracellular matrix. *Biochim Biophys Acta* 2004; **1654**: 13-22 [PMID: 14984764 DOI: 10.1016/j.bbcan.2003.07.002]
 - 42 **Ames JJ**, Contois L, Caron JM, Tweedie E, Yang X, Friesel R, Vary C, Brooks PC. Identification of an Endogenously Generated Cryptic Collagen Epitope (XL313) That May Selectively Regulate Angiogenesis by an Integrin Yes-associated Protein (YAP) Mechano-transduction Pathway. *J Biol Chem* 2016; **291**: 2731-2750 [PMID: 26668310 DOI: 10.1074/jbc.M115.669614]
 - 43 **Schultz GS**, Wysocki A. Interactions between extracellular matrix and growth factors in wound healing. *Wound Repair Regen* 2009; **17**: 153-162 [PMID: 19320882 DOI: 10.1111/j.1524-475X.2009.00466.x]
 - 44 **McBeath R**, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev Cell* 2004; **6**: 483-495 [PMID: 15068789 DOI: 10.1016/s1534-5807(04)00075-9]
 - 45 **Klein EA**, Yin L, Kothapalli D, Castagnino P, Byfield FJ, Xu T, Levental I, Hawthorne E, Janmey PA, Assoian RK. Cell-cycle control by physiological matrix elasticity and in vivo tissue stiffening. *Curr Biol* 2009; **19**: 1511-1518 [PMID: 19765988 DOI: 10.1016/j.cub.2009.07.069]
 - 46 **Engler AJ**, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell* 2006; **126**: 677-689 [PMID: 16923388 DOI: 10.1016/j.cell.2006.06.044]

- 47 **Cruz-Acuña R**, García AJ. Synthetic hydrogels mimicking basement membrane matrices to promote cell-matrix interactions. *Matrix Biol* 2017; **57-58**: 324-333 [PMID: 27283894 DOI: 10.1016/j.matbio.2016.06.002]
- 48 **Lutfikhuizen DT**, Harmsen MC, Van Luyn MJ. Cellular and molecular dynamics in the foreign body reaction. *Tissue Eng* 2006; **12**: 1955-1970 [PMID: 16889525 DOI: 10.1089/ten.2006.12.1955]
- 49 **Zhang S**. Fabrication of novel biomaterials through molecular self-assembly. *Nat Biotechnol* 2003; **21**: 1171-1178 [PMID: 14520402 DOI: 10.1038/nbt874]
- 50 **Liu CY**, Apuzzo ML, Tirrell DA. Engineering of the extracellular matrix: working toward neural stem cell programming and neurorestoration--concept and progress report. *Neurosurgery* 2003; **52**: 1154-65; discussion 1165 [PMID: 12699561]
- 51 **Lutolf MP**, Weber FE, Schmoekel HG, Schense JC, Kohler T, Müller R, Hubbell JA. Repair of bone defects using synthetic mimetics of collagenous extracellular matrices. *Nat Biotechnol* 2003; **21**: 513-518 [PMID: 12704396 DOI: 10.1038/nbt818]
- 52 **Zisch AH**, Lutolf MP, Ehrbar M, Raeber GP, Rizzi SC, Davies N, Schmökel H, Bezuidenhout D, Djonov V, Zilla P, Hubbell JA. Cell-demanded release of VEGF from synthetic, biointeractive cell ingrowth matrices for vascularized tissue growth. *FASEB J* 2003; **17**: 2260-2262 [PMID: 14563693 DOI: 10.1096/fj.02-1041fje]
- 53 **Lutolf MP**, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat Biotechnol* 2005; **23**: 47-55 [PMID: 15637621 DOI: 10.1038/nbt1055]
- 54 **Drinnan CT**, Zhang G, Alexander MA, Pulido AS, Suggs LJ. Multimodal release of transforming growth factor- β 1 and the BB isoform of platelet derived growth factor from PEGylated fibrin gels. *J Control Release* 2010; **147**: 180-186 [PMID: 20381553 DOI: 10.1016/j.jconrel.2010.03.026]
- 55 **Gjorevski N**, Sachs N, Manfrin A, Giger S, Bragina ME, Ordóñez-Morán P, Clevers H, Lutolf MP. Designer matrices for intestinal stem cell and organoid culture. *Nature* 2016; **539**: 560-564 [PMID: 27851739 DOI: 10.1038/nature20168]
- 56 **Drury JL**, Mooney DJ. Hydrogels for tissue engineering: scaffold design variables and applications. *Biomaterials* 2003; **24**: 4337-4351 [PMID: 12922147 DOI: 10.1016/s0142-9612(03)00340-5]
- 57 **Geckil H**, Xu F, Zhang X, Moon S, Demirci U. Engineering hydrogels as extracellular matrix mimics. *Nanomedicine (Lond)* 2010; **5**: 469-484 [PMID: 20394538 DOI: 10.2217/nmm.10.12]
- 58 **Vega SL**, Kwon MY, Burdick JA. Recent advances in hydrogels for cartilage tissue engineering. *Eur Cell Mater* 2017; **33**: 59-75 [PMID: 28138955 DOI: 10.22203/eCM.v033a05]
- 59 **Lindberg K**, Badylak SF. Porcine small intestinal submucosa (SIS): a bioscaffold supporting in vitro primary human epidermal cell differentiation and synthesis of basement membrane proteins. *Burns* 2001; **27**: 254-266 [PMID: 11311519 DOI: 10.1016/s0305-4179(00)00113-3]
- 60 **Dewez JL**, Lhoest JB, Detrait E, Berger V, Dupont-Gillain CC, Vincent LM, Schneider YJ, Bertrand P, Rouxhet PG. Adhesion of mammalian cells to polymer surfaces: from physical chemistry of surfaces to selective adhesion on defined patterns. *Biomaterials* 1998; **19**: 1441-1445 [PMID: 9794515 DOI: 10.1016/s0142-9612(98)00055-6]
- 61 **Whitesides GM**, Ostuni E, Takayama S, Jiang X, Ingber DE. Soft lithography in biology and biochemistry. *Annu Rev Biomed Eng* 2001; **3**: 335-373 [PMID: 11447067 DOI: 10.1146/annurev.bioeng.3.1.335]
- 62 **Di Cio S**, Bøggild TML, Connelly J, Sutherland DS, Gautrot JE. Differential integrin expression regulates cell sensing of the matrix nanoscale geometry. *Acta Biomater* 2017; **50**: 280-292 [PMID: 27940195 DOI: 10.1016/j.actbio.2016.11.069]
- 63 **Sill TJ**, von Recum HA. Electrospinning: applications in drug delivery and tissue engineering. *Biomaterials* 2008; **29**: 1989-2006 [PMID: 18281090 DOI: 10.1016/j.biomaterials.2008.01.011]
- 64 **Nagata S**, Hanayama R, Kawane K. Autoimmunity and the clearance of dead cells. *Cell* 2010; **140**: 619-630 [PMID: 20211132 DOI: 10.1016/j.cell.2010.02.014]
- 65 **Manfredi AA**, Capobianco A, Bianchi ME, Rovere-Querini P. Regulation of dendritic- and T-cell fate by injury-associated endogenous signals. *Crit Rev Immunol* 2009; **29**: 69-86 [PMID: 19348611 DOI: 10.1615/critrevimmunol.v29.i1.30]
- 66 **Brown BN**, Valentin JE, Stewart-Akers AM, McCabe GP, Badylak SF. Macrophage phenotype and remodeling outcomes in response to biologic scaffolds with and without a cellular component. *Biomaterials* 2009; **30**: 1482-1491 [PMID: 19121538 DOI: 10.1016/j.biomaterials.2008.11.040]
- 67 **Keane TJ**, Londono R, Turner NJ, Badylak SF. Consequences of ineffective decellularization of biologic scaffolds on the host response. *Biomaterials* 2012; **33**: 1771-1781 [PMID: 22137126 DOI: 10.1016/j.biomaterials.2011.10.054]
- 68 **Emami A**, Talaei-Khozani T, Vojdani Z, Zarei Fard N. Comparative assessment of the efficiency of various decellularization agents for bone tissue engineering. *J Biomed Mater Res B Appl Biomater* 2021; **109**: 19-32 [PMID: 32627321 DOI: 10.1002/jbm.b.34677]
- 69 **Gratzer PF**, Harrison RD, Woods T. Matrix alteration and not residual sodium dodecyl sulfate cytotoxicity affects the cellular repopulation of a decellularized matrix. *Tissue Eng* 2006; **12**: 2975-2983 [PMID: 17518665 DOI: 10.1089/ten.2006.12.2975]
- 70 **Dai Q**, Jiang W, Huang F, Song F, Zhang J, Zhao H. Recent Advances in Liver Engineering With Decellularized Scaffold. *Front Bioeng Biotechnol* 2022; **10**: 831477 [PMID: 35223793 DOI: 10.3389/fbioe.2022.831477]
- 71 **Dang LH**, Tseng Y, Tseng H, Hung SH. Partial Decellularization for Segmental Tracheal Scaffold Tissue Engineering: A Preliminary Study in Rabbits. *Biomolecules* 2021; **11** [PMID: 34200705 DOI: 10.3390/biom11060866]
- 72 **Shen W**, Berning K, Tang SW, Lam YW. Rapid and Detergent-Free Decellularization of Cartilage. *Tissue Eng Part C Methods* 2020; **26**: 201-206 [PMID: 32126898 DOI: 10.1089/ten.TEC.2020.0008]
