Review

Journal of Tissue Engineering
Volume 15: 1–31
© The Author(s) 2024
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/20417314241300386
journals.sagepub.com/home/tej

Decellularized extracellular matrix for organoid and engineered organ culture

Xiaoxu Guo[1*], Boxun Liu[2*], Yi Zhang[2], Sousan Cheong[1], Tao Xu[3,4], Feng Lu[1] and Yunfan He[1]

Abstract

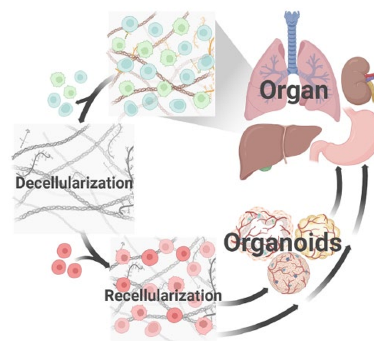
The repair and regeneration of tissues and organs using engineered biomaterials has attracted great interest in tissue engineering and regenerative medicine. Recent advances in organoids and engineered organs technologies have enabled scientists to generate 3D tissue that recapitulate the structural and functional characteristics of native organs, opening up new avenues in regenerative medicine. The matrix is one of the most important aspects for improving organoids and engineered organs construction. However, the clinical application of these techniques remained a big challenge because current commercial matrix does not represent the complexity of native microenvironment, thereby limiting the optimal regenerative capacity. Decellularized extracellular matrix (dECM) is expected to maintain key native matrix biomolecules and is believed to hold enormous potential for regenerative medicine applications. Thus, it is worth investigating whether the dECM can be used as matrix for improving organoid and engineered organs construction. In this review, the characteristics of dECM and its preparation method were summarized. In addition, the present review highlights the applications of dECM in the fabrication of organoids and engineered organs.

Keywords

Organoid, decellularized extracellular matrix, tissue engineering, biofabrication

Date received: 11 July 2024; accepted: 1 November 2024

Graphical Abstract (Created with bioRender.com)



[1]The Department of Plastic and Cosmetic Surgery, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong, China
[2]Research and Development Department, Huamei Biotech Co. Ltd., Shenzhen, China
[3]Tsinghua Shenzhen International Graduate School, Tsinghua University, Shenzhen, People's Republic of China
[4]Bio-intelligent Manufacturing and Living Matter Bioprinting Center, Research Institute of Tsinghua University in Shenzhen, Tsinghua University, Shenzhen, People's Republic of China

*These authors contributed equally to this work.

Corresponding authors:
Tao Xu, Center for Bio-intelligent Manufacturing and Living Matter Bioprinting, Research Institute of Tsinghua University in Shenzhen, Tsinghua University, No. 39 Gaoxin South Ninth Road, Shenzhen, Guangdong 518057, China.
Email: drtaoxu@yeah.net

Feng Lu, The Department of Plastic and Cosmetic Surgery, Nanfang Hospital, Southern Medical University, 1838 Guangzhou North Road, Guangzhou, Guangdong 510515, China.
Email: doctorlufeng@hotmail.com

Yunfan He, The Department of Plastic and Cosmetic Surgery, Nanfang Hospital, Southern Medical University, 1838 Guangzhou North Road, Guangzhou, Guangdong 510515, China.
Email: doctorheyunfan@hotmail.com

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

Introduction

The repair and replacement of damaged tissue or organs are driving an expanding need worldwide. However, there is a critical shortage of organs available for transplantation and the post-transplant immunological rejection still remains a significant concern. In the United States alone, the annual expenditure for diseases related to tissue defects and organ failure exceeds $400 billion.[1] Recently, extracellular matrix (ECM)-derived bio-scaffolds due to their excellent biocompatibility have drawed increasing interest in tissue engineering and regenerative medicine due to their excellent biocompatibility, biological activity, and mechanical properties. As a biological three-dimensional (3D) network, ECM plays a critical role in cell signaling, function, phenotype, and morphology, providing a beneficial microenvironment for cellular homeostasis, growth, tissue formation, and repair.[2,3] Each tissue or organ has its unique ECM, and the interactions between cells and ECM are crucial in regulating cell behavior, function, and fate.[4,5] Due to the complex biological properties and 3D ultrastructure of native ECM, replicating them using traditional manufacturing methods and biomaterials is often challenging.[6]

Decellularized extracellular matrix (dECM) materials refer to biomaterials formed by removing immunogenic cellular components from human or animal organs/tissues using decellularization techniques. dECM minimizes changes to ultrastructure and composition and provides an excellent 3D microenvironment for subsequent cell seeding, thereby opening new avenues for tissue engineering.[7] In clinical practice, the use of dECM is progressively being adopted to support tissue repair and transplantation therapies.[8–10] A variety of commercially available dECM products have been developed, including Alloderm® and GraftJacket®, which are derived from human dermis and used for repairing skin wounds as well as tendon and ligament injuries. Additionally, OASIS®, sourced from porcine small intestinal submucosa, is utilized for skin wound healing, while Prima™ Plus and CardioCel®, derived from porcine and bovine cardiac tissues, are employed in cardiac valve repairs.[11–16] The emergence of these products underscores the versatility and potential of dECM-based biomaterials in clinical applications. Currently, tissue engineering materials derived from dECM have been widely applied in the fabrication of engineered tissue and organ-like constructs, including the gastrointestinal tract, kidneys, liver, lungs, pancreas, uterus, bone,[17–19] cartilage,[20–24] heart,[25–29] skin,[30,31] brain,[32,33] esophagus,[34] and oral cavity,[35] among others. Compared to traditional two-dimensional culture models, organoids represent an innovative technology that can recapitulate the entire physiological processes of an organism. They possess advantages such as a closer resemblance to physiological cell composition and behavior, a more stable genome, and suitability for biological transfection and high-throughput screening. In contrast to animal models, organoid models offer simpler operation and can be utilized to study mechanisms underlying disease onset and progression. Therefore, they hold broad application prospects in various fields including organ development, precision medicine, regenerative medicine, drug screening, gene editing, and disease modeling. Organoids refer to tissue-like structures formed through the in vitro 3D culture of adult stem cells (ASCs) or pluripotent stem cells (PSCs) with a certain spatial organization. While not exact replicas of human organs, organoids can mimic real organs in structure and function, enabling the closest possible simulation of in vivo tissue structure and function as well as long-term stable passaging. Currently, the cultivation of most organoids relies on Matrigel, a commercial matrix derived from the Engelbreth-Holm-Swarm mouse sarcoma, comprising a variety of elements including laminin, type IV collagen, and growth factors. However, in organoid culture, there can be instances where morphogenetic control during the self-assembly process is suboptimal. Possible reasons for this include the lack of tissue-specific cell types, typically the absence of matrix, vasculature, and immune components, as well as potential limitations in nutrient supply leading to cell death and accumulation within the central and luminal cavities of organoids. Matrigel also has several other drawbacks in orgnoid culture.[36] Compared to porcine and human-derived dECM, Matrigel exhibits greater compositional differences from natural human ECM.[37,38] Additionally, Matrigel poses potential antigenicity and potential risks of harboring animal pathogens that could infect macrophages and impact the immune system (e.g. lactate dehydrogenase-elevating virus).[39,40] It is well established that ECM and dECM provide essential environments for cell growth, proliferation, and differentiation, possessing tissue specificity and containing rich regulatory factors. The mechanical properties of both ECM and dECM, including stiffness and viscoelasticity, are also crucial for maintaining the structural integrity and functionality of organoids. Numerous studies have utilized tissue-specific dECM for culturing organoids. For example, human intestinal dECM has been employed to construct intestinal organoids,[41,42] and porcine or human liver dECM has been used to develop hepatic and cholangiocyte organoids.[43–45] Further specific applications are discussed in Section “dECM-based materials for organoids engineering and organ” of this review. Consequently, there is an increasing emphasis on elucidating the interactions among ECM, dECM, organogenesis, stem cells, and the surrounding cellular microenvironment to optimize organoid culture and enhance their potential applications in regenerative medicine and disease modeling. This optimization encompasses not only the selection of appropriate ECM and dECM components and the modulation of their mechanical properties but also the regulation of cellular behavior

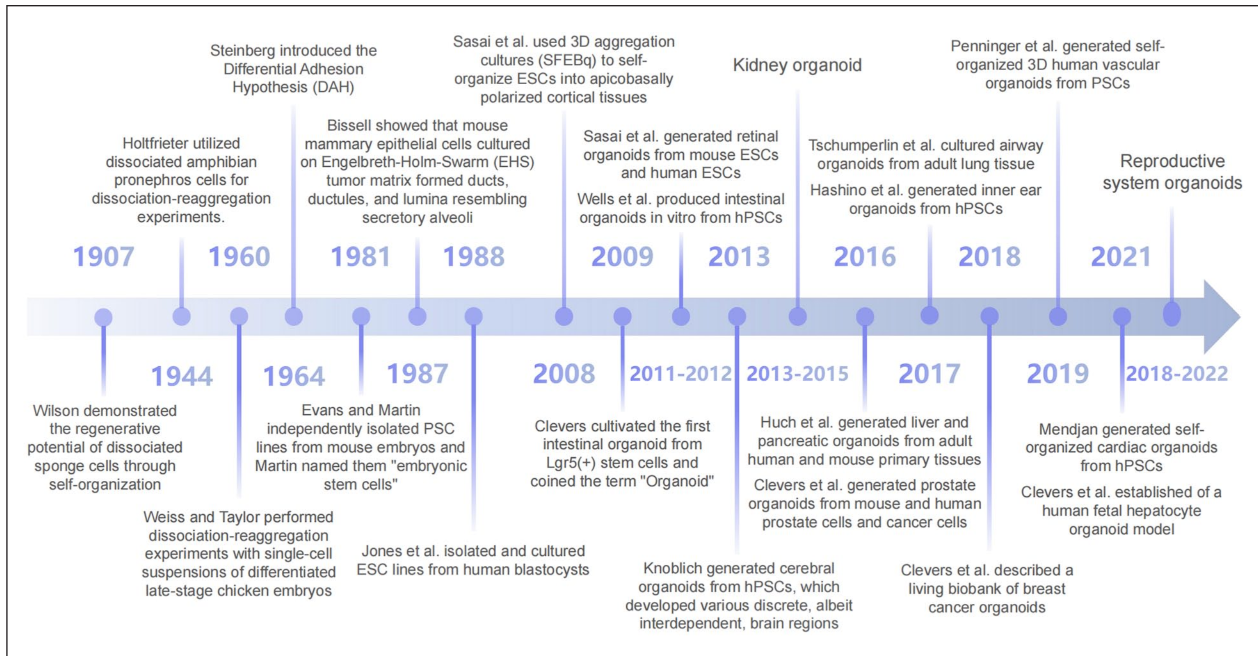


Figure 1. Historical overview of organoid development. This figure presents key historical events in the development of organoid theories and cultivation research. 1944,[57] 1960,[58] 1964,[59] 1987,[60,61] 2008,[62] 2011–2012,[63–65] 2013,[66] 2013–2015,[67–70] 2016,[71,72] 2017,[73,74] 2018,[75] 2019.[76]

through biochemical signals and physical environmental factors. Currently, bioengineering of whole organ culture based on dECM scaffolds is getting popular, and dECM-based organoids also have the potential to develop into engineered organs.