- 73 **Lin CH**, Hsia K, Su CK, Chen CC, Yeh CC, Ma H, Lu JH. Sonication-Assisted Method for Decellularization of Human Umbilical Artery for Small-Caliber Vascular Tissue Engineering. *Polymers (Basel)* 2021; **13** [PMID: 34067495 DOI: 10.3390/polym13111699]
- 74 **Suss PH**, Ribeiro VST, Motooka CE, de Melo LC, Tuon FF. Comparative study of decellularization techniques to obtain natural extracellular matrix scaffolds of human peripheral-nerve allografts. *Cell Tissue Bank* 2022; **23**: 511-520 [PMID: 34767141 DOI: 10.1007/s10561-021-09977-x]
- 75 **Yusof F**, Sha'ban M, Azhim A. Development of decellularized meniscus using closed sonication treatment system:

- potential scaffolds for orthopedics tissue engineering applications. *Int J Nanomedicine* 2019; **14**: 5491-5502 [PMID: 31410000 DOI: 10.2147/IJN.S207270]
- 76 **Manalastas TM**, Dugos N, Ramos G, Mondragon JM. Effect of Decellularization Parameters on the Efficient Production of Kidney Bioscaffolds. *Appl Biochem Biotechnol* 2021; **193**: 1239-1251 [PMID: 32418019 DOI: 10.1007/s12010-020-03338-2]
- 77 **Tao M**, Liang F, He J, Ye W, Javed R, Wang W, Yu T, Fan J, Tian X, Wang X, Hou W, Ao Q. Decellularized tendon matrix membranes prevent post-surgical tendon adhesion and promote functional repair. *Acta Biomater* 2021; **134**: 160-176 [PMID: 34303866 DOI: 10.1016/j.actbio.2021.07.038]
- 78 **Cheng J**, Wang C, Gu Y. Combination of freeze-thaw with detergents: A promising approach to the decellularization of porcine carotid arteries. *Biomed Mater Eng* 2019; **30**: 191-205 [PMID: 30741667 DOI: 10.3233/BME-191044]
- 79 **Pulver**, Shevtsov A, Leybovich B, Artyuhov I, Maleev Y, Peregodov A. Production of organ extracellular matrix using a freeze-thaw cycle employing extracellular cryoprotectants. *Cryo Letters* 2014; **35**: 400-406 [PMID: 25397955]
- 80 **Mattei G**, Di Patria V, Tirella A, Alaimo A, Elia G, Corti A, Paolicchi A, Ahluwalia A. Mechanostructure and composition of highly reproducible decellularized liver matrices. *Acta Biomater* 2014; **10**: 875-882 [PMID: 24184179 DOI: 10.1016/j.actbio.2013.10.023]
- 81 **Syed O**, Walters NJ, Day RM, Kim HW, Knowles JC. Evaluation of decellularization protocols for production of tubular small intestine submucosa scaffolds for use in oesophageal tissue engineering. *Acta Biomater* 2014; **10**: 5043-5054 [PMID: 25173840 DOI: 10.1016/j.actbio.2014.08.024]
- 82 **Guimaraes AB**, Correia AT, Alves BP, Da Silva RS, Martins JK, Pêgo-Fernandes PM, Xavier NS, Dolhnikoff M, Cardoso PFG. Evaluation of a Physical-Chemical Protocol for Porcine Tracheal Decellularization. *Transplant Proc* 2019; **51**: 1611-1613 [PMID: 31155202 DOI: 10.1016/j.transproceed.2019.01.042]
- 83 **Visscher DO**, Lee H, van Zuijlen PPM, Helder MN, Atala A, Yoo JJ, Lee SJ. A photo-crosslinkable cartilage-derived extracellular matrix bioink for auricular cartilage tissue engineering. *Acta Biomater* 2021; **121**: 193-203 [PMID: 33227486 DOI: 10.1016/j.actbio.2020.11.029]
- 84 **Changchen W**, Hongquan W, Bo Z, Leilei X, Haiyue J, Bo P. The characterization, cytotoxicity, macrophage response and tissue regeneration of decellularized cartilage in costal cartilage defects. *Acta Biomater* 2021; **136**: 147-158 [PMID: 34563726 DOI: 10.1016/j.actbio.2021.09.031]
- 85 **Weng J**, Chen B, Xie M, Wan X, Wang P, Zhou X, Zhou Z, Mei J, Wang L, Huang D, Wang Z, Chen C. Rabbit thyroid extracellular matrix as a 3D bioscaffold for thyroid bioengineering: a preliminary in vitro study. *Biomed Eng Online* 2021; **20**: 18 [PMID: 33563294 DOI: 10.1186/s12938-021-00856-w]
- 86 **Uygun BE**, Soto-Gutierrez A, Yagi H, Izamis ML, Guzzardi MA, Shulman C, Milwid J, Kobayashi N, Tilles A, Berthiaume F, Hertl M, Nahmias Y, Yarmush ML, Uygun K. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. *Nat Med* 2010; **16**: 814-820 [PMID: 20543851 DOI: 10.1038/nm.2170]
- 87 **Hussein KH**, Park KM, Kang KS, Woo HM. Heparin-gelatin mixture improves vascular reconstruction efficiency and hepatic function in bioengineered livers. *Acta Biomater* 2016; **38**: 82-93 [PMID: 27134015 DOI: 10.1016/j.actbio.2016.04.042]
- 88 **Devalliere J**, Chen Y, Dooley K, Yarmush ML, Uygun BE. Improving functional re-endothelialization of acellular liver scaffold using REDV cell-binding domain. *Acta Biomater* 2018; **78**: 151-164 [PMID: 30071351 DOI: 10.1016/j.actbio.2018.07.046]
- 89 **Ko IK**, Peng L, Peloso A, Smith CJ, Dhal A, Deegan DB, Zimmerman C, Clouse C, Zhao W, Shupe TD, Soker S, Yoo JJ, Atala A. Bioengineered transplantable porcine livers with re-endothelialized vasculature. *Biomaterials* 2015; **40**: 72-79 [PMID: 25433603 DOI: 10.1016/j.biomaterials.2014.11.027]
- 90 **Baptista PM**, Siddiqui MM, Lozier G, Rodriguez SR, Atala A, Soker S. The use of whole organ decellularization for the generation of a vascularized liver organoid. *Hepatology* 2011; **53**: 604-617 [PMID: 21274881 DOI: 10.1002/hep.24067]
- 91 **Neishabouri A**, Soltani Khaboushan A, Daghigh F, Kajbafzadeh AM, Majidi Zolbin M. Decellularization in Tissue Engineering and Regenerative Medicine: Evaluation, Modification, and Application Methods. *Front Bioeng Biotechnol* 2022; **10**: 805299 [PMID: 35547166 DOI: 10.3389/fbioe.2022.805299]
- 92 **Yang J**, Dang H, Xu Y. Recent advancement of decellularization extracellular matrix for tissue engineering and biomedical application. *Artif Organs* 2022; **46**: 549-567 [PMID: 34855994 DOI: 10.1111/aor.14126]
- 93 **Cox B**, Emili A. Tissue subcellular fractionation and protein extraction for use in mass-spectrometry-based proteomics. *Nat Protoc* 2006; **1**: 1872-1878 [PMID: 17487171 DOI: 10.1038/nprot.2006.273]
- 94 **Xu CC**, Chan RW, Tirunagari N. A biodegradable, acellular xenogeneic scaffold for regeneration of the vocal fold lamina propria. *Tissue Eng* 2007; **13**: 551-566 [PMID: 17518602 DOI: 10.1089/ten.2006.0169]
- 95 **Moffat D**, Ye K, Jin S. Decellularization for the retention of tissue niches. *J Tissue Eng* 2022; **13**: 20417314221101151 [PMID: 35620656 DOI: 10.1177/20417314221101151]
- 96 **Kajbafzadeh AM**, Javan-Farazmand N, Monajemzadeh M, Baghayee A. Determining the optimal decellularization and sterilization protocol for preparing a tissue scaffold of a human-sized liver tissue. *Tissue Eng Part C Methods* 2013; **19**: 642-651 [PMID: 23270591 DOI: 10.1089/ten.TEC.2012.0334]
- 97 **Coronado RE**, Somaraki-Cormier M, Natesan S, Christy RJ, Ong JL, Halff GA. Decellularization and Solubilization of Porcine Liver for Use as a Substrate for Porcine Hepatocyte Culture: Method Optimization and Comparison. *Cell Transplant* 2017; **26**: 1840-1854 [PMID: 29390876 DOI: 10.1177/0963689717742157]
- 98 **Abaci A**, Guvendiren M. Designing Decellularized Extracellular Matrix-Based Bioinks for 3D Bioprinting. *Adv Health Mater* 2020; **9**: e2000734 [PMID: 32691980 DOI: 10.1002/adhm.202000734]
- 99 **Flynn LE**. The use of decellularized adipose tissue to provide an inductive microenvironment for the adipogenic differentiation of human adipose-derived stem cells. *Biomaterials* 2010; **31**: 4715-4724 [PMID: 20304481 DOI: 10.1016/j.biomaterials.2010.02.046]
- 100 **Brown BN**, Freund JM, Han L, Rubin JP, Reing JE, Jeffries EM, Wolf MT, Tottey S, Barnes CA, Ratner BD, Badylak SF. Comparison of three methods for the derivation of a biologic scaffold composed of adipose tissue extracellular matrix.

- Tissue Eng Part C Methods* 2011; **17**: 411-421 [PMID: 21043998 DOI: 10.1089/ten.TEC.2010.0342]
- 101 **Zhao C**, Li Y, Peng G, Lei X, Zhang G, Gao Y. Decellularized liver matrix-modified chitosan fibrous scaffold as a substrate for C3A hepatocyte culture. *J Biomater Sci Polym Ed* 2020; **31**: 1041-1056 [PMID: 32162599 DOI: 10.1080/09205063.2020.1738690]
- 102 **Alaby Pinheiro Faccioli L**, Suhett Dias G, Hoff V, Lemos Dias M, Ferreira Pimentel C, Hochman-Mendez C, Braz Parente D, Labrunie E, Souza Mourão PA, Rogério de Oliveira Salvalaggio P, Goldberg AC, Campos de Carvalho AC, Dos Santos Goldenberg RC. Optimizing the Decellularized Porcine Liver Scaffold Protocol. *Cells Tissues Organs* 2022; **211**: 385-394 [PMID: 33040059 DOI: 10.1159/000510297]
- 103 **Lorvellec M**, Scottoni F, Crowley C, Fiadeiro R, Maghsoudlou P, Pellegata AF, Mazzacuva F, Gjinovci A, Lyne AM, Zulini J, Little D, Mosaku O, Kelly D, De Coppi P, Gissen P. Mouse decellularised liver scaffold improves human embryonic and induced pluripotent stem cells differentiation into hepatocyte-like cells. *PLoS One* 2017; **12**: e0189586 [PMID: 29261712 DOI: 10.1371/journal.pone.0189586]
- 104 **Maghsoudlou P**, Georgiades F, Smith H, Milan A, Shangaris P, Urbani L, Loukogeorgakis SP, Lombardi B, Mazza G, Hagen C, Sebire NJ, Turmaine M, Eaton S, Olivo A, Godovac-Zimmermann J, Pinzani M, Gissen P, De Coppi P. Optimization of Liver Decellularization Maintains Extracellular Matrix Micro-Architecture and Composition Predisposing to Effective Cell Seeding. *PLoS One* 2016; **11**: e0155324 [PMID: 27159223 DOI: 10.1371/journal.pone.0155324]
- 105 **Maurer P**, Hohenester E. Structural and functional aspects of calcium binding in extracellular matrix proteins. *Matrix Biol* 1997; **15**: 569-80; discussion 581 [PMID: 9138289 DOI: 10.1016/s0945-053x(97)90033-0]
- 106 **Klebe RJ**. Isolation of a collagen-dependent cell attachment factor. *Nature* 1974; **250**: 248-251 [PMID: 4859375 DOI: 10.1038/250248a0]
- 107 **Lehr EJ**, Rayat GR, Chiu B, Churchill T, McGann LE, Coe JY, Ross DB. Decellularization reduces immunogenicity of sheep pulmonary artery vascular patches. *J Thorac Cardiovasc Surg* 2011; **141**: 1056-1062 [PMID: 20637475 DOI: 10.1016/j.jtcvs.2010.02.060]
- 108 **Ahmed E**, Saleh T, Yu L, Song SH, Park KM, Kwak HH, Woo HM. Decellularized extracellular matrix-rich hydrogel-silver nanoparticle mixture as a potential treatment for acute liver failure model. *J Biomed Mater Res A* 2020; **108**: 2351-2367 [PMID: 32415903 DOI: 10.1002/jbm.a.36988]
- 109 **Everwien H**, Keshi E, Hillebrandt KH, Ludwig B, Weinhart M, Tang P, Beierle AS, Napierala H, Gassner JM, Seiffert N, Moosburner S, Geisel D, Reutzel-Selke A, Strücker B, Pratschke J, Haep N, Sauer IM. Engineering an endothelialized, endocrine Neo-Pancreas: Evaluation of islet functionality in an ex vivo model. *Acta Biomater* 2020; **117**: 213-225 [PMID: 32949822 DOI: 10.1016/j.actbio.2020.09.022]
- 110 **Wu G**, Wu D, Lo J, Wang Y, Wu J, Lu S, Xu H, Zhao X, He Y, Li J, Demirci U, Wang S. A bioartificial liver support system integrated with a DLM/GelMA-based bioengineered whole liver for prevention of hepatic encephalopathy via enhanced ammonia reduction. *Biomater Sci* 2020; **8**: 2814-2824 [PMID: 32307491 DOI: 10.1039/c9bm01879d]
- 111 **Prasertung I**, Kanokpanont S, Bunaprasert T, Thanakit V, Damrongsakkul S. Development of acellular dermis from porcine skin using periodic pressurized technique. *J Biomed Mater Res B Appl Biomater* 2008; **85**: 210-219 [PMID: 17853423 DOI: 10.1002/jbm.b.30938]
- 112 **Olsen JV**, Ong SE, Mann M. Trypsin cleaves exclusively C-terminal to arginine and lysine residues. *Mol Cell Proteomics* 2004; **3**: 608-614 [PMID: 15034119 DOI: 10.1074/mcp.T400003-MCP200]
- 113 **Grauss RW**, Hazekamp MG, Oppenhuizen F, van Munsteren CJ, Gittenberger-de Groot AC, DeRuiter MC. Histological evaluation of decellularised porcine aortic valves: matrix changes due to different decellularisation methods. *Eur J Cardiothorac Surg* 2005; **27**: 566-571 [PMID: 15784352 DOI: 10.1016/j.ejcts.2004.12.052]
- 114 **Wang Z**, Sun F, Lu Y, Zhang B, Zhang G, Shi H. Rapid Preparation Method for Preparing Tracheal Decellularized Scaffolds: Vacuum Assistance and Optimization of DNase I. *ACS Omega* 2021; **6**: 10637-10644 [PMID: 34056217 DOI: 10.1021/acsomega.0c06247]
- 115 **Ramm R**, Goecke T, Theodoridis K, Hoeffler K, Sarikouch S, Findeisen K, Ciubotaru A, Cebotari S, Tudorache I, Haverich A, Hilfiker A. Decellularization combined with enzymatic removal of N-linked glycans and residual DNA reduces inflammatory response and improves performance of porcine xenogeneic pulmonary heart valves in an ovine in vivo model. *Xenotransplantation* 2020; **27**: e12571 [PMID: 31769101 DOI: 10.1111/xen.12571]
- 116 **Conconi MT**, De Coppi P, Di Liddo R, Vigolo S, Zanon GF, Parnigotto PP, Nussdorfer GG. Tracheal matrices, obtained by a detergent-enzymatic method, support in vitro the adhesion of chondrocytes and tracheal epithelial cells. *Transpl Int* 2005; **18**: 727-734 [PMID: 15910302 DOI: 10.1111/j.1432-2277.2005.00082.x]
- 117 **Keane TJ**, Swinehart IT, Badylak SF. Methods of tissue decellularization used for preparation of biologic scaffolds and in vivo relevance. *Methods* 2015; **84**: 25-34 [PMID: 25791470 DOI: 10.1016/j.ymeth.2015.03.005]
- 118 **Goddard ET**, Hill RC, Barrett A, Betts C, Guo Q, Maller O, Borges VF, Hansen KC, Schedin P. Quantitative extracellular matrix proteomics to study mammary and liver tissue microenvironments. *Int J Biochem Cell Biol* 2016; **81**: 223-232 [PMID: 27771439 DOI: 10.1016/j.biocel.2016.10.014]
- 119 **Woods T**, Gratzner PF. Effectiveness of three extraction techniques in the development of a decellularized bone-anterior cruciate ligament-bone graft. *Biomaterials* 2005; **26**: 7339-7349 [PMID: 16023194 DOI: 10.1016/j.biomaterials.2005.05.066]
- 120 **Cartmell JS**, Dunn MG. Effect of chemical treatments on tendon cellularity and mechanical properties. *J Biomed Mater Res* 2000; **49**: 134-140 [PMID: 10559756 DOI: 10.1002/(sici)1097-4636(200001)49:1<134::aid-jbm17>3.0.co;2-d]
- 121 **LoPresti ST**, Brown BN. Effect of Source Animal Age upon Macrophage Response to Extracellular Matrix Biomaterials. *J Immunol Regen Med* 2018; **1**: 57-66 [PMID: 30101208 DOI: 10.1016/j.regen.2018.03.004]
- 122 **Sicari BM**, Johnson SA, Siu BF, Crapo PM, Daly KA, Jiang H, Medberry CJ, Tottey S, Turner NJ, Badylak SF. The effect of source animal age upon the in vivo remodeling characteristics of an extracellular matrix scaffold. *Biomaterials* 2012; **33**: 5524-5533 [PMID: 22575834 DOI: 10.1016/j.biomaterials.2012.04.017]
- 123 **Ozcebe SG**, Baheccioglu G, Yue XS, Zorlutuna P. Effect of cellular and ECM aging on human iPSC-derived cardiomyocyte performance, maturity and senescence. *Biomaterials* 2021; **268**: 120554 [PMID: 33296796 DOI: 10.1016/j.biomaterials.2020.120554]