This review emphasizes the preparation of dECM-derived biomaterials from tissues/organs and the necessity of post-decellularization processes such as sterilization, as well as their applications in organoid and organ engineering dECM scaffolds are considered promising biomaterials as bioinks for 3D bioprinting due to their capability to transition from pre-gel solutions to hydrogels, which is also discussed herein.

Organoids

Single cells or small clusters of cells proliferate and reorganize to form complex and structured cellular assemblies that mimic the functional attributes of specific organs, termed organoids. The study of organoids traces back to the early 20th century (Figure 1). In 1907, Wilson demonstrated that dissociated sponge cells possess the inherent capability to self-organize and regenerate entire organisms.[46] The foundational work for organoid technology was laid in 1981 when Evans and Martin independently isolated pluripotent stem cell lines from mouse embryos, with Martin naming them "embryonic stem cells".[47,48] By 1998, Jones et al. had successfully isolated and cultured embryonic stem cell lines from human blastocysts, expanding the possibilities for using stem cells to culture organoids.[49] Significant advancements were made in the 2000s. In 2009, Professor Hans Clevers coined the term "Organoid" and he cultivated the first intestinal organoid from Lgr5(+) stem cells derived from mouse intestines.[50] Recent years have seen rapid developments in organoid research, including Mendjan's generation of self-organized cardiac organoids from human pluripotent stem cells (hPSCs) in 2021[51] and Clevers et al.'s establishment of a human fetal hepatocyte organoid model.[52] Significant advancements in reproductive system organoids were also made from 2018 to 2022.[53–56]

Organoids can be classified into ASC-derived organoids and PSC-derived organoids, according to the cellular origin. ASCs can be directly harvested from human tissues, simplifying the cultivation protocols for organoids. However, for adult organs like the heart, where tissue-specific cells with stem cell properties may not be obtainable, the generation of organoids using ASCs might not be feasible. Since human ASCs are sourced from adult tissues rather than embryonic sources, their use is ethically uncontroversial. Compared to PSC-derived organoids, ASC-derived organoids more closely resemble mature adult tissues, making them better suited for modeling adult tissue repair. They can also be expanded ex vivo over extended periods while maintaining genetic stability.[77,78] PSC-derived organoids can be cultivated using embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). ESCs, originating from the inner cell mass, are

capable of differentiating into all three germ layers, exhibiting robust self-renewal and pluripotent differentiation capabilities.[79] iPSCs can also differentiate into all three germline cell types,[80] and can be reprogrammed from patient-derived somatic cells using small molecules or transcription factors to generate organoids. iPSC-derived organoids, with their complex cellular compositions, are often used to study early organogenesis, although they typically do not expand further once they reach their differentiation endpoint.[77,81,82] Organoids from various sources possess the capacity to include high-functioning cell populations, encapsulating both the function and structure of the original organs. The cells within organoids follow the differentiation trajectories of the organs, and can also develop vascular-like structures similar to those of the host, potentially enabling long-term survival post-transplantation.[83] Utilizing organoids for model construction offers advantages of reduced model development time, personalization, and genetic editing capabilities.

In the cultivation and application of organoids, a critical challenge lies in developing mature organoids that possess the functional and physiological properties of native organs, essential for tissue repair and biological function reconstruction. Advances in bioengineering have enabled the standardized, programmable, and scalable cultivation of organoids. For instance, the use of photolithography or micropore technology to fabricate 3D porous plates allows cells to form aggregates with specific parameters through micropore arrays, enhancing the consistency of organoid modeling.[84,85] Bioreactors increasingly facilitate the large-scale, high-quality, efficient, and homogeneous cultivation of organoids. Bioreactor systems, by employing rotational forces, enhance the uniform diffusion of internal materials and support the growth of 3D structures,[86] offering a cost-effective solution with higher morphological stability of the cultivated organoids and the potential for large-scale and homogeneous organoid cultivation.[87,88] The development of hydrogel matrices with high biocompatibility, which can dynamically mimic the physiological ECM due to their unique 3D structures and editable properties, has also become a focal point of research. Organoids cultivated in specific hydrogels outperform those lacking in vivo-like microenvironment conditions.[89] Utilizing cell-containing hydrogels as bio-inks for 3D bioprinting allows for the construction of organoids. This technique holds tremendous potential for accurately and reproducibly creating complex microstructures, crucial for cultivating organoids that closely resemble the structure and function of original organs.[90,91]

Current organoid models are limited in maturity and functionality, exhibiting only certain functions of the tissues and failing to replicate the complete physiological lineage of cell types, maturity, and function of their respective original organs. As organoids increase in size, nutrient supply to cells located at the center of the organoid becomes restricted, which can lead to severe cell death. This issue is more likely to occur in densely structured organoids (such as the brain) and those forming cavitated structures (such as bile ducts and pancreas).[92] The majority of organoid models are derived from a single type of stem cells and lack tissue-specific cell types, including niche-specific stromal cells, immune cells, vascularization, innervation, or microbiomes. These cell types play a crucial role in establishing a specific microenvironment, enabling organoid models to more effectively mimic the physiological responses of real tissues. Additionally, studies on cell behavior within organoids derived from stem cells remain scarce. Consequently, even under the same experimental conditions, cultivated organoids exhibit high heterogeneity and significant variability in phenotypic characteristics (such as shape, size, and cellular composition), which is disadvantageous for high-throughput screening experiments and research requiring high spatial and temporal resolution imaging.[93,94] Optimizing the interactions between tissue-specific cells and the ECM, as well as local physical and biochemical microenvironment characteristics, can significantly reduce batch-to-batch heterogeneity.[95] Synthetic matrices can be used to control the composition and stiffness of the culture medium. Currently, a promising approach is the use of dECM that provides a matrix rich in tissue-specific regulatory factors, offering a better environment for the growth, proliferation, and differentiation of stem cells and organoids.[38,96]

Decellularization extracellular matrix

dECM is a promising natural biomaterial prepared from human or animal tissues or organs through decellularization.[97] dECM retains the 3D structure and biological properties of ECM, exhibiting bioactivity, biocompatibility, and non-immunogenicity.[98,99] It preserves numerous cell growth factors, such as fibroblast growth factor, transforming growth factor, and hepatocyte growth factor,[100] which can enhance the growth, migration, proliferation, differentiation, and angiogenesis of seeded cells. This "real-time interaction" with seeded cells can reshape the structure of tissues and organs, playing a crucial role in tissue and organ regeneration and functional repair. Therefore, various scaffolds and materials based on dECM have garnered increasing attention in recent years.

The origin of ECM

ECM can be derived from three primary sources: human tissues, cell cultures, and animal tissues. ECM from human sources significantly reduces the risk of immune rejection associated with xenogenic ECM, such as the response to α-gal epitopes.[101] Moreover, human-derived ECM best mimics the structural and functional properties of native tissues and organs, offering superior biocompatibility and

clinical efficacy. Therefore, human tissues are the preferred source.[102] Ideally, tissues from young, healthy donors are the best source, but human tissue is relatively scarce and its availability is limited, especially for younger, healthier tissues.[103] Cadaveric tissue can be used as donors but availability is again limited.[104] Cadaveric skin, sold as Alloderm and GraftJacket®,[105,106] is used clinically for wound treatment. Tissues associated with birth, such as the placenta, umbilical cord, and fetal membranes, are often discarded after delivery, making them more abundant and accessible. Studies have shown that ECM derived from placental tissues possesses favorable immunogenic and regenerative properties, containing various growth factors (e.g. epidermal growth factor, TGF-β, VEGF) that support the growth and differentiation of diverse cell types.[107,108] For instance, research by Murchison et al. demonstrated that placental ECM hydrogels can serve as suitable substrates for hiPSCs differentiation into the three embryonic layers.[109] Wang et al. utilized placental dECM hydrogels to construct spinal cord-like organoids that are more stable and mature than those developed using Matrigel.[110]

Human ECMs secreted by human cells culture can also be applied in tissue engineering and regenerative medicine (TERM).[111] For example, Gonzalez-Fernandez et al. enhanced the survival and proliferation of mesenchymal stromal cells (MSCs) by incorporating ECM secreted during the spheroid formation of MSCs. When these ECM-loaded spheroids were encapsulated in inductive alginate gels, their osteogenic differentiation capability was enhanced.[112] Wan et al. found that dECM obtained from MSCs differentiated into osteoblasts exhibited stronger osteoinductive effects on reseeded MSCs.[113] Furthermore, Choi et al. demonstrated that ECM secreted by decellularized human iPSC-derived chondrocytes enhanced chondrogenesis of hiPSCs and promoted cartilage repair when specific dECM was implanted into cartilage defects in rat knees.[114] These studies and concepts provide a promising avenue for isolating cell-type-specific ECMs, though yield remains limited. Some researchers are focusing on using stable cell lines to produce these ECMs, minimizing batch-to-batch variations due to different donors, thereby offering new insights into these challenges.[115]

ECM from animal tissues is more abundant, but shares many limitations with Matrigel derived from murine sources. Antigenic glycans found in animal tissues, such as α-gal and Neu5Gc, can lead to chronic rejection and calcification issues. Additionally, certain protein components (e.g. protein antigens in bovine bioprosthetic valves) might trigger immune responses, and the presence of porcine endogenous retrovirus (PERVs) genes poses potential immunological and pathogenic risks in clinical applications.[40,116] Nonetheless, due to their availability, animal-derived ECMs remain the most commonly used resources. Compared to Matrigel, tissues from cattle and pigs share greater structural

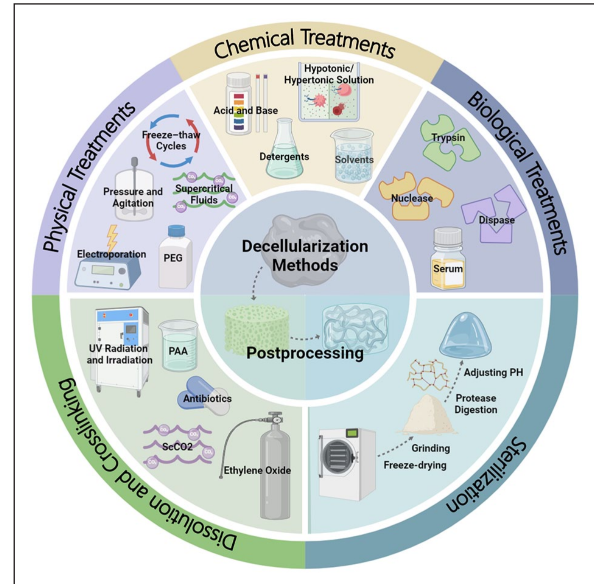


Figure 2. Overview of common decellularization techniques and post-treatment processes. Decellularization of target tissues is effectively achieved through chemical, physical, or enzymatic methods, with combinations of these approaches often being employed. The harvested dECM materials are subjected to sterilization procedures to eliminate potential microbial contamination, reduce toxicity, and enhance biocompatibility. Inevitably, these processes can impact the structure and functionality of the dECM, which is frequently addressed by employing solubilization and crosslinking methods for improvement. (Created with bioRender.com).

similarity with human tissues, particularly pigs, gaining increasing attention and research focus. During decellularization, optimizing methods to reduce immunogenicity, such as the removal of α-gal epitopes, helps lower the risk of immune rejection. Therefore, the careful selection of animal sources is crucial to ensure protein structures are closely related to human tissues, alongside effective antigen removal and decellularization processes to mitigate risks.[45,117–119]

Decellularization methods

Effective decellularization maximally removes all cellular and nuclear components from tissues or organs, reducing or eliminating immune rejection reactions and providing a foundation for cell development of new tissues and organs (Figure 2).[116] Therefore, various factors such as cell type, tissue density, thickness, and lipid content determine the effectiveness of tissue decellularization methods.[120]

Various decellularization methods involve the use of chemical, physical, or enzymatic approaches to remove cells and genetic material residues from the target tissue (Table 1). Different methods are often combined to more effectively eliminate cells and genetic material residues from the scaffold. Physical methods include temperature, pressure, supercritical fluids, polyethylene glycol, and

Table 1. Commonly decellularization methods and the effects.