- 124 **Hu M**, Bi H, Moffat D, Blystone M, DeCostanza P, Alayi T, Ye K, Hathout Y, Jin S. Proteomic and Bioinformatic Analysis of Decellularized Pancreatic Extracellular Matrices. *Molecules* 2021; **26** [PMID: 34771149 DOI: 10.3390/molecules26216740]
- 125 **Crapo PM**, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. *Biomaterials* 2011; **32**: 3233-3243 [PMID: 21296410 DOI: 10.1016/j.biomaterials.2011.01.057]
- 126 **Zheng MH**, Chen J, Kirilak Y, Willers C, Xu J, Wood D. Porcine small intestine submucosa (SIS) is not an acellular collagenous matrix and contains porcine DNA: possible implications in human implantation. *J Biomed Mater Res B Appl Biomater* 2005; **73**: 61-67 [PMID: 15736287 DOI: 10.1002/jbm.b.30170]
- 127 **Caralt M**, Uzarski JS, Iacob S, Obergfell KP, Berg N, Bijonowski BM, Kiefer KM, Ward HH, Wandinger-Ness A, Miller WM, Zhang ZJ, Abecassis MM, Wertheim JA. Optimization and critical evaluation of decellularization strategies to develop renal extracellular matrix scaffolds as biological templates for organ engineering and transplantation. *Am J Transplant* 2015; **15**: 64-75 [PMID: 25403742 DOI: 10.1111/ajt.12999]
- 128 **Fischer I**, Westphal M, Rossbach B, Bethke N, Hariharan K, Ullah I, Reinke P, Kurtz A, Stachelscheid H. Comparative characterization of decellularized renal scaffolds for tissue engineering. *Biomed Mater* 2017; **12**: 045005 [PMID: 28396578 DOI: 10.1088/1748-605X/aa6c6d]
- 129 **Mouliřová V**, Jiřík M, Schindler C, Červenková L, Pálek R, Rosendorf J, Arlt J, Bolek L, Šušová S, Nietzsche S, Liřka V, Dahmen U. Novel morphological multi-scale evaluation system for quality assessment of decellularized liver scaffolds. *J Tissue Eng* 2020; **11**: 2041731420921121 [PMID: 32523667 DOI: 10.1177/2041731420921121]
- 130 **Sun WQ**, Leung P. Calorimetric study of extracellular tissue matrix degradation and instability after gamma irradiation. *Acta Biomater* 2008; **4**: 817-826 [PMID: 18334308 DOI: 10.1016/j.actbio.2008.02.006]
- 131 **Tao M**, Ao T, Mao X, Yan X, Javed R, Hou W, Wang Y, Sun C, Lin S, Yu T, Ao Q. Sterilization and disinfection methods for decellularized matrix materials: Review, consideration and proposal. *Bioact Mater* 2021; **6**: 2927-2945 [PMID: 33732964 DOI: 10.1016/j.bioactmat.2021.02.010]
- 132 **Mendes GC**, Brandão TR, Silva CL. Ethylene oxide sterilization of medical devices: a review. *Am J Infect Control* 2007; **35**: 574-581 [PMID: 17980234 DOI: 10.1016/j.ajic.2006.10.014]
- 133 **Thier R**, Bolt HM. Carcinogenicity and genotoxicity of ethylene oxide: new aspects and recent advances. *Crit Rev Toxicol* 2000; **30**: 595-608 [PMID: 11055837 DOI: 10.1080/10408440008951121]
- 134 **Clapp PA**, Davies MJ, French MS, Gilbert BC. The bactericidal action of peroxides; an E.P.R. spin-trapping study. *Free Radic Res* 1994; **21**: 147-167 [PMID: 7981786 DOI: 10.3109/10715769409056566]
- 135 **Hodde J**, Hiles M. Virus safety of a porcine-derived medical device: evaluation of a viral inactivation method. *Biotechnol Bioeng* 2002; **79**: 211-216 [PMID: 12115437 DOI: 10.1002/bit.10281]
- 136 **Goszyła C**, Ladd MR, Werts A, Fulton W, Johnson B, Sodhi C, Hackam DJ. A Comparison of Sterilization Techniques for Production of Decellularized Intestine in Mice. *Tissue Eng Part C Methods* 2020; **26**: 67-79 [PMID: 31802699 DOI: 10.1089/ten.TEC.2019.0219]
- 137 **Linley E**, Denyer SP, McDonnell G, Simons C, Maillard JY. Use of hydrogen peroxide as a biocide: new consideration of its mechanisms of biocidal action. *J Antimicrob Chemother* 2012; **67**: 1589-1596 [PMID: 22532463 DOI: 10.1093/jac/dks129]
- 138 **Shah DD**, Zhang J, Hsieh MC, Sundaram S, Maity H, Mallela KMG. Effect of Peroxide- Versus Alkoxy-Induced Chemical Oxidation on the Structure, Stability, Aggregation, and Function of a Therapeutic Monoclonal Antibody. *J Pharm Sci* 2018; **107**: 2789-2803 [PMID: 30075161 DOI: 10.1016/j.xphs.2018.07.024]
- 139 **Poornejad N**, Nielsen JJ, Morris RJ, Gassman JR, Reynolds PR, Roeder BL, Cook AD. Comparison of four decontamination treatments on porcine renal decellularized extracellular matrix structure, composition, and support of human renal cortical tubular epithelium cells. *J Biomater Appl* 2016; **30**: 1154-1167 [PMID: 26589294 DOI: 10.1177/0885328215615760]
- 140 **Kim MK**, Jeong W, Lee SM, Kim JB, Jin S, Kang HW. Decellularized extracellular matrix-based bio-ink with enhanced 3D printability and mechanical properties. *Biofabrication* 2020; **12**: 025003 [PMID: 31783385 DOI: 10.1088/1758-5090/ab5d80]
- 141 **Lewis PL**, Su J, Yan M, Meng F, Glaser SS, Alpini GD, Green RM, Sosa-Pineda B, Shah RN. Complex bile duct network formation within liver decellularized extracellular matrix hydrogels. *Sci Rep* 2018; **8**: 12220 [PMID: 30111800 DOI: 10.1038/s41598-018-30433-6]
- 142 **Yu C**, Ma X, Zhu W, Wang P, Miller KL, Stupin J, Koroleva-Maharajh A, Hairabedian A, Chen S. Scanningless and continuous 3D bioprinting of human tissues with decellularized extracellular matrix. *Biomaterials* 2019; **194**: 1-13 [PMID: 30562651 DOI: 10.1016/j.biomaterials.2018.12.009]
- 143 **Chen C**, Pla-Palacín I, Baptista PM, Shang P, Oosterhoff LA, van Wolferen ME, Penning LC, Geijsen N, Spee B. Hepatocyte-like cells generated by direct reprogramming from murine somatic cells can repopulate decellularized livers. *Biotechnol Bioeng* 2018; **115**: 2807-2816 [PMID: 29959867 DOI: 10.1002/bit.26784]
- 144 **Wang X**, Cui J, Zhang BQ, Zhang H, Bi Y, Kang Q, Wang N, Bie P, Yang Z, Wang H, Liu X, Haydon RC, Luu HH, Tang N, Dong J, He TC. Decellularized liver scaffolds effectively support the proliferation and differentiation of mouse fetal hepatic progenitors. *J Biomed Mater Res A* 2014; **102**: 1017-1025 [PMID: 23625886 DOI: 10.1002/jbm.a.34764]
- 145 **Butter A**, Aliyev K, Hillebrandt KH, Raschzok N, Kluge M, Seiffert N, Tang P, Napierala H, Muhamma AI, Reutzel-Selke A, Andreou A, Pratschke J, Sauer IM, Struecker B. Evolution of graft morphology and function after recellularization of decellularized rat livers. *J Tissue Eng Regen Med* 2018; **12**: e807-e816 [PMID: 27957815 DOI: 10.1002/term.2383]
- 146 **Matuska AM**, McFetridge PS. The effect of terminal sterilization on structural and biophysical properties of a decellularized collagen-based scaffold; implications for stem cell adhesion. *J Biomed Mater Res B Appl Biomater* 2015; **103**: 397-406 [PMID: 24895116 DOI: 10.1002/jbm.b.33213]
- 147 **Moradi L**, Mohammadi Jobania B, Jafarnezhad-Ansariha F, Ghorbani F, Esmail-Pour R, Majidi Zolbina M, Kajbafzadeh AM. Evaluation of different sterilization methods for decellularized kidney tissue. *Tissue Cell* 2020; **66**: 101396 [PMID: 32933719 DOI: 10.1016/j.tice.2020.101396]

- 148 **Hussein KH**, Park KM, Teotia PK, Hong SH, Yang SR, Park SM, Ahn C, Woo HM. Sterilization using electrolyzed water highly retains the biological properties in tissue-engineered porcine liver scaffold. *Int J Artif Organs* 2013; **36**: 781-792 [PMID: [24338653](#) DOI: [10.5301/ijao.5000246](#)]
- 149 **Bonenfant NR**, Sokocevic D, Wagner DE, Borg ZD, Lathrop MJ, Lam YW, Deng B, Desarno MJ, Ashikaga T, Loi R, Weiss DJ. The effects of storage and sterilization on de-cellularized and re-cellularized whole lung. *Biomaterials* 2013; **34**: 3231-3245 [PMID: [23380353](#) DOI: [10.1016/j.biomaterials.2013.01.031](#)]
- 150 **Khajavi M**, Hashemi M, Kalalinia F. Recent advances in optimization of liver decellularization procedures used for liver regeneration. *Life Sci* 2021; **281**: 119801 [PMID: [34229008](#) DOI: [10.1016/j.lfs.2021.119801](#)]
- 151 **Lin YQ**, Wang LR, Wang JT, Pan LL, Zhu GQ, Liu WY, Braddock M, Zheng MH. New advances in liver decellularization and recellularization: innovative and critical technologies. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 1183-1191 [PMID: [26220044](#) DOI: [10.1586/17474124.2015.1058155](#)]
- 152 **De Kock J**, Ceelen L, De Spiegelaere W, Casteleyn C, Claes P, Vanhaecke T, Rogiers V. Simple and quick method for whole-liver decellularization: a novel in vitro three-dimensional bioengineering tool? *Arch Toxicol* 2011; **85**: 607-612 [PMID: [21512802](#) DOI: [10.1007/s00204-011-0706-1](#)]
- 153 **Bühler NE**, Schulze-Osthoff K, Königsrainer A, Schenk M. Controlled processing of a full-sized porcine liver to a decellularized matrix in 24 h. *J Biosci Bioeng* 2015; **119**: 609-613 [PMID: [25468420](#) DOI: [10.1016/j.jbiosc.2014.10.019](#)]
- 154 **Mazza G**, Rombouts K, Rennie Hall A, Urbani L, Vinh Luong T, Al-Akkad W, Longato L, Brown D, Maghsoudlou P, Dhillon AP, Fuller B, Davidson B, Moore K, Dhar D, De Coppi P, Malago M, Pinzani M. Decellularized human liver as a natural 3D-scaffold for liver bioengineering and transplantation. *Sci Rep* 2015; **5**: 13079 [PMID: [26248878](#) DOI: [10.1038/srep13079](#)]
- 155 **Nari GA**, Cid M, Comín R, Reyna L, Juri G, Taborda R, Salvatierra NA. Preparation of a three-dimensional extracellular matrix by decellularization of rabbit livers. *Rev Esp Enferm Dig* 2013; **105**: 138-143 [PMID: [23735020](#) DOI: [10.4321/s1130-01082013000300004](#)]
- 156 **Shupe T**, Williams M, Brown A, Willenberg B, Petersen BE. Method for the decellularization of intact rat liver. *Organogenesis* 2010; **6**: 134-136 [PMID: [20885860](#) DOI: [10.4161/org.6.2.11546](#)]
- 157 **Mirmalek-Sani SH**, Sullivan DC, Zimmerman C, Shupe TD, Petersen BE. Immunogenicity of decellularized porcine liver for bioengineered hepatic tissue. *Am J Pathol* 2013; **183**: 558-565 [PMID: [23747949](#) DOI: [10.1016/j.ajpath.2013.05.002](#)]
- 158 **Struecker B**, Hillebrandt KH, Voitl R, Butter A, Schmuck RB, Reutzel-Selke A, Geisel D, Joehrens K, Pickerodt PA, Raschzok N, Puhl G, Neuhaus P, Pratschke J, Sauer IM. Porcine liver decellularization under oscillating pressure conditions: a technical refinement to improve the homogeneity of the decellularization process. *Tissue Eng Part C Methods* 2015; **21**: 303-313 [PMID: [25164028](#) DOI: [10.1089/ten.TEC.2014.0321](#)]
- 159 **Hillebrandt K**, Polenz D, Butter A, Tang P, Reutzel-Selke A, Andreou A, Napierala H, Raschzok N, Pratschke J, Sauer IM, Struecker B. Procedure for Decellularization of Rat Livers in an Oscillating-pressure Perfusion Device. *J Vis Exp* 2015; e53029 [PMID: [26327608](#) DOI: [10.3791/53029](#)]
- 160 **Struecker B**, Butter A, Hillebrandt K, Polenz D, Reutzel-Selke A, Tang P, Lippert S, Leder A, Rohn S, Geisel D, Denecke T, Aliyev K, Jöhrens K, Raschzok N, Neuhaus P, Pratschke J, Sauer IM. Improved rat liver decellularization by arterial perfusion under oscillating pressure conditions. *J Tissue Eng Regen Med* 2017; **11**: 531-541 [PMID: [25185781](#) DOI: [10.1002/term.1948](#)]
- 161 **Ren H**, Shi X, Tao L, Xiao J, Han B, Zhang Y, Yuan X, Ding Y. Evaluation of two decellularization methods in the development of a whole-organ decellularized rat liver scaffold. *Liver Int* 2013; **33**: 448-458 [PMID: [23301992](#) DOI: [10.1111/liv.12088](#)]
- 162 **Wu Q**, Bao J, Zhou YJ, Wang YJ, Du ZG, Shi YJ, Li L, Bu H. Optimizing perfusion-decellularization methods of porcine livers for clinical-scale whole-organ bioengineering. *Biomed Res Int* 2015; **2015**: 785474 [PMID: [25918720](#) DOI: [10.1155/2015/785474](#)]
- 163 **Sabetkish S**, Kajbafzadeh AM, Sabetkish N, Khorramirouz R, Akbarzadeh A, Seyedian SL, Pasalar P, Orangian S, Beigi RS, Aryan Z, Akbari H, Tavangar SM. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix liver scaffolds. *J Biomed Mater Res A* 2015; **103**: 1498-1508 [PMID: [25045886](#) DOI: [10.1002/jbm.a.35291](#)]
- 164 **Soto-Gutierrez A**, Zhang L, Medberry C, Fukumitsu K, Faulk D, Jiang H, Reing J, Gramignoli R, Komori J, Ross M, Nagaya M, Lagasse E, Stolz D, Strom SC, Fox IJ, Badylak SF. A whole-organ regenerative medicine approach for liver replacement. *Tissue Eng Part C Methods* 2011; **17**: 677-686 [PMID: [21375407](#) DOI: [10.1089/ten.TEC.2010.0698](#)]
- 165 **Yagi H**, Fukumitsu K, Fukuda K, Kitago M, Shinoda M, Obara H, Itano O, Kawachi S, Tanabe M, Coudriet GM, Piganelli JD, Gilbert TW, Soto-Gutierrez A, Kitagawa Y. Human-scale whole-organ bioengineering for liver transplantation: a regenerative medicine approach. *Cell Transplant* 2013; **22**: 231-242 [PMID: [22943797](#) DOI: [10.3727/096368912X654939](#)]
- 166 **Park KM**, Hussein KH, Hong SH, Ahn C, Yang SR, Park SM, Kweon OK, Kim BM, Woo HM. Decellularized Liver Extracellular Matrix as Promising Tools for Transplantable Bioengineered Liver Promotes Hepatic Lineage Commitments of Induced Pluripotent Stem Cells. *Tissue Eng Part A* 2016; **22**: 449-460 [PMID: [26801816](#) DOI: [10.1089/ten.TEA.2015.0313](#)]
- 167 **Geerts S**, Ozer S, Jaramillo M, Yarmush ML, Uygun BE. Nondestructive Methods for Monitoring Cell Removal During Rat Liver Decellularization. *Tissue Eng Part C Methods* 2016; **22**: 671-678 [PMID: [27169332](#) DOI: [10.1089/ten.TEC.2015.0571](#)]
- 168 **Barakat O**, Abbasi S, Rodriguez G, Rios J, Wood RP, Ozaki C, Holley LS, Gauthier PK. Use of decellularized porcine liver for engineering humanized liver organ. *J Surg Res* 2012; **173**: e11-e25 [PMID: [22099595](#) DOI: [10.1016/j.jss.2011.09.033](#)]
- 169 **Shi Y**, Su J, Roberts AI, Shou P, Rabson AB, Ren G. How mesenchymal stem cells interact with tissue immune responses. *Trends Immunol* 2012; **33**: 136-143 [PMID: [22227317](#) DOI: [10.1016/j.it.2011.11.004](#)]
- 170 **Brown C**, McKee C, Bakshi S, Walker K, Hakman E, Halassy S, Svinarich D, Dodds R, Govind CK, Chaudhry GR.