Decellularization methods	Mechanism	Impact on cells and ECM	Ref.
Physical treatments			
Freeze–thaw cycles	Repeatedly forming ice crystals inside the cells to disrupt the cell membrane.	Inducing cell lysis and ECM disruption, with incomplete separation of cellular remnants; rapid freezing process causing ultrastructural changes in ECM, the use of cryoprotectants can reduce ECM damage.	123,124
Pressure or mechanical force	Inducing cell rupture to remove cellular debris.	Minimizing the exposure time to harsh reagents to disrupt the ultrastructure and mechanical properties of ECM.	125,126
Mechanical agitation	Enhancing the efficiency of chemical agents and removal of cellular material.	Disrupting the ultrastructure of ECM, in combination with chemical or biological decellularization methods.	120
Supercritical CO_2-based extraction	Pressure can induce cell rupture, thereby enhancing the efficiency of chemical agents and removal of cellular material.	Short duration, minimal alteration of ECM mechanical properties, enabling complete removal of cells.	127–129
Electroporation	Disrupting the cell membrane in combination with other methods.	It can preserve the ultrastructure of ECM and is suitable for smaller organs.	130
Chemical treatments			
Acid and base	Disrupting the cytoplasm or nucleic acids, leading to protein denaturation.	It has sterilizing capabilities, can cause denaturation of collagen, eliminate growth factors, and alter ECM mechanical properties.	125,126
Nonionic detergents—Triton X-100	Disrupting the lipid-lipid and lipid-protein connections of the cell membrane, with minimal disruption of protein-protein connections.	A relatively mild detergent suitable for use on thinner tissues and organ sites, capable of completely removing cellular remnants with minimal impact on ECM ultrastructure, potentially resulting in minor loss of collagen and GAGs and relaxation of the collagen network.	131,132
Ionic detergents—sodium dodecyl sulfate (SDS)	Enhanced solubilization of the plasma membrane and nuclear envelope.	Potent detergents are capable of dissolving cellular membranes, removing cellular components from dense tissues, and depleting collagen and GAGs. This process may result in a reduction of growth factor content within ECM.	132,133
Poly (ethylene glycol) (PEG): PEG with different molecular weights	Incomplete separation of cellular remnants.	Inducing cell lysis and ECM disruption can avoid the use of detergents, but cannot be used alone for decellularization.	134–136
Zwitterionic detergents—CHAPS	Balancing the effects of both ionic and nonionic detergents.	Suitable for thin tissues, this approach is more effective at removing cellular components compared to nonionic detergents, while preserving ECM more effectively than ionic detergents.	131,137
Hypotonic/hypertonic solution	Utilizing osmotic pressure to disrupt cell integrity and DNA-protein interactions.	Capable of effectively lysing cells, but exhibiting suboptimal removal of cellular debris.	138,139
Solvents—alcohols/acetone	Cell disruption is achieved through the dehydrating action or by dissolving and removing lipids with the use of alcohols and acetone.	Alcohols and acetone can effectively remove cellular components and heat sources from dense tissues, facilitate the removal of genetic material, and cause the denaturation of collagen and other ECM proteins, resulting in a harder texture of the scaffold.	140,141
Acetic acid	The cytoplasm is solubilized, nucleic acids are disrupted, and proteins are denatured.	The process preserves GAGs while selectively removing collagen proteins.	142
Peroxyacetic acid	Nucleic acids are solubilized and subsequently removed.	The impact on the composition and structure of ECM is minimal.	143,144
Biological treatments			
Trypsin	Hydrolyzes the C-terminal peptide bonds of arginine and lysine, disrupting protein-protein interactions.	Prolonged processing can compromise the ultrastructure of ECM, damage elastin and collagen, and exhibit weaker removal of GAGs compared to detergents, rendering it unsuitable for sensitive tissues such as the cornea.	145,146
Nucleases (RNase and DNase)	Catalyzes the hydrolysis of DNA and RNA.	Effectively removes cells by degrading nucleic acids, but is difficult to eliminate from tissues and can induce an immune response; typically used in conjunction with detergents.	131,147
Dispase	Hydrolyzes peptides associated with basement membrane proteins.	The removal of fibronectin and type IV collagen is an initial step in the decellularization process to eliminate epithelial and endothelial cells; it is often combined with other decellularization techniques.	125,126
Sera	Serum nucleases catalyze the degradation of DNA and RNA.	Serum nucleases effectively facilitate cell removal while preserving tissue transparency; however, the use of non-human serum carries the risk of cross-species pathogen transmission.	148,149
Chelators—EDTA/EGTA	Divalent cations, such as Ca^{2+} and Mg^{2+}, are essential for cellular adhesion to collagen and fibronectin. Chelating agents disrupt cell-ECM adhesion by binding to these divalent cations.	In conjunction with proteases, such as trypsin, chelating agents can potentiate the decellularization process. Alone, they lack the capacity to decellularize tissues effectively.	125,126,146

electroporation. The basic principle is the mechanical disruption of cell membrane structures in the tissue, leading to cell death, followed by decellularization through methods such as solution washing, nucleic acid, and lipid removal. Chemical methods are the most commonly used and include acids, alkalis, detergents, low and high osmotic solutions, and various chemical solvents, primarily to dissolve cell membranes and degrade DNA. Biological methods mainly involve various enzymes and chelating agents, which can selectively hydrolyze various proteins and genetic material. However, residual enzymes may cause adverse reactions,[121,122], so they are often used in combination with other chemical and biological methods.

The ultimate goal of decellularization is to produce two primary end products: a complete organ scaffold or specific dECM materials. Whole organ decellularization aims to preserve not only the three-dimensional architecture of the organ but also its vascular network, facilitating subsequent recellularization and the development of transplantable human organs. The most commonly employed method is perfusion decellularization, which utilizes custom bioreactors to perfuse reagents through the organ's native vasculature. These reagents include ionic and non-ionic surfactants (such as SDS and Triton X-100) and enzymes (such as DNase). The perfusion duration and reagent concentration are adjusted according to the type and size of the organ.[150–152] Whole organ decellularization techniques are predominantly developed in research institutions and have not yet seen widespread commercialization. The future direction involves improving decellularization efficiency and enhancing scalability, automation, and standardization to better serve regenerative medicine and organ transplantation. Commonly used dECM materials include dECM scaffolds and hydrogels. dECM scaffolds are frequently employed in applications that requires to maintain the tissue/organ's original shapes and structures. By designing the diameter and distribution of microfibers, the architecture of the ECM network can be systematically manipulated to induce specific cellular functions. This is particularly useful in studies involving tissues/organs with multilayered or multi-regional characteristics, diverse cell types, and functional features such as glands and ducts. On the other hand, dECM hydrogels, which require processes such as solubilization and cross-linking, possess high water content and offer greater design flexibility and tunability. Cross-linking can enhance their gelation kinetics and mechanical properties. These hydrogels are well-suited for minimally invasive applications, such as injections, and are often used in scenarios requiring more irregular shapes or where precise shape conformity is not critical. In summary, dECM scaffolds and hydrogels each have their own advantages and the choice depends on specific requirements and application objectives. For preparing dECM materials, the decellularization process must not only remove cellular content and nuclear material while preserving the native ECM structure, but also eliminate potential contaminants or residual detergents. However, different decellularization protocols can still impact the immunogenicity and functionality of the final product. For instance, damage-associated molecular patterns (DAMPs) are danger signals released upon cell and ECM damage during graft processing or implantation. Incomplete or harsh decellularization protocols may leave residual nuclear and mitochondrial DNA, as well as fragmented components like hyaluronic acid and fibronectin, which are renowned DAMPs. These remnants can interact with the innate immune system post-transplantation, potentially leading to graft damage or even transplant failure. Therefore, selecting appropriate decellularization and postprocessing protocols and further developing new methods is crucial. Additionally, the dECM may be digested, degraded, or extracted to create coatings or thermoreversible gels as required. This process can be divided into three main stages: the washing phase, sterilization phase, and processing phase. The washing phase typically employs detergents, enzymes, or denaturing agents to lyse cells and degrade animal tissue. This is followed by rinsing with agents such as PBS to remove residual detergents from the dECM, as these contaminants are often cytotoxic and can inhibit cell proliferation during recellularization. The methods summarized above are commonly combined and can be optimized by adjusting concentration, time, and other parameters to enhance decellularization outcomes.[125] We consider the sterilization and processing phases as the "postprocesses of dECM," which will be introduced in the following discussion.

Postprocesses of dECM

Postprocesses of dECM to improve biocompatibility before application is necessary. Although methods such as acid-base or certain solvents can effectively remove cellular components and have sterilization effects, they may not achieve ideal sterilization due to inadequate penetration, and over-penetration may damage dECM components. Therefore, appropriate disinfection and sterilization processes can be selected based on different sources, decellularization methods, and application scenarios.

Common physical methods include UV radiation and irradiation. UV radiation is simple and easy to perform,[153] and can be used for surface and environmental disinfection. However, it has weak penetration and is usually used in combination with other disinfection methods.[27,154] Irradiation sterilization can directly damage and induce the production of peroxides and free radicals from water molecules, disrupting the genetic material, enzymes, and proteins of microbes, thereby impairing their metabolic functions.[155,156] Ethylene oxide (EtO) sterilization inactivates microbial macromolecules,[157] representing a well-established method. However, EtO is toxic and soluble in

water, leading to potential residual toxicity post-sterilization, necessitating weeks for complete sterilization. It is noteworthy that the use of EtO sterilization is not recommended for loose and porous freeze-dried dECM due to these concerns.[156,158] Peroxyacetic acid (PAA) concentrations of 0.001%, 0.003%, and 0.3% exhibit bactericidal, fungicidal, and sporicidal activities, respectively, with their degradation products being non-toxic and thus safer, while minimally impacting the dECM proteins and its physicochemical properties.[159,160] Antibiotics can help reduce contamination during the decellularization process,[161] yet their efficacy is limited and may leave microbial residues, necessitating subsequent sterility and antibiotic residue testing for dECM.[162–164] Supercritical carbon dioxide ($ScCO_2$) presents a promising approach with its superior penetration and solubility features, offering an eco-friendly method for disinfection and sterilization. Utilizing $ScCO_2$ can reduce the time required to produce the final dECM and is beneficial for its long-term preservation.[22,128,165] It is well-known that disinfection can kill or inhibit the activity of pathogenic microorganisms, but it may not completely eliminate all microorganisms, such as bacterial spores. Sterilization, on the other hand, eradicates all forms of microorganisms, including viruses, fungi, and spores, achieving a sterile state. Disinfection is more convenient and cost-effective, making it suitable for some laboratory-based tests, but it is not appropriate for clinical applications. Ethanol and UV are common disinfection methods; 70%–90% ethanol or isopropanol is frequently used for surface disinfection of materials, and thinner dECM materials may be treated with UV irradiation or radiation. However, for clinical application, sterilization of dECM materials is required. EtO and autoclaving are the routine use of sterilization methods in clinical applications. For dECM materials with high structural integrity, peracetic acid and $ScCO_2$ are recommended. For heat-sensitive dECM materials, EtO and PAA are considered appropriate options.[166] Both disinfection and sterilization may alter the physical and chemical properties of dECM to some extent (e.g. certain collagen cross-linking and macromolecular chain breakage) or lead to the presence of residual sterilizing agents. After all, successful application of dECM materials in clinics require both safety and efficiency. Further studies are needed to explore the clinically acceptable sterilization methods for various dECM materials.