- Mesenchymal stem cells: Cell therapy and regeneration potential. *J Tissue Eng Regen Med* 2019; **13**: 1738-1755 [PMID: 31216380 DOI: 10.1002/term.2914]
- 171 **Bale SS**, Golberg I, Jindal R, McCarty WJ, Luitje M, Hegde M, Bhushan A, Usta OB, Yarmush ML. Long-term coculture strategies for primary hepatocytes and liver sinusoidal endothelial cells. *Tissue Eng Part C Methods* 2015; **21**: 413-422 [PMID: 25233394 DOI: 10.1089/ten.TEC.2014.0152]
- 172 **Kojima H**, Yasuchika K, Fukumitsu K, Ishii T, Ogiso S, Miyauchi Y, Yamaoka R, Kawai T, Katayama H, Yoshitoshi-Uebayashi EY, Kita S, Yasuda K, Sasaki N, Komori J, Uemoto S. Establishment of practical recellularized liver graft for blood perfusion using primary rat hepatocytes and liver sinusoidal endothelial cells. *Am J Transplant* 2018; **18**: 1351-1359 [PMID: 29338127 DOI: 10.1111/ajt.14666]
- 173 **Ogiso S**, Yasuchika K, Fukumitsu K, Ishii T, Kojima H, Miyauchi Y, Yamaoka R, Komori J, Katayama H, Kawai T, Yoshitoshi EY, Kita S, Yasuda K, Uemoto S. Efficient recellularisation of decellularised whole-liver grafts using biliary tree and foetal hepatocytes. *Sci Rep* 2016; **6**: 35887 [PMID: 27767181 DOI: 10.1038/srep35887]
- 174 **Bao J**, Shi Y, Sun H, Yin X, Yang R, Li L, Chen X, Bu H. Construction of a portal implantable functional tissue-engineered liver using perfusion-decellularized matrix and hepatocytes in rats. *Cell Transplant* 2011; **20**: 753-766 [PMID: 21054928 DOI: 10.3727/096368910X536572]
- 175 **Anderson BD**, Nelson ED, Joo D, Amiot BP, Katane AA, Mendenhall A, Steiner BG, Stumbras AR, Nelson VL, Palumbo RN, Gilbert TW, Davidow DS, Ross JJ, Nyberg SL. Functional characterization of a bioengineered liver after heterotopic implantation in pigs. *Commun Biol* 2021; **4**: 1157 [PMID: 34620986 DOI: 10.1038/s42003-021-02665-2]
- 176 **Bao J**, Wu Q, Sun J, Zhou Y, Wang Y, Jiang X, Li L, Shi Y, Bu H. Hemocompatibility improvement of perfusion-decellularized clinical-scale liver scaffold through heparin immobilization. *Sci Rep* 2015; **5**: 10756 [PMID: 26030843 DOI: 10.1038/srep10756]
- 177 **Chen Y**, Devalliere J, Bulutoglu B, Yarmush ML, Uygun BE. Repopulation of intrahepatic bile ducts in engineered rat liver grafts. *Technology (Singap World Sci)* 2019; **7**: 46-55 [PMID: 31388515 DOI: 10.1142/S2339547819500043]
- 178 **Joplin R**. Isolation and culture of biliary epithelial cells. *Gut* 1994; **35**: 875-878 [PMID: 8063212 DOI: 10.1136/gut.35.7.875]
- 179 **Sampaziotis F**, de Brito MC, Geti I, Bertero A, Hannan NR, Vallier L. Directed differentiation of human induced pluripotent stem cells into functional cholangiocyte-like cells. *Nat Protoc* 2017; **12**: 814-827 [PMID: 28333915 DOI: 10.1038/nprot.2017.011]
- 180 **Lee MO**, Moon SH, Jeong HC, Yi JY, Lee TH, Shim SH, Rhee YH, Lee SH, Oh SJ, Lee MY, Han MJ, Cho YS, Chung HM, Kim KS, Cha HJ. Inhibition of pluripotent stem cell-derived teratoma formation by small molecules. *Proc Natl Acad Sci U S A* 2013; **110**: E3281-E3290 [PMID: 23918355 DOI: 10.1073/pnas.1303669110]
- 181 **Kratochvil MJ**, Seymour AJ, Li TL, Paşca SP, Kuo CJ, Heilshorn SC. Engineered materials for organoid systems. *Nat Rev Mater* 2019; **4**: 606-622 [PMID: 33552558 DOI: 10.1038/s41578-019-0129-9]
- 182 **Marsee A**, Roos FJM, Verstegen MMA; HPB Organoid Consortium, Gehart H, de Koning E, Lemaigre F, Forbes SJ, Peng WC, Huch M, Takebe T, Vallier L, Clevers H, van der Laan LJW, Spee B. Building consensus on definition and nomenclature of hepatic, pancreatic, and biliary organoids. *Cell Stem Cell* 2021; **28**: 816-832 [PMID: 33961769 DOI: 10.1016/j.stem.2021.04.005]
- 183 **Huch M**, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, Ellis E, van Wenum M, Fuchs SA, de Ligt J, van de Wetering M, Sasaki N, Boers SJ, Kemperman H, de Jonge J, Ijzermans JN, Nieuwenhuis EE, Hoekstra R, Strom S, Vries RR, van der Laan LJ, Cuppen E, Clevers H. Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell* 2015; **160**: 299-312 [PMID: 25533785 DOI: 10.1016/j.cell.2014.11.050]
- 184 **Sampaziotis F**, Justin AW, Tysoe OC, Sawiak S, Godfrey EM, Upponi SS, Gieseck RL 3rd, de Brito MC, Berntsen NL, Gómez-Vázquez MJ, Ortmann D, Yiangou L, Ross A, Bargehr J, Bertero A, Zonneveld MCF, Pedersen MT, Pawlowski M, Valestrand L, Madrigal P, Georgakopoulos N, Pirmadjid N, Skeldon GM, Casey J, Shu W, Materek PM, Snijders KE, Brown SE, Rimland CA, Simonic I, Davies SE, Jensen KB, Zilbauer M, Gelson WTH, Alexander GJ, Sinha S, Hannan NRF, Wynn TA, Karlsen TH, Melum E, Markaki AE, Saeb-Parsy K, Vallier L. Reconstruction of the mouse extrahepatic biliary tree using primary human extrahepatic cholangiocyte organoids. *Nat Med* 2017; **23**: 954-963 [PMID: 28671689 DOI: 10.1038/nm.4360]
- 185 **Soroka CJ**, Assis DN, Alrabadi LS, Roberts S, Cusack L, Jaffe AB, Boyer JL. Bile-Derived Organoids From Patients With Primary Sclerosing Cholangitis Recapitulate Their Inflammatory Immune Profile. *Hepatology* 2019; **70**: 871-882 [PMID: 30561836 DOI: 10.1002/hep.30470]
- 186 **Sampaziotis F**, Muraro D, Tysoe OC, Sawiak S, Beach TE, Godfrey EM, Upponi SS, Brevini T, Wesley BT, Garcia-Bernardo J, Mahbubani K, Canu G, Gieseck R 3rd, Berntsen NL, Mulcahy VL, Crick K, Fear C, Robinson S, Swift L, Gambardella L, Bargehr J, Ortmann D, Brown SE, Osnato A, Murphy MP, Corbett G, Gelson WTH, Mells GF, Humphreys P, Davies SE, Amin I, Gibbs P, Sinha S, Teichmann SA, Butler AJ, See TC, Melum E, Watson CJ, Saeb-Parsy K, Vallier L. Cholangiocyte organoids can repair bile ducts after transplantation in the human liver. *Science* 2021; **371**: 839-846 [PMID: 33602855 DOI: 10.1126/science.aaz6964]
- 187 **Rimland CA**, Tilson SG, Morell CM, Tomaz RA, Lu WY, Adams SE, Georgakopoulos N, Otaizo-Carrasquero F, Myers TG, Ferdinand JR, Gieseck RL 3rd, Sampaziotis F, Tysoe OC, Ross A, Kraiczky JM, Wesley B, Muraro D, Zilbauer M, Oniscu GC, Hannan NRF, Forbes SJ, Saeb-Parsy K, Wynn TA, Vallier L. Regional Differences in Human Biliary Tissues and Corresponding In Vitro-Derived Organoids. *Hepatology* 2021; **73**: 247-267 [PMID: 3222998 DOI: 10.1002/hep.31252]
- 188 **Willemsse J**, Roos FJM, Voegt IJ, Schurink IJ, Bijvelds M, de Jonge HR, van der Laan LJW, de Jonge J, Verstegen MMA. Scaffolds obtained from decellularized human extrahepatic bile ducts support organoids to establish functional biliary tissue in a dish. *Biotechnol Bioeng* 2021; **118**: 836-851 [PMID: 33118611 DOI: 10.1002/bit.27613]
- 189 **Roos FJM**, Wu H, Willemsse J, Lieshout R, Albarinos LAM, Kan YY, Poley JW, Bruno MJ, de Jonge J, Bártfai R, Marks H, IJzermans JNM, Verstegen MMA, van der Laan LJW. Cholangiocyte organoids from human bile retain a local phenotype and can repopulate bile ducts in vitro. *Clin Transl Med* 2021; **11**: e566 [PMID: 34954911 DOI: 10.1002/ctm2.566]

- 190 **Meng F**, Almohanna F, Altuhami A, Assiri AM, Broering D. Vasculature reconstruction of decellularized liver scaffolds via gelatin-based re-endothelialization. *J Biomed Mater Res A* 2019; **107**: 392-402 [PMID: 30508280 DOI: 10.1002/jbm.a.36551]
- 191 **Shaheen MF**, Joo DJ, Ross JJ, Anderson BD, Chen HS, Huebert RC, Li Y, Amiot B, Young A, Zlochiver V, Nelson E, Mounajjed T, Dietz AB, Michalak G, Steiner BG, Davidow DS, Paradise CR, van Wijnen AJ, Shah VH, Liu M, Nyberg SL. Sustained perfusion of revascularized bioengineered livers heterotopically transplanted into immunosuppressed pigs. *Nat Biomed Eng* 2020; **4**: 437-445 [PMID: 31611679 DOI: 10.1038/s41551-019-0460-x]
- 192 **Kim DH**, Ahn J, Kang HK, Kim MS, Kim NG, Kook MG, Choi SW, Jeon NL, Woo HM, Kang KS. Development of highly functional bioengineered human liver with perfusable vasculature. *Biomaterials* 2021; **265**: 120417 [PMID: 32987272 DOI: 10.1016/j.biomaterials.2020.120417]
- 193 **Devillard CD**, Marquette CA. Vascular Tissue Engineering: Challenges and Requirements for an Ideal Large Scale Blood Vessel. *Front Bioeng Biotechnol* 2021; **9**: 721843 [PMID: 34671597 DOI: 10.3389/fbioe.2021.721843]
- 194 **Nishibe T**, Kondo Y, Muto A, Dardik A. Optimal prosthetic graft design for small diameter vascular grafts. *Vascular* 2007; **15**: 356-360 [PMID: 18053420 DOI: 10.2310/6670.2007.00053]
- 195 **Lampridis S**, George SJ. Nonautologous Grafts in Coronary Artery Bypass Surgery: A Systematic Review. *Ann Thorac Surg* 2021; **112**: 2094-2103 [PMID: 33340520 DOI: 10.1016/j.athoracsur.2020.11.028]
- 196 **Weinberg CB**, Bell E. A blood vessel model constructed from collagen and cultured vascular cells. *Science* 1986; **231**: 397-400 [PMID: 2934816 DOI: 10.1126/science.2934816]
- 197 **L'Heureux N**, Pâquet S, Labbé R, Germain L, Auger FA. A completely biological tissue-engineered human blood vessel. *FASEB J* 1998; **12**: 47-56 [PMID: 9438410 DOI: 10.1096/fasebj.12.1.47]
- 198 **Shin'oka T**, Imai Y, Ikada Y. Transplantation of a tissue-engineered pulmonary artery. *N Engl J Med* 2001; **344**: 532-533 [PMID: 11221621 DOI: 10.1056/nejm200102153440717]
- 199 **Lawson JH**, Glickman MH, Ilzecki M, Jakimowicz T, Jaroszynski A, Peden EK, Pilgrim AJ, Prichard HL, Guziewicz M, Przywara S, Szmidi J, Turek J, Witkiewicz W, Zapotoczny N, Zubilewicz T, Niklason LE. Bioengineered human acellular vessels for dialysis access in patients with end-stage renal disease: two phase 2 single-arm trials. *Lancet* 2016; **387**: 2026-2034 [PMID: 27203778 DOI: 10.1016/S0140-6736(16)00557-2]
- 200 **Helms F**, Lau S, Aper T, Zippusch S, Klingenberg M, Haverich A, Wilhelmi M, Böer U. A 3-Layered Bioartificial Blood Vessel with Physiological Wall Architecture Generated by Mechanical Stimulation. *Ann Biomed Eng* 2021; **49**: 2066-2079 [PMID: 33483842 DOI: 10.1007/s10439-021-02728-9]
- 201 **Kobayashi J**, Kikuchi A, Aoyagi T, Okano T. Cell sheet tissue engineering: Cell sheet preparation, harvesting/manipulation, and transplantation. *J Biomed Mater Res A* 2019; **107**: 955-967 [PMID: 30684395 DOI: 10.1002/jbm.a.36627]
- 202 **Mertsching H**, Hansmann J. Bioreactor technology in cardiovascular tissue engineering. *Adv Biochem Eng Biotechnol* 2009; **112**: 29-37 [PMID: 19290496 DOI: 10.1007/978-3-540-69357-4_2]
- 203 **Homan KA**, Gupta N, Kroll KT, Kolesky DB, Skylar-Scott M, Miyoshi T, Mau D, Valerius MT, Ferrante T, Bonventre JV, Lewis JA, Morizane R. Flow-enhanced vascularization and maturation of kidney organoids in vitro. *Nat Methods* 2019; **16**: 255-262 [PMID: 30742039 DOI: 10.1038/s41592-019-0325-y]
- 204 **Krawiec JT**, Vorp DA. Adult stem cell-based tissue engineered blood vessels: a review. *Biomaterials* 2012; **33**: 3388-3400 [PMID: 22306022 DOI: 10.1016/j.biomaterials.2012.01.014]
- 205 **Cho SW**, Lim SH, Kim IK, Hong YS, Kim SS, Yoo KJ, Park HY, Jang Y, Chang BC, Choi CY, Hwang KC, Kim BS. Small-diameter blood vessels engineered with bone marrow-derived cells. *Ann Surg* 2005; **241**: 506-515 [PMID: 15729075 DOI: 10.1097/01.sla.0000154268.12239.ed]
- 206 **Zhao Y**, Zhang S, Zhou J, Wang J, Zhen M, Liu Y, Chen J, Qi Z. The development of a tissue-engineered artery using decellularized scaffold and autologous ovine mesenchymal stem cells. *Biomaterials* 2010; **31**: 296-307 [PMID: 19819544 DOI: 10.1016/j.biomaterials.2009.09.049]
- 207 **Kaushal S**, Amiel GE, Guleserian KJ, Shapira OM, Perry T, Sutherland FW, Rabkin E, Moran AM, Schoen FJ, Atala A, Soker S, Bischoff J, Mayer JE Jr. Functional small-diameter neovessels created using endothelial progenitor cells expanded ex vivo. *Nat Med* 2001; **7**: 1035-1040 [PMID: 11533707 DOI: 10.1038/nm0901-1035]
- 208 **Borschel GH**, Huang YC, Calve S, Arruda EM, Lynch JB, Dow DE, Kuzon WM, Dennis RG, Brown DL. Tissue engineering of recellularized small-diameter vascular grafts. *Tissue Eng* 2005; **11**: 778-786 [PMID: 15998218 DOI: 10.1089/ten.2005.11.778]
- 209 **Ma X**, He Z, Li L, Liu G, Li Q, Yang D, Zhang Y, Li N. Development and in vivo validation of tissue-engineered, small-diameter vascular grafts from decellularized aortae of fetal pigs and canine vascular endothelial cells. *J Cardiothorac Surg* 2017; **12**: 101 [PMID: 29178903 DOI: 10.1186/s13019-017-0661-x]
- 210 **Dahan N**, Sarig U, Bronshtein T, Baruch L, Karram T, Hoffman A, Machluf M. Dynamic Autologous Reendothelialization of Small-Caliber Arterial Extracellular Matrix: A Preclinical Large Animal Study. *Tissue Eng Part A* 2017; **23**: 69-79 [PMID: 27784199 DOI: 10.1089/ten.TEA.2016.0126]
- 211 **Badylak SF**. The extracellular matrix as a biologic scaffold material. *Biomaterials* 2007; **28**: 3587-3593 [PMID: 17524477 DOI: 10.1016/j.biomaterials.2007.04.043]
- 212 **Agarwal T**, Maiti TK, Ghosh SK. Decellularized caprine liver-derived biomimetic and pro-angiogenic scaffolds for liver tissue engineering. *Mater Sci Eng C Mater Biol Appl* 2019; **98**: 939-948 [PMID: 30813101 DOI: 10.1016/j.msec.2019.01.037]
- 213 **Pan MX**, Hu PY, Cheng Y, Cai LQ, Rao XH, Wang Y, Gao Y. An efficient method for decellularization of the rat liver. *J Formos Med Assoc* 2014; **113**: 680-687 [PMID: 23849456 DOI: 10.1016/j.jfma.2013.05.003]
- 214 **Ahmed E**, Saleh T, Yu L, Kwak HH, Kim BM, Park KM, Lee YS, Kang BJ, Choi KY, Kang KS, Woo HM. Micro and ultrastructural changes monitoring during decellularization for the generation of a biocompatible liver. *J Biosci Bioeng* 2019; **128**: 218-225 [PMID: 30904455 DOI: 10.1016/j.jbiosc.2019.02.007]
- 215 **Badylak SF**, Hoppo T, Nieponice A, Gilbert TW, Davison JM, Jobe BA. Esophageal preservation in five male patients after endoscopic inner-layer circumferential resection in the setting of superficial cancer: a regenerative medicine

- approach with a biologic scaffold. *Tissue Eng Part A* 2011; **17**: 1643-1650 [PMID: 21306292 DOI: 10.1089/ten.TEA.2010.0739]
- 216 **Gilot GJ**, Alvarez-Pinzon AM, Barcksdale L, Westerdahl D, Krill M, Peck E. Outcome of Large to Massive Rotator Cuff Tears Repaired With and Without Extracellular Matrix Augmentation: A Prospective Comparative Study. *Arthroscopy* 2015; **31**: 1459-1465 [PMID: 25891222 DOI: 10.1016/j.arthro.2015.02.032]
- 217 **Quarti A**, Nardone S, Colaneri M, Santoro G, Pozzi M. Preliminary experience in the use of an extracellular matrix to repair congenital heart diseases. *Interact Cardiovasc Thorac Surg* 2011; **13**: 569-572 [PMID: 21979987 DOI: 10.1510/icvts.2011.280016]
- 218 **Kimmel H**, Rahn M, Gilbert TW. The clinical effectiveness in wound healing with extracellular matrix derived from porcine urinary bladder matrix: a case series on severe chronic wounds. *J Am Col Certif Wound Spec* 2010; **2**: 55-59 [PMID: 24527148 DOI: 10.1016/j.jcws.2010.11.002]
- 219 **Soler JA**, Gidwani S, Curtis MJ. Early complications from the use of porcine dermal collagen implants (Permacol) as bridging constructs in the repair of massive rotator cuff tears. A report of 4 cases. *Acta Orthop Belg* 2007; **73**: 432-436 [PMID: 17939470]
- 220 **Rüffer A**, Purbojo A, Cicha I, Glöckler M, Potapov S, Dittrich S, Cesnjevar RA. Early failure of xenogenous decellularised pulmonary valve conduits--a word of caution! *Eur J Cardiothorac Surg* 2010; **38**: 78-85 [PMID: 20219384 DOI: 10.1016/j.ejcts.2010.01.044]
- 221 **Massaro MS**, Pálek R, Rosendorf J, Červenková L, Liška V, Moulisová V. Decellularized xenogeneic scaffolds in transplantation and tissue engineering: Immunogenicity versus positive cell stimulation. *Mater Sci Eng C Mater Biol Appl* 2021; **127**: 112203 [PMID: 34225855 DOI: 10.1016/j.msec.2021.112203]