After decellularization and sterilization processes, organs and tissues inevitably undergo changes in ECM shape, biomechanical properties, and collagen fiber exposure. These alterations not only hinder the preservation of the native ECM structure but also adversely affect cell growth and penetration into internal regions, thereby limiting the infiltration and diffusion of oxygen and nutrients. Solubilization and crosslinking are therefore recommended to tune the mechanical properties and physical characteristics of ECM scaffolds. The primary purpose of dissolution is to break down proteins, glycoproteins, and other macromolecules within the dECM and enhance their solubility in water. Additionally, this process can partially dissolve residual cellular components, further reducing immunogenicity. In contrast, the main purpose of crosslinking is to enhance the mechanical strength and stability of the dECM, retard the degradation rate, and mask exposed antigenic sites to reduce immunogenicity. Decellularized tissues are typically digested using chemical and biological agents, such as proteases, and then subjected to physical agitation to achieve the desired concentration of a gel-like solution. Upon termination of the digestion process, the dissolved dECM proteins spontaneously reassemble through intramolecular cross-linking.[167,168] This approach maximally preserves the corresponding bioactivity of dECM materials, reduces purification steps, and retains essential growth factors and small molecule peptides present in natural ECM.

Crosslinking enhances the material's mechanical strength and maintains a stable 3D network structure. Glutaraldehyde is a commonly used chemical crosslinking agent for collagen-based materials, which can reduce calcification and preserving tissue mechanical properties.[169,170] Additionally, ethylene glycol and genipin can serve as crosslinking agents with reduced cytotoxicity.[171,172] Studies have also found that weak crosslinking with genipin and modification with fibronectin and fibrinogen coatings yield excellent cell viability.[173] Furthermore, a poly (2-hydroxyethyl methacrylate) (pHEMA) coating for materials may offer better biocompatibility, anti-calcification, and anti-thrombotic properties than glutaraldehyde.[174] Photooxidation crosslinking offers the advantages of being non-toxic, causing minimal calcification and inflammation, preventing ECM degradation, promoting regeneration of collagen and glycosaminoglycans, and cell infiltration.[175]

The application of solubilization and crosslinking techniques in the processing of dECM has distinct focuses. Solubilization is primarily employed to reduce scaffold stiffness, whereas crosslinking is used to enhance the mechanical properties and stability of the scaffold. Consequently, these techniques are often combined as complementary steps to optimize the final dECM material. However, some challenges remain. Solubilization may lead to the loss of certain ECM components, such as elastin and GAGs, potentially affecting the scaffold's mechanical properties and biocompatibility. Although crosslinking can slow the degradation rate of dECM, providing sufficient time for angiogenesis and new ECM synthesis, extensive crosslinking—especially with chemical crosslinkers—may increase the M1/M2 macrophage polarization ratio, leading to chronic inflammation and foreign body reactions. Therefore, the degradation rate of dECM scaffolds must be finely tuned to balance stability and functional remodeling, minimizing fibrosis and chronic immune responses. In general, while the decellularization process aims to remove

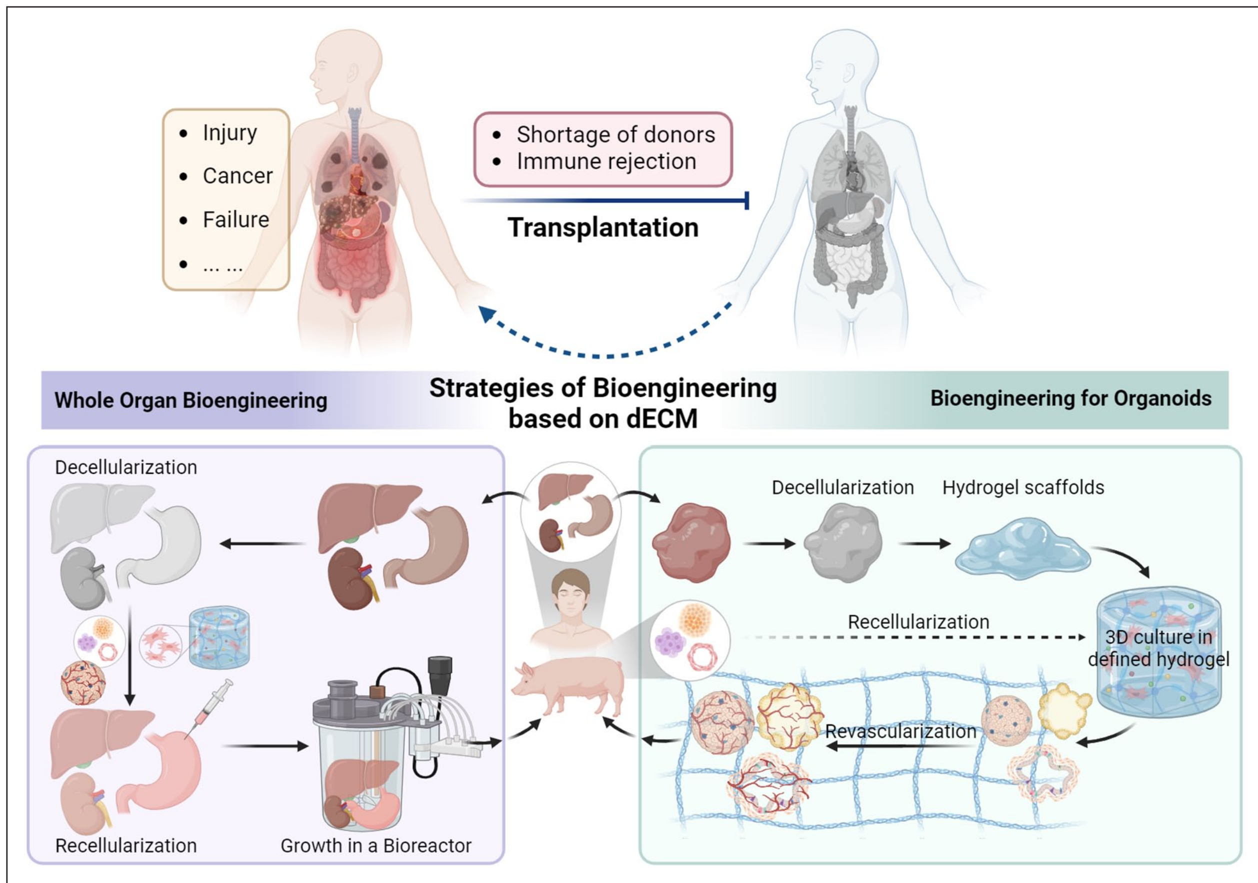


Figure 3. Overview of the application and research progress in organoid and engineered organs based on dECM materials. dECM materials exhibit tissue and organ specificity and can be enhanced with relevant proteins and cellular molecules to promote the development and construction of organoid models that closely mimic natural conditions. The utilization of stem cells and organoids to populate whole-organ decellularized scaffolds, followed by cultivation in bioreactors, has significantly advanced the field of whole-organ bioengineering. With the rapid progress of these technologies, they demonstrate immense potential for applications in regenerative medicine and organ transplantation, offering promising avenues for addressing current challenges in these fields. (Created with bioRender.com).

cellular and nuclear components to reduce immunogenicity, incomplete removal may lead to immune reactions post-implantation. Residual DNA and cellular proteins, such as vimentin, could trigger immune responses. Selective antigen removal and solubilization are two key methods for reducing the immunogenicity of dECM. However, excessively harsh methods should be avoided as they may damage the ultrastructure of dECM or even increase its immunogenicity.

dECM-based materials for organoids engineering and organ systems

The construction of organoids and engineered organs necessitates three fundamental components: seeded cells, culture medium, and matrix. Seeding cells primarily include ESCs, iPSCs, ASCs, and tumor-derived cells. The culture medium is tailored to the specific characteristics, tissue features, and genotype of different types of organoids, requiring specific additives such as growth factors and signaling pathway inhibitors to optimize culturing conditions. The matrix provides structural support and certain nutritional components for the cultivation of organoids. Currently, the most common matrices are animal-derived commercial hydrogels, such as Matrigel, which have significantly advanced the development of organoids in recent years. However, their complex composition and performance instability remain limitations that restrict the clinical application of cultured organoids. An ideal organoid matrix should possess excellent mechanical properties, high biocompatibility, non-toxicity, and non-immunogenicity. Moreover, it should support and enhance cellular activities, mimic the specific microenvironments of various cells, and interact favorably with cells to facilitate component renewal.[176] Utilizing dECM derived from tissue-specific ECM is a promising approach in matrix design, displaying tissue and organ specificity, and can be enhanced with relevant proteins and cellular molecules to foster the development and construction of organoid models that closely mimic natural conditions. Figure 3

provides a summary of the advancements and applications in the field of organoids and engineered organs utilizing dECM materials.

dECM materials for gastrointestinal organoids and engineered organs

For gastrointestinal diseases, particularly those prone to cancer cell dissemination, the current standard treatment involves surgical intervention, known as “en bloc” resection. This encompasses the removal of not only the affected organs but also the concomitant resection of the mesentery containing lymphatic vessels and lymph nodes. Following partial or total organ resection, defects in the gastrointestinal tract are typically compensated by adjacent organs, such as the reconstruction of the esophagus and stomach using the stomach and jejunum. However, adverse sequelae such as loss of reservoir function, impaired anti-reflux barrier, dumping syndrome, and drastic changes in food intake and nutritional status often impact the postoperative course of the disease. The use of tissue-engineered materials obtained from the patient’s own body or other healthy donors for the construction of gastrointestinal-like organs represents an ideal approach for repairing or regenerating tissue or organ defects resulting from disease, surgical resection, and trauma.[177] Three-dimensional microscale organoids of the gastrointestinal tract, referred to as gastrointestinal-like organs, have been established through the in vitro culture system by embedding stem cells into ECM materials.[178]

Currently, the cultivation of most gastrointestinal organoids relies on Matrigel. Due to the aforementioned limitations associated with Matrigel, there is growing interest in utilizing dECM derived from porcine gastric and intestinal tissues for the cultivation of gastrointestinal organoids. The dECM hydrogels extracted from these tissues have been found to possess physiological characteristics and mechanical properties similar to commercially available gels, capable of supporting the development and function of gastrointestinal organoids, thereby enabling long-term passaging and transplantation (Figure 4). The gel maintains appropriate hardness, which is foundational for the formation, survival, and differentiation of gastrointestinal organoids. In comparison to Matrigel, the dECM hydrogel exhibits higher abundance and diversity of collagen subtypes and proteoglycans, upregulation of ECM and cell proliferation-related genes, specific enrichment of key ECM proteins associated with organoid formation, as well as the preservation of numerous exosomal proteins involved in cell adhesion. Therefore, dECM hydrogels derived from porcine gastric and intestinal mucosa/submucosa can support the survival and growth of human gastrointestinal organoids, and even hepatic and pancreatic organoids, both in vitro and in vivo, maintaining structural and characteristic protein expression at the molecular level. They can also serve as a tool for the growth of cells derived from human organoids with stable transcriptomic features and for the delivery of organoids into the body.[38,179] Functional loss of the small intestine due to disease or injury hinders adequate nutrient absorption and may lead to malnutrition and dehydration, a condition known as intestinal failure (IF). Short bowel syndrome (SBS) describes a state of malabsorption that typically occurs following extensive small bowel resection and is the most common cause of chronic irreversible IF (SBS-IF).[180,181] The applicability of small-scale organoid units in extensive SBS-IF is limited. A proposed approach involves the engineering of a human intestinal mucosal transplant by seeding patient-derived organoids and intestinal fibroblasts onto a scaffold derived from decellularized human and patient-derived intestines, forming a functional human intestinal organoid in vitro, which can be utilized for functional analysis and in vivo transplantation.[41,42] This approach represents the first use of pure primary human-sourced cells and biomaterials for bioengineered transplants, closely resembling true organ nanoarchitecture and physiology. The variability of fluid dynamics and its impact on epithelial morphology, as well as the ability to capture neuro-muscular peristaltic activity, are future directions for improving and developing this approach.[43,182]

dECM materials for renal organoids and engineered organs

The kidneys are a pair of bean-shaped organs in vertebrates, responsible for the elimination of waste products and uremic toxins from the bloodstream, contributing significantly to maintaining internal balance. It has been established that a significant portion of renal diseases are caused by or involve alterations in ECM, for example, fibrosis; however, current therapeutic approaches of treating ECM changes in kidneys are still limited. Notably, recurrent acute kidney injury can lead to irreversible kidney damage, progressing to chronic kidney disease (CKD) and potentially culminating in end-stage renal failure. In advanced stages of chronic kidney failure, the kidneys are unable to function properly, and current mainstay clinical treatments rely on lifelong dialysis or kidney transplantation. However, dialysis is costly, adds to the psychological burden of patients, and is associated with complications such as cardiovascular diseases and infections. Kidney transplantation is an effective treatment for end-stage kidney disease but faces a chronic shortage of kidney donors. Kidney organoids represent a potential tool for producing transplantable kidneys. The decellularization and recellularization of kidneys into kidney organoids provide a solid foundation (Figure 5). Kidney dECM preserves the complex structure, composition, and microenvironment of natural kidney tissue, supporting the self-organization and differentiation of stem cells or progenitor cells into

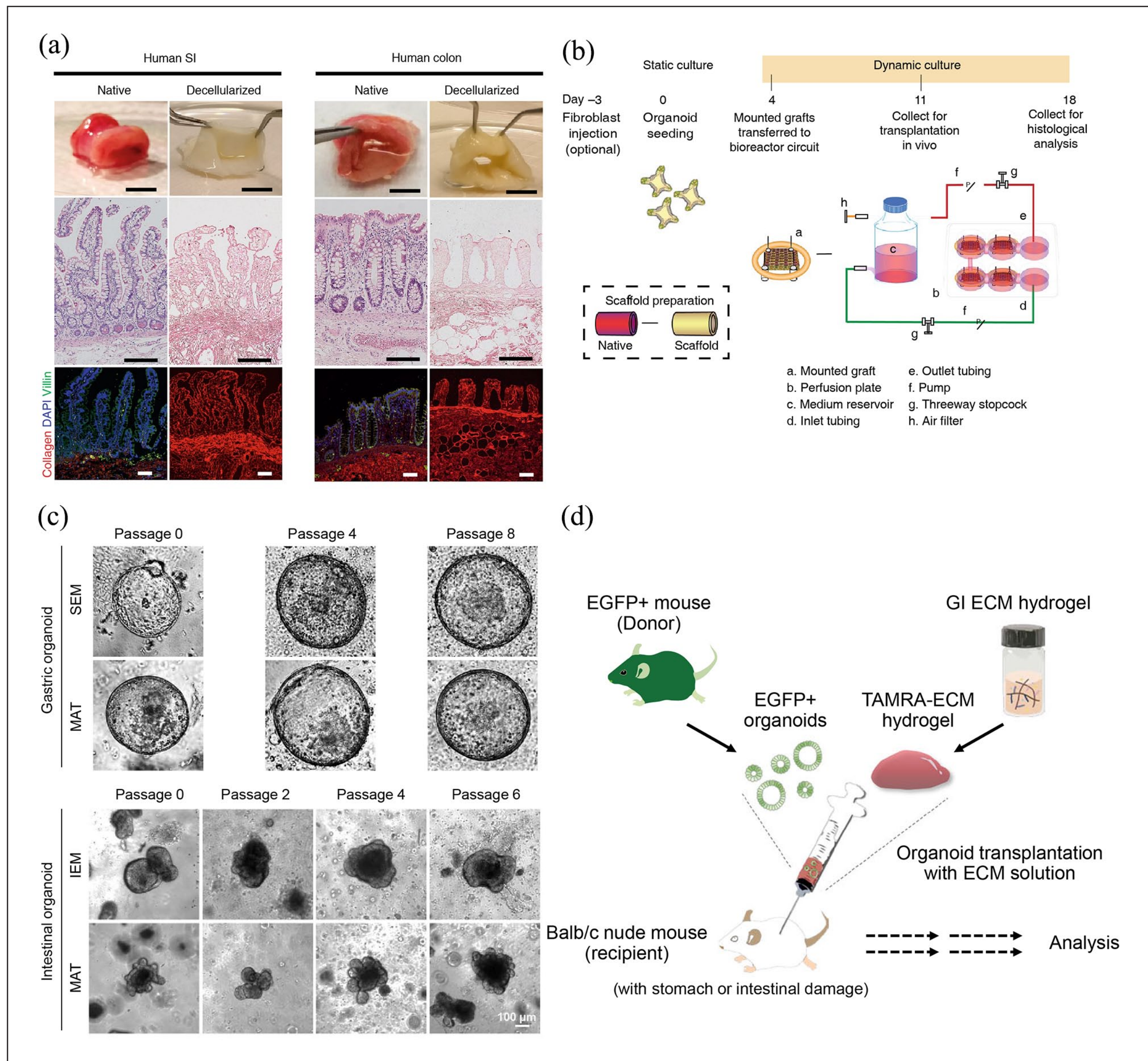


Figure 4. Application of dECM materials in gastrointestinal organoids and engineered organs culture: (a) decellularization characterization of human SI and colon scaffolds, (b) a schematic representation of a strategy for the vitro preparation of functional human jejunal mucosal grafts using a bioreactor, (c) brightfield images of gastrointestinal organoids cultured in decellularized stomach-derived ECM (SEM) or decellularized intestine-derived ECM (IEM) hydrogels and Matrigel, (d) a schematic diagram illustrating the application of dECM hydrogels carrying GI organoids as grafts for treating acute epithelial injuries in mouse models of the stomach and intestine.
Images adapted with permission from Meran et al.[41] and Kim et al.[38]

specific kidney cell types, such as nephrons, collecting ducts, and stromal cells, closely resembling the cellular diversity found in natural kidneys.[183–185] Different decellularization protocols can yield materials with varying histological properties,[186] human cell re-endothelialization degrees,[150] varying degrees of preserved microvascular integrity and functionality, thereby influencing the differentiation of various embryonic stem cells (ESCs) into renal lineages.[187–189] This offers new insights into the importance of optimizing decellularization protocols.

Kidney dECM is currently considered one of the most promising biomaterials for constructing renal organoids and bioartificial kidneys.[190,192] However, insufficient recellularization of scaffolds in vivo still challenges the applications of kidney dECM. Kidney is a highly vascularized organ and complete vascular structures is crucial for recellularization of engineered kidney. Kim et al. have utilized kidney dECM hydrogels to culture renal organoids derived from hPSCs, which have a broad vascular network and self-derived ECs, as well as a more mature glomerular

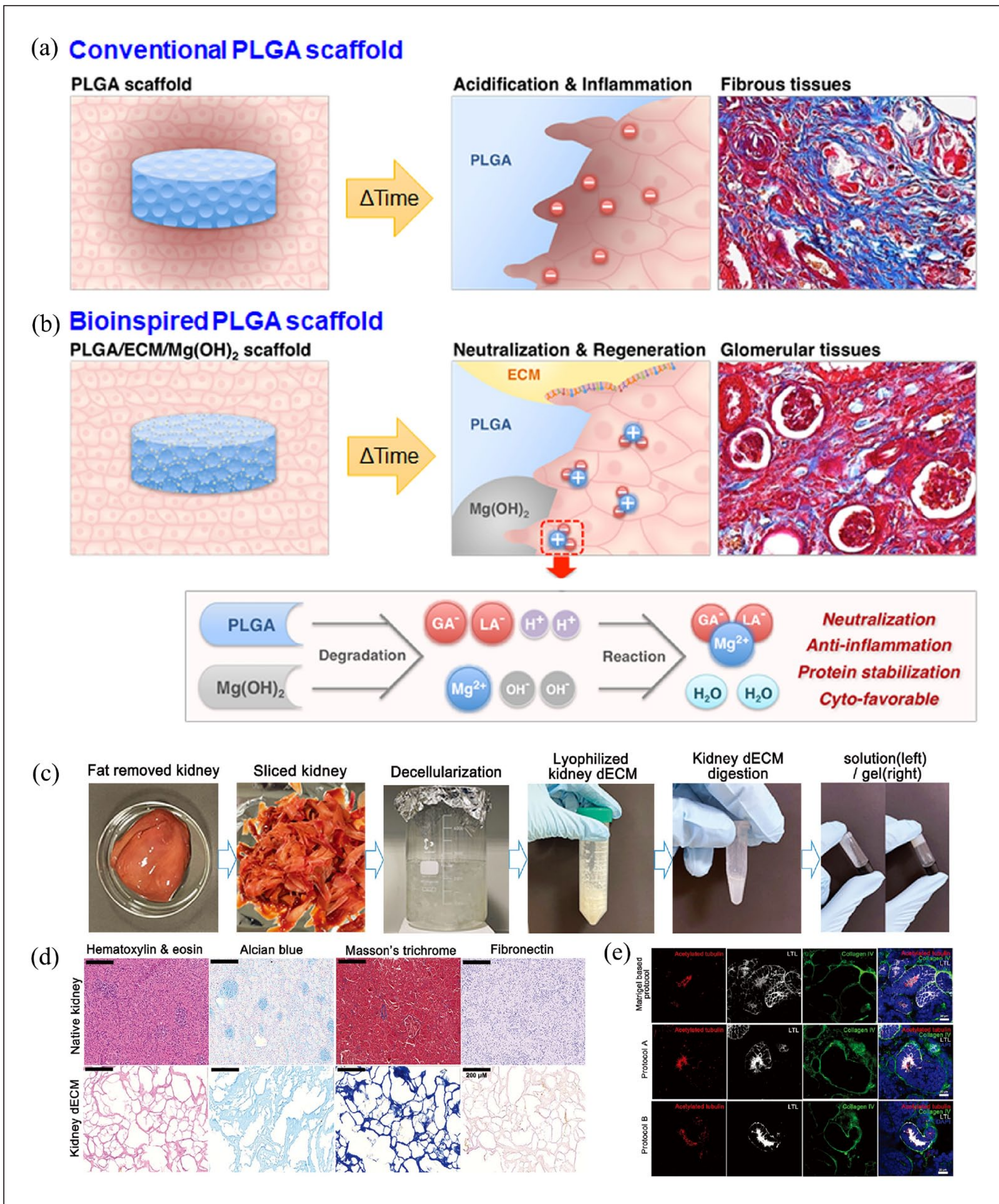


Figure 5. Application of dECM materials in renal organoids and engineered organs culture. Schematic illustration of (a) conventional and (b) dECM-derived bioinspired scaffold for renal tissue regeneration, highlighting their biological and chemical mechanisms, (c) the decellularization and gelation processes of porcine kidney and (d) characterization, (e) renal organoids cultured on kidney dECM exhibit more mature tubular structures
Images adapted with permission from Kim et al.[190] and Lih et al.[191]

development pattern and higher similarity to human kidneys. Transplanting renal organoids into the kidneys of mice accelerated the recruitment of ECs from the host mouse kidneys, maintaining vascular integrity through more organized slit diaphragm-like structures.[190] Researchers have established an in vitro induction protocol for the generation of stromal progenitors (SPs) from mouse PSCs. When these induced SPs are combined with

two distinct types of induced parenchymal progenitors (nephron progenitors and ureteric buds), the resultant organoids, entirely derived from PSCs, are capable of recapitulating complex renal structures, with multiple types of stromal cells interspersed among differentiating nephrons and branching ureteric buds. This approach holds promise for better replicating the intricate architecture of organs. However, the proximal tubules (PT), responsible for the majority of renal reabsorption functions, remain immature in renal organoids, with limited expression of key transporters critical for nephron functionality. Vanslambrouck et al. have shown that enhanced specification of metanephric nephron progenitors leads to elongated and radially aligned proximal nephrons, featuring distinct S1–S3 PT cell types. Compared to standard renal organoid protocols, this approach yields organoids with improved PT maturity, exhibiting an increase in the expression of a range of critical transporters, which translates to enhanced functionality as measured by substrate uptake and transport.[193,194]

Recent years have also seen progress in kidney fabrication via bioprinting. Combining 3D bioprinting with renal organoid technology allows for the rapid, high-throughput generation of renal organoids with reproducible cell numbers and cell type ratios, facilitating easier access to oxygen and nutrients due to the organoid's structural integrity.[183] However, generating complex organs like the kidney via 3D bioprinting presents several challenges. Firstly, the kidney's structure is complex, composed of both macrostructures such as renal arteries, veins, and ureters, and microstructures like nephrons and the stroma that occupies the space between them. Secondly, with over 20 different cell types in the kidney, selecting and correctly positioning these cells is challenging. Moreover, choosing suitable biomaterials to preserve the printed kidney's structure and function warrants careful consideration.

dECM materials for hepatic organoids and engineered organs

The liver is a vital organ for metabolism and homeostasis in the body. The high mortality rate associated with end-stage liver disease (ESLD) poses a significant global public health challenge. According to data from the World Health Organization, liver disease ranks as the 12th most common cause of death worldwide. Currently, orthotopic liver transplantation (OLT) is the most effective therapeutic option to enhance the survival and life quality of ESLD patients. Whole-organ bioengineering and regenerative medicine represent promising new technologies that could alleviate liver shortages by increasing the number of organs available for transplantation (Figure 6). Functionally bioengineered livers (FBLs) are a promising alternative to OLT, with recent studies reporting successful in situ transplantation of FBLs prepared using rat whole decellularized liver scaffolds (DLSs) in rats subjected to total hepatectomy, effectively prolonging survival.[195] Transplantation of human liver organoids co-cultured in decellularized natural liver scaffolds into rabbits subjected to bile duct ligation improved short-term survival and liver function.[196] Decellularized liver scaffolds have been successfully prepared from a variety of species, including rats, pigs, dogs, and humans, with porcine-derived decellularized materials being widely utilized.[197] In recent years, due to the broad availability of porcine organs and their compatibility with human liver size and structure, decellularized porcine livers have attracted increasing interest for constructing engineered liver.[118,119,198,199] Ansari et al. have indicated that a combination of saponin, sodium deoxycholate, and deionized water perfused through the hepatic artery and portal vein may yield decellularized scaffolds with an intact vascular system and preserved ECM.[119] Faccioli et al. described a protocol promising complete decellularization of an entire porcine liver within 3 days, resulting in a scaffold that preserves tissue integrity, supports cell adhesion, and produces vital hepatic proteins such as albumin, demonstrating functional viability.[118]

In the field of liver regenerative medicine, ex vivo preservation of the liver on organ perfusion devices is essential for liver and bile duct reconstruction and repair, with the use of stem cells or hepato-biliary cells being indispensable. Functional ductal organoids (FDOs) and bile duct organoids are valuable sources of hepato-biliary cells for application, as these organoids can be generated from relatively small liver biopsies and yield a large number of genetically stable cells, which are crucial for the construction of liver organoids and organ engineering.[44,202] Recent research has successfully constructed FDOs with well-developed biliary networks from primary biliary cells isolated from mouse bile ducts in rat DLSs.[200] Other studies have cultured differentiated organoids showing uniform differentiation and even polarization using porcine liver scaffolds and effective recellularization. Additional research has successfully reconstructed a hollow intrahepatic duct-like organ with a single-layered cell membrane within a decellularized 3D scaffold of the liver. Prior to differentiation, this organ exhibited characteristics resembling bile duct cells and retained the potential for proliferation and stem cell differentiation. Introduction of the hepatic duct-like organ into the bile duct network of rat-derived dECM led to successful recellularization and reconstruction of intrahepatic bile ducts, maintaining bile duct cell properties, exhibiting major bile duct functions, and reconstructing a ductal tree-like network with luminal structures. Furthermore, it was observed that when the hepatic duct-like organ underwent recellularization in conjunction with primary liver cells, it maintained its characteristics as bile duct cells, thus providing a more accurate simulation of the natural cellular distribution within the liver.[201]

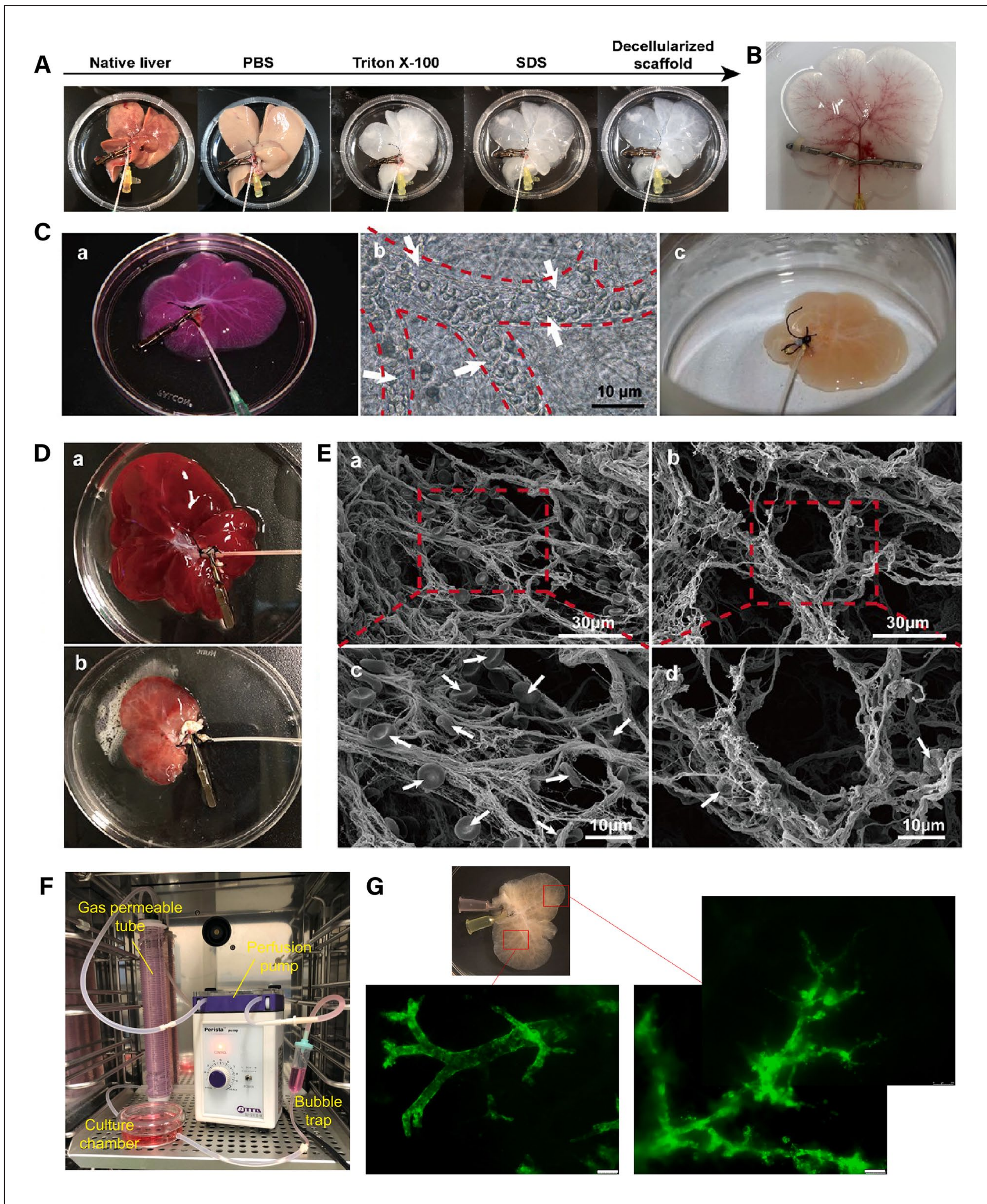


Figure 6. Application of dECM materials in hepatic organoids and engineered organs culture: (A) The preparation process of DLSs and (B) the morphology of their intrahepatic bile duct tree. (C) The vascularization process of DLSs. (D) Macroscopic images and (E) SEM images of DLSs (a) and vascularized liver scaffolds (VLSs) (b) after blood perfusion. (F) A photograph of the perfusion culture apparatus placed in a CO_2 incubator, utilized for the recellularization of bile ducts in DLSs. (G) Reconstruction of biliary tree-like structures was achieved through the recellularization of bile ducts using liver ductal organoids.
Images adapted with permission from Guo et al.[195], Chen et al.[200], and Tomofuji et al.[201]

dECM materials for pulmonary organoids and engineered organs

Respiratory diseases rank as the third leading cause of mortality globally, with lung transplantation facing similar challenges of donor shortage and the necessity for lifelong immunosuppression.[203] Decellularized lung scaffolds and hydrogels are increasingly utilized in lung and lung organoid bioengineering through decellularization and recellularization processes (Figure 7); however, a fully functional pulmonary organ has yet to be realized. Numerous studies have employed stem cells from various sources, including induced iPSCs, ASCs, and ESCs, to cultivate lung organoid models.

Researchers have induced these stem cells to differentiate into lung-specific cell types, such as tracheal, bronchial, and alveolar cells, by employing specific culture conditions and signaling pathways, including the use of particular growth factors (e.g. Fibroblast Growth Factor (FGF)-2, FGF-10, FGF-7) and cytokines.[206–208] Additionally, Leiby et al. have proposed an organoid model utilizing decellularized lung slices (preserving alveolar, airway, and vascular substructures for cell interaction within a physiologically relevant 3D environment) for the multilinear recellularization to generate engineered lung tissue, comprising alveolar-like structures with multiple cell lineages. This organoid model facilitates the study of cell-cell and cell-matrix interactions within the alveoli, as well as the biochemical signaling regulating type II alveolar epithelial cells and their microenvironment.[208] Güney et al. introduced a novel scaffold material, Agar-Matrigel scaffold (Agrigel), forming a mechanically tunable ECM with adjustable viscoelasticity and stiffness, capable of cultivating long-lived, contractile lung tubule organoids, termed bronchotubules. This method enables the long-term stable culture of the bronchotubule system, offering possibilities for simulating different bronchial diseases' microenvironments, such as COPD and IPF.[204] Valdoz et al. discovered that soluble ECM and dECM in the 3D suspension culture of lung organoids, by enhancing carbohydrate metabolism and preventing cell dissociation, improved cell viability. The cultured lung organoids developed luminal structures, showcasing viable gas exchange units, branching formation, perfusable vascular systems, and luminal structures maintained for approximately 70 days.[209]

One of the goals of lung organ engineering is to generate functional capillaries with healthy microvascular walls to maintain blood passage in the capillaries and restore the natural barrier properties between the vasculature and airway compartments.[210] However, current recellularization techniques struggle to achieve complete endothelial coverage.[151,211] A theoretical model has been constructed to calculate quantitative values for pulmonary capillary recruitment and barrier hydraulic conductivity. It was found that in native tissues, increasing pulmonary artery perfusion to normal cardiac output resulted in elevated average capillary pressure and diameter, rather than an increase in microvascular/capillary recruitment. Conversely, in decellularized tissues, increased perfusion rates led to increased microvascular recruitment without affecting the size and fluid dynamics of a given capillary flow path, at least for the tested perfusion rates. This suggests that decellularization may lead to changes in the microvascular hemodynamics of the lung.[211] Excitingly, Nichols et al. reported the formation of normal microvasculature and systemic support within bioengineered lungs (BEL). Following the transplantation of BEL into porcine subjects, it was observed that the vasculature continued to develop, expressing a range of markers associated with vascular function, including CD31, ERG1, eNOS, and ACE. Additionally, within two weeks, BELs developed a vascular system through collateral circulation, showing no signs of rejection; however, pulmonary artery and vein anastomosis was not achieved.[205] Of course, there are many aspects that require further research, such as achieving engineered barrier functions with ultra-low levels of capillary-alveolar hydraulic conductivity to mimic natural whole-organ barrier functions. Even with high levels of cellularization, natural levels of hydraulic conductivity require endothelial tubulization and organized tight junction formation, design criteria that thus far are challenging to achieve ex vivo.[211] Additionally, the anastomosis of the bronchial circulation and lymphatic system to establish functional vascular circulation has yet to be realized.

dECM materials for pancreatic organoids and engineered organs

Diabetes is a global health condition necessitating long-term management and monitoring. Currently, pancreatic or islet transplantation represents the a reliable treatment modality for achieving glucose homeostasis in patients with Type I diabetes, with intraportal islet transplantation standing as the standard approach for β-cell replacement. Organ decellularization and recellularization are emerging as a promising solution to address the scarcity of viable organs available for transplantation by offering potential alternative sources of donor organs.[212–214] (Figure 8). The microenvironment is crucial for the regeneration of islet-like organoids from stem cells, with organoids comprising all major endocrine cell types, including α, β, δ, and pancreatic polypeptide cells, assembled into tissue structures akin to human islets. Studies have utilized a natural material derived from decellularized rat pancreatic extracellular matrix (dpECM) to induce the self-assembly of human islet-like organoids during the pancreatic differentiation of iPSCs. Exposure of iPSCs to dpECM during differentiation resulted in significant upregulation of critical pancreatic transcription factors such as PDX-1, MAFA, and NKX6.1, and prompted the production of all principal

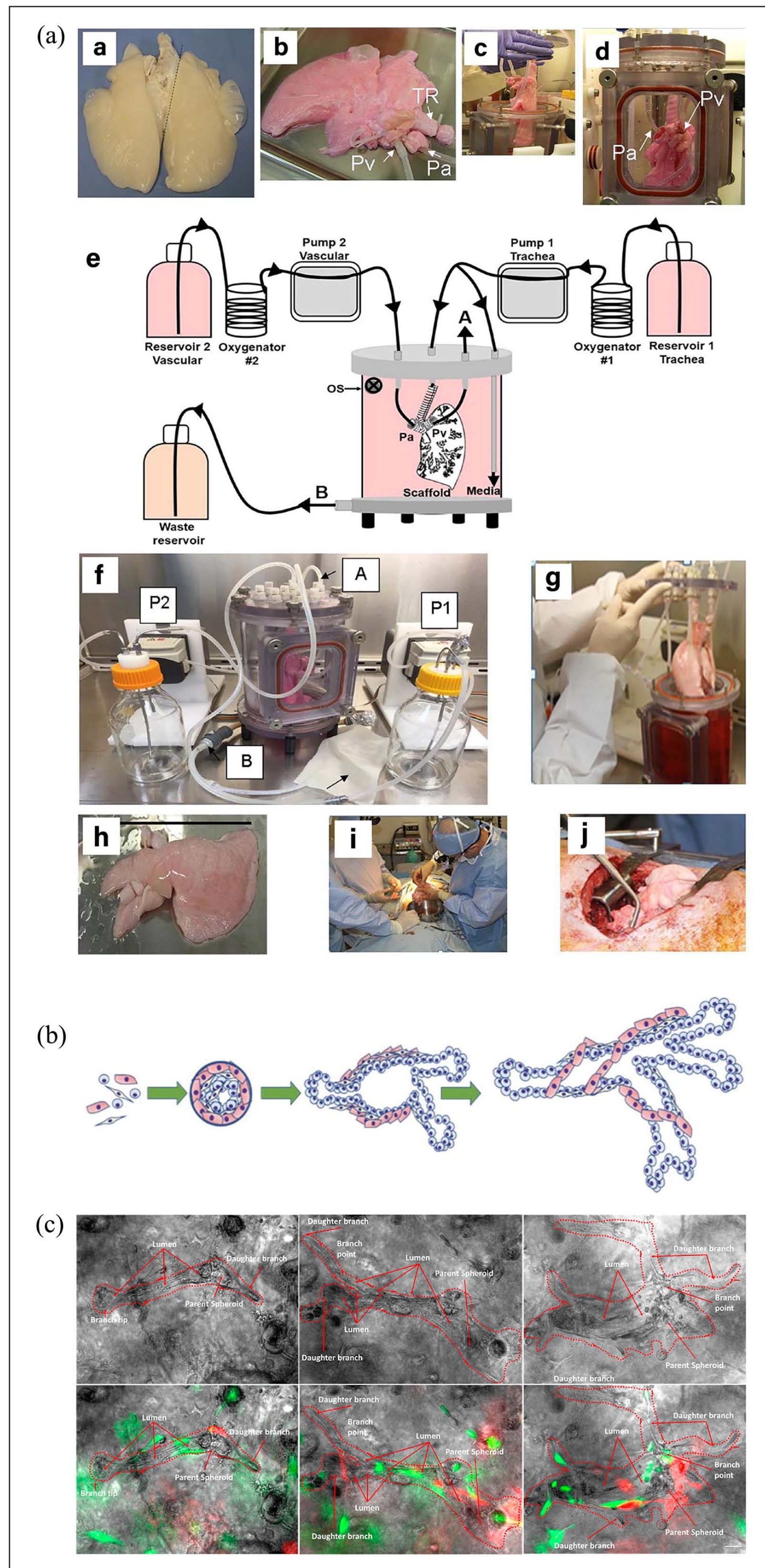


Figure 7. Application of dECM materials in pulmonary organoids and engineered organs culture: (a) procedural workflow for the production and transplantation of bioengineered lung into a large animal model, (b) schematic representation, and (c) images of branching morphogenesis over a 20-day culture period, illustrating the promotion of airway-like tubular morphology in lung organoids through epithelial-mesenchymal interactions and the physicochemical properties of the ECM.
Images adapted with permission from Güney et al.[204] and Nichols et al.[205]

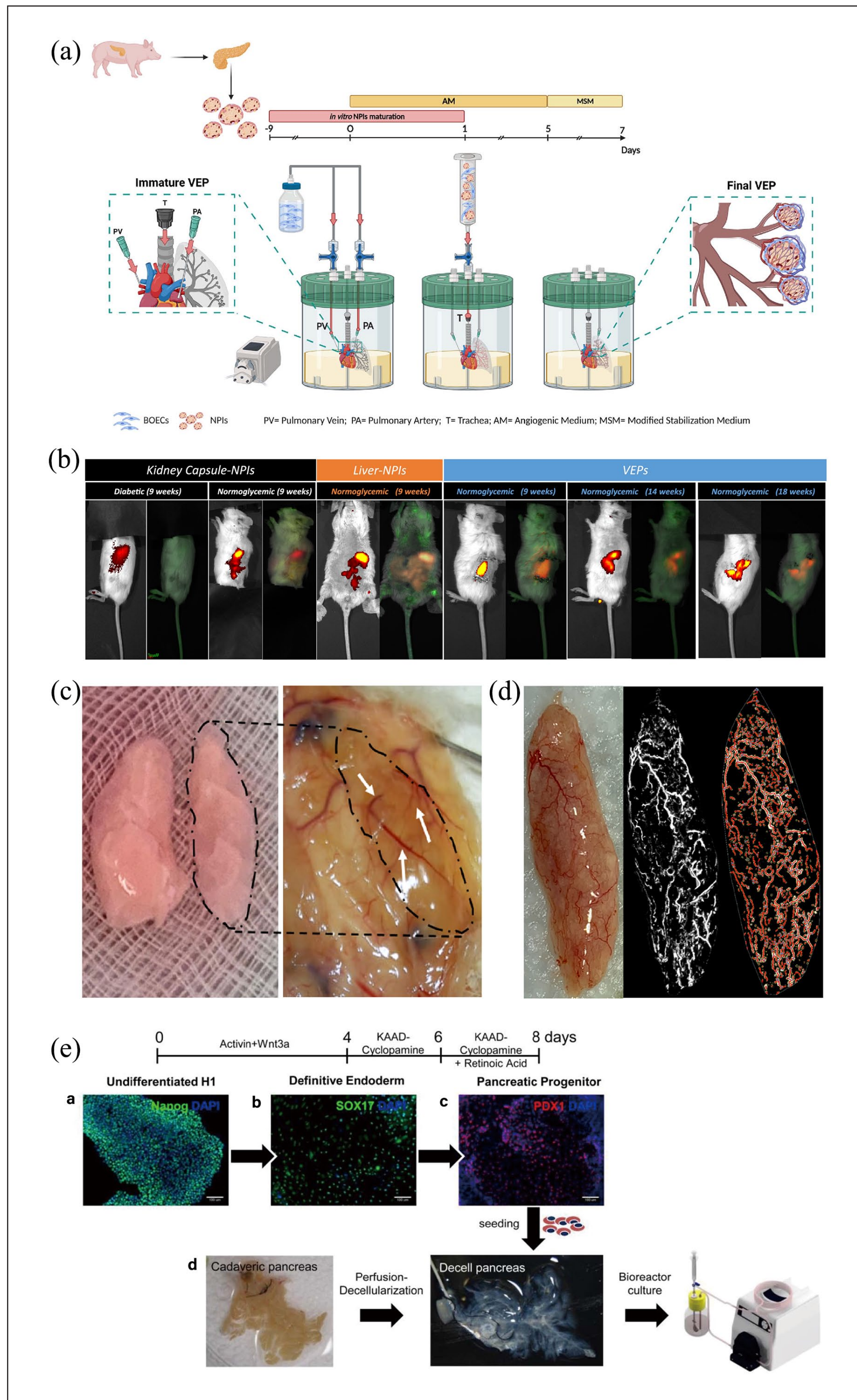


Figure 8. Application of dECM materials in pancreatic organoids and engineered organs culture: (a) schematic diagram of a custom bioreactor and the assembly steps for vascularized endocrine pancreas (VEP), (b) long-term in vivo analysis following VEP transplantation, (c) pre-transplant VEP imaging, along with gross pathological assessment, and (d) vascular density analysis at 9 weeks post-transplantation, (e) schematic representation of the protocol for decellularized mouse pancreas perfusion and the recellularization of directed differentiation into PP using hPSCs within a bioreactor.
Images adapted with permission from Citro et al.[117] and Goh et al.[215]

hormones, including insulin, glucagon, somatostatin, and pancreatic polypeptide from stem cell-derived organoids.[215] Further research, through bioinformatics analysis of collagen derived from decellularized pancreatic ECM, has for the first time demonstrated that ECM-derived peptides dictate the fate of iPSCs, giving rise to endocrine progenitor cells and subsequent islet-like organoids.[216]

Recent studies have indicated the potential of dECM from mouse, rat, pig, sheep, and human pancreata to generate usable decellularized pancreatic scaffolds.[102,103,217–223] These studies have optimized various decellularization protocols for pancreatic tissue to generate dECM scaffolds with improved 3D structural preservation, GAG retention, and cellular biocompatibility. Goh et al. has investigated the differentiation of hPSC into pancreatic progenitor (PP) lineage cells and their reseeding into decellularized organ scaffolds, showing a more favorable outcome for cell proliferation and reconstitution of pancreatic organ functionality. Interestingly, it was observed that reseeding decellularized liver scaffolds with hPSC-PP cells yielded more promising results compared to pancreatic scaffolds. Through chemical complementation and bioreactor cultivation, these hPSC-PP cells demonstrated robust growth throughout the entire liver scaffold, with the engrafted cells exhibiting endocrine functionality. This enhanced performance in the liver scaffold may be attributed to its higher vascular density, which facilitates more uniform support for cell reseeding. Additionally, the liver's vascular architecture allows for easier perfusion, thereby promoting nutrient delivery and waste removal within the 3D reconstructed organ. Furthermore, the liver may serve as a potential supportive site for pancreatic islet transplantation.[215] Recellularization and refunctionalization of native dECM scaffolds using mesenchymal stem cells from recipients could potentially restore organ structure and function while reducing adverse immunological reactions during transplantation.[218] Citro et al. have combined decellularized lung scaffolds as a platform for β-cell replacement with neonatal pig islets and EC s derived from healthy subjects to construct endocrine pancreatic organs with immunoprotected and vascularized features. This technique not only promotes the maturation of neonatal pig islets ex vivo but also sustains immediate function in vivo post-transplantation for over 18 weeks.[117] Islet infusion into the portal vein remains the preferred method for islet transplantation, despite almost immediate loss of islet quality post-implantation. Therefore, methods that avoid direct intravascular placement are needed, with the omentum emerging as a promising extrahepatic site. Berkova et al. compared different methods of decellularization of the pancreas, including perfusion through the pancreatic duct, gastric artery, portal vein, and a novel method via the splenic vein, aiming to provide a more suitable microenvironment for islet survival in the omental flap. They found that pancreatic perfusion via the splenic vein may offer a smaller ECM scaffold, which aids in the transplantation of islets into the omentum.[224]

dECM materials for uterine organoids and engineered organs

Numerous intractable uterine pathologies, leading to reproductive disorders, as well as conditions such as uterine failure, may be amenable to treatments involving uterine organoids, bioengineered reconstruction of the uterus and its tissues, and uterine transplantation.[225–227] Daryabari et al. have provided the first efficacious perfusion-based decellularization protocol for obtaining human uterine whole-organ scaffolds. Employing a peristaltic pump to perfuse SDS through the uterine artery generates a scaffold that contains ECM components similar to the native human uterus.[228] A combined physical and chemical method for uterine decellularization has also been reported to more effectively remove cellular remnants and detergents, while preserving the scaffold's three-dimensional structure and mechanical properties.[229] Studies have demonstrated that combining decellularized extracellular matrix derived from the uterus with chorionic villus mesenchymal stem cells for uterine restoration can lead to efficient live births and regenerate functional uterine tissue in rats with severe uterine damage. The reconstructable uterine material (RUM) can be tailored into different states (such as liquid RUM and solid RUM) and shapes (e.g. rectangular, triangular prism, and cube), effectively preventing intrauterine adhesions, promoting endometrial regeneration and myometrial collagen reconstruction, and accelerating wound healing through the construction of physical barriers and secretion of cellular factors, thus achieving effective uterine restoration.[230,231]

Recellularization of dECM with MSCs can create a conducive regenerative microenvironment by increasing anti-inflammatory interleukin-10 levels, enhancing the number of FOXP3+ regulatory T cells and CD163+ M2 macrophages, and reducing the number of CD8+ cytotoxic T cells[232] (Figure 9(a)–(c)). Currently, most human endometrial organoids (hEOs) are cultured in 3D microenvironments that are significantly different from the human endometrium, limiting their applicability in bioengineering. Gómez-Álvarez et al. have introduced a hybrid endometrial-derived hydrogel that combines the rigidity of PuraMatrix (PM) with the bioactive components of natural scaffolds and the interactive properties of decellularized porcine endometrial extracellular matrix (EndoECM) hydrogel. Due to the biochemical similarity of this hydrogel to natural tissues, it has enhanced the differentiation efficiency of hEOs, demonstrated excellent in vivo stability, and exhibited xenobiocompatibility in mice within two weeks.[233] Additionally, hydrogels developed from decellularized human and bovine endometrium have been shown to support the growth of mouse and human endometrial organoids, displaying proteomic profiles more akin to native tissues than organoids cultured in Matrigel, with the solid form of scaffolds being suitable for supporting high-throughput cell culture-based applications[234] (Figure 9(d)–(g)).

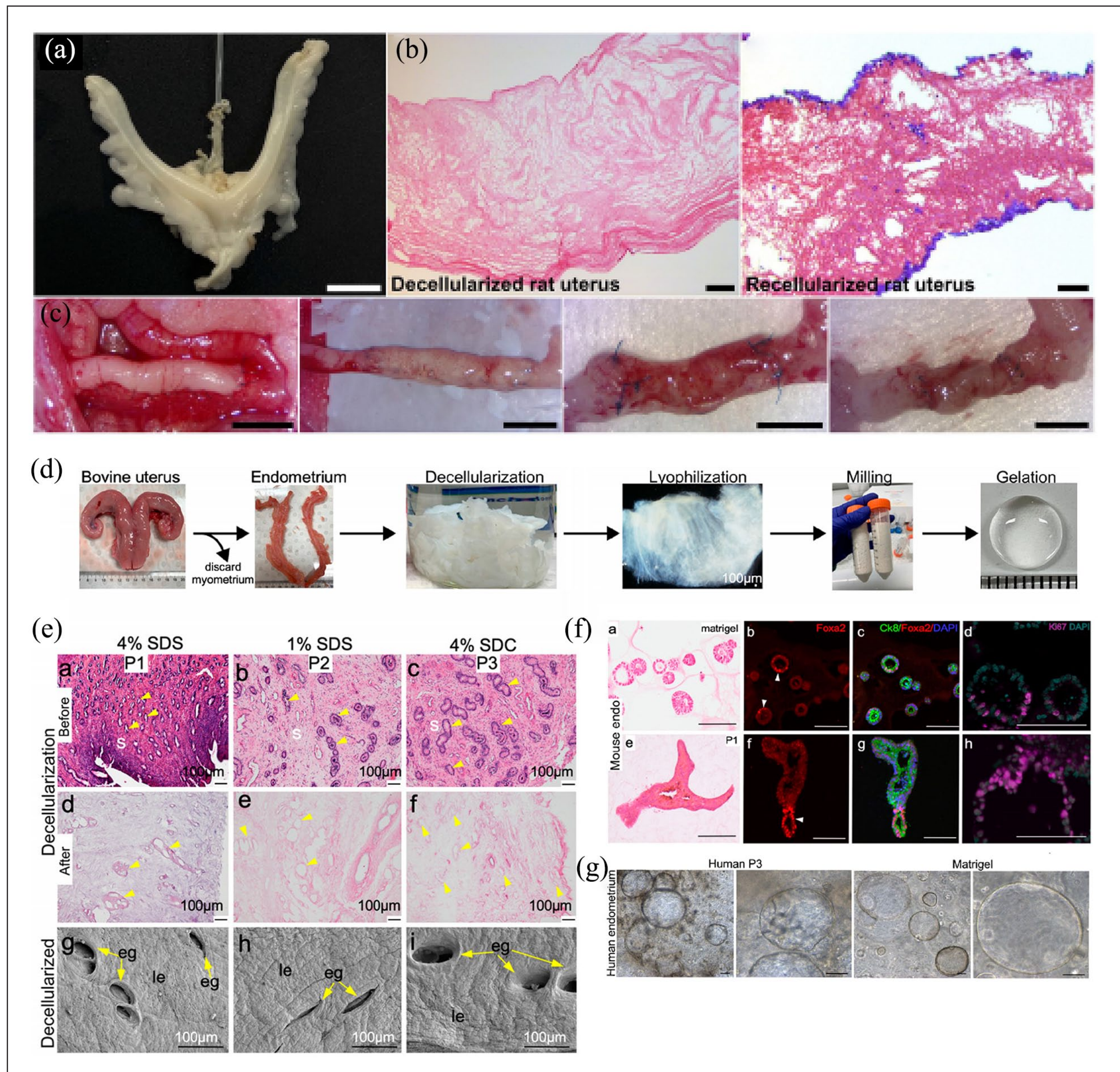


Figure 9. Application of dECM materials in uterine organoids and engineered organs culture: (a and b) decellularization and recellularization of rat uterus via vascular perfusion, followed by (c) graft evaluation at 14, 30, and 120 days post-transplantation, (d) overview of the key steps in the preparation of dECM hydrogel from bovine endometrium, (e) histological images of natural and decellularized endometrial tissue sections, (f and g) Endometrial dECM hydrogel supports the culture of human and mouse uterine organoids.
Images adapted with permission from Sehic et al.[232] and Jamaluddin et al.[234]

dECM materials for neural and other fast developing organoids and engineered organs

Diseases in the field of neuroscience remain difficult to cure. A major difficulty is the lack of ideal model systems that can help to the discovery of effective therapies for complex central nervous system (CNS) disorders. Human CNS organoids, including brain and spinal cord organoids, have garnered significant attention due to their closer anatomical and physiological resemblance to the human brain and spinal cord[235] (Figure 10). Spinal cord injury often results in severe loss of motor and sensory functions