- 222 **Cramer MC**, Badylak SF. Extracellular Matrix-Based Biomaterials and Their Influence Upon Cell Behavior. *Ann Biomed Eng* 2020; **48**: 2132-2153 [PMID: 31741227 DOI: 10.1007/s10439-019-02408-9]
- 223 **Badylak SF**, Gilbert TW. Immune response to biologic scaffold materials. *Semin Immunol* 2008; **20**: 109-116 [PMID: 18083531 DOI: 10.1016/j.smim.2007.11.003]
- 224 **Mora-Solano C**, Collier JH. Engaging adaptive immunity with biomaterials. *J Mater Chem B* 2014; **2**: 2409-2421 [PMID: 24729870 DOI: 10.1039/c3tb21549k]
- 225 **Brown BN**, Londono R, Tottey S, Zhang L, Kukla KA, Wolf MT, Daly KA, Reing JE, Badylak SF. Macrophage phenotype as a predictor of constructive remodeling following the implantation of biologically derived surgical mesh materials. *Acta Biomater* 2012; **8**: 978-987 [PMID: 22166681 DOI: 10.1016/j.actbio.2011.11.031]
- 226 **Badylak SF**, Valentin JE, Ravindra AK, McCabe GP, Stewart-Akers AM. Macrophage phenotype as a determinant of biologic scaffold remodeling. *Tissue Eng Part A* 2008; **14**: 1835-1842 [PMID: 18950271 DOI: 10.1089/ten.tea.2007.0264]
- 227 **Londono R**, Dziki JL, Haljasmaa E, Turner NJ, Leifer CA, Badylak SF. The effect of cell debris within biologic scaffolds upon the macrophage response. *J Biomed Mater Res A* 2017; **105**: 2109-2118 [PMID: 28263432 DOI: 10.1002/jbm.a.36055]
- 228 **Valentin JE**, Badylak JS, McCabe GP, Badylak SF. Extracellular matrix bioscaffolds for orthopaedic applications. A comparative histologic study. *J Bone Joint Surg Am* 2006; **88**: 2673-2686 [PMID: 17142418 DOI: 10.2106/jbjs.E.01008]
- 229 **White LJ**, Taylor AJ, Faulk DM, Keane TJ, Saldin LT, Reing JE, Swinehart IT, Turner NJ, Ratner BD, Badylak SF. The impact of detergents on the tissue decellularization process: A ToF-SIMS study. *Acta Biomater* 2017; **50**: 207-219 [PMID: 27993639 DOI: 10.1016/j.actbio.2016.12.033]
- 230 **Hutter H**, Vogel BE, Plenefisch JD, Norris CR, Proenca RB, Spieth J, Guo C, Mastwal S, Zhu X, Scheel J, Hedgecock EM. Conservation and novelty in the evolution of cell adhesion and extracellular matrix genes. *Science* 2000; **287**: 989-994 [PMID: 10669422 DOI: 10.1126/science.287.5455.989]
- 231 **Keane TJ**, Badylak SF. The host response to allogeneic and xenogeneic biological scaffold materials. *J Tissue Eng Regen Med* 2015; **9**: 504-511 [PMID: 24668694 DOI: 10.1002/term.1874]
- 232 **Huleihel L**, Bartolacci JG, Dziki JL, Vorobyov T, Arnold B, Scarritt ME, Pineda Molina C, LoPresti ST, Brown BN, Naranjo JD, Badylak SF. Matrix-Bound Nanovesicles Recapitulate Extracellular Matrix Effects on Macrophage Phenotype. *Tissue Eng Part A* 2017; **23**: 1283-1294 [PMID: 28580875 DOI: 10.1089/ten.TEA.2017.0102]
- 233 **Beattie AJ**, Gilbert TW, Guyot JP, Yates AJ, Badylak SF. Chemoattraction of progenitor cells by remodeling extracellular matrix scaffolds. *Tissue Eng Part A* 2009; **15**: 1119-1125 [PMID: 18837648 DOI: 10.1089/ten.tea.2008.0162]
- 234 **Agmon G**, Christman KL. Controlling stem cell behavior with decellularized extracellular matrix scaffolds. *Curr Opin Solid State Mater Sci* 2016; **20**: 193-201 [PMID: 27524932 DOI: 10.1016/j.cossms.2016.02.001]
- 235 **Ott HC**, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, Taylor DA. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nat Med* 2008; **14**: 213-221 [PMID: 18193059 DOI: 10.1038/nm1684]
- 236 **Wainwright JM**, Czajka CA, Patel UB, Freytes DO, Tobita K, Gilbert TW, Badylak SF. Preparation of cardiac extracellular matrix from an intact porcine heart. *Tissue Eng Part C Methods* 2010; **16**: 525-532 [PMID: 19702513 DOI: 10.1089/ten.TEC.2009.0392]
- 237 **Bruyneel AAN**, Carr CA. Ambiguity in the Presentation of Decellularized Tissue Composition: The Need for Standardized Approaches. *Artif Organs* 2017; **41**: 778-784 [PMID: 27925237 DOI: 10.1111/aor.12838]
- 238 **Ferng AS**, Connell AM, Marsh KM, Qu N, Medina AO, Bajaj N, Palomares D, Iwanski J, Tran PL, Lotun K, Johnson K, Khalpey Z. Acellular porcine heart matrices: whole organ decellularization with 3D-bioscaffold & vascular preservation. *J Clin Transl Res* 2017; **3**: 260-270 [PMID: 30873477]
- 239 **Alexanian RA**, Mahapatra K, Lang D, Vaidyanathan R, Markandeya YS, Gill RK, Zhai AJ, Dhillon A, Lea MR, Abozeid S, Schmuck EG, Raval AN, Eckhardt LL, Glukhov AV, Lalit PA, Kamp TJ. Induced cardiac progenitor cells repopulate decellularized mouse heart scaffolds and differentiate to generate cardiac tissue. *Biochim Biophys Acta Mol Cell Res* 2020; **1867**: 118559 [PMID: 31634503 DOI: 10.1016/j.bbamcr.2019.118559]
- 240 **Ross EA**, Williams MJ, Hamazaki T, Terada N, Clapp WL, Adin C, Ellison GW, Jorgensen M, Batich CD. Embryonic stem cells proliferate and differentiate when seeded into kidney scaffolds. *J Am Soc Nephrol* 2009; **20**: 2338-2347 [PMID: 19729441 DOI: 10.1681/ASN.2008111196]
- 241 **Nakayama KH**, Batchelder CA, Lee CI, Tarantal AF. Decellularized rhesus monkey kidney as a three-dimensional

- scaffold for renal tissue engineering. *Tissue Eng Part A* 2010; **16**: 2207-2216 [PMID: 20156112 DOI: 10.1089/ten.tea.2009.0602]
- 242 **Sullivan DC**, Mirmalek-Sani SH, Deegan DB, Baptista PM, AbouShwareb T, Atala A, Yoo JJ. Decellularization methods of porcine kidneys for whole organ engineering using a high-throughput system. *Biomaterials* 2012; **33**: 7756-7764 [PMID: 22841923 DOI: 10.1016/j.biomaterials.2012.07.023]
- 243 **Orlando G**, Farney AC, Iskandar SS, Mirmalek-Sani SH, Sullivan DC, Moran E, AbouShwareb T, De Coppi P, Wood KJ, Stratta RJ, Atala A, Yoo JJ, Soker S. Production and implantation of renal extracellular matrix scaffolds from porcine kidneys as a platform for renal bioengineering investigations. *Ann Surg* 2012; **256**: 363-370 [PMID: 22691371 DOI: 10.1097/SLA.0b013e31825a02ab]
- 244 **Song JJ**, Guyette JP, Gilpin SE, Gonzalez G, Vacanti JP, Ott HC. Regeneration and experimental orthotopic transplantation of a bioengineered kidney. *Nat Med* 2013; **19**: 646-651 [PMID: 23584091 DOI: 10.1038/nm.3154]
- 245 **Ott HC**, Clippinger B, Conrad C, Schuetz C, Pomerantseva I, Ikonomou L, Kotton D, Vacanti JP. Regeneration and orthotopic transplantation of a bioartificial lung. *Nat Med* 2010; **16**: 927-933 [PMID: 20628374 DOI: 10.1038/nm.2193]
- 246 **Petersen TH**, Calle EA, Zhao L, Lee EJ, Gui L, Raredon MB, Gavrilov K, Yi T, Zhuang ZW, Breuer C, Herzog E, Niklason LE. Tissue-engineered lungs for in vivo implantation. *Science* 2010; **329**: 538-541 [PMID: 20576850 DOI: 10.1126/science.1189345]
- 247 **Price AP**, England KA, Matson AM, Blazar BR, Panoskaltsis-Mortari A. Development of a decellularized lung bioreactor system for bioengineering the lung: the matrix reloaded. *Tissue Eng Part A* 2010; **16**: 2581-2591 [PMID: 20297903 DOI: 10.1089/ten.TEA.2009.0659]
- 248 **Song JJ**, Kim SS, Liu Z, Madsen JC, Mathisen DJ, Vacanti JP, Ott HC. Enhanced in vivo function of bioartificial lungs in rats. *Ann Thorac Surg* 2011; **92**: 998-1005; discussion 1005 [PMID: 21871290 DOI: 10.1016/j.athoracsur.2011.05.018]
- 249 **Hung SH**, Su CH, Lin SE, Tseng H. Preliminary experiences in trachea scaffold tissue engineering with segmental organ decellularization. *Laryngoscope* 2016; **126**: 2520-2527 [PMID: 26928374 DOI: 10.1002/lary.25932]
- 250 **Minami T**, Ishii T, Yasuchika K, Fukumitsu K, Ogiso S, Miyauchi Y, Kojima H, Kawai T, Yamaoka R, Oshima Y, Kawamoto H, Kotaka M, Yasuda K, Osafune K, Uemoto S. Novel hybrid three-dimensional artificial liver using human induced pluripotent stem cells and a rat decellularized liver scaffold. *Regen Ther* 2019; **10**: 127-133 [PMID: 31032388 DOI: 10.1016/j.reth.2019.03.002]



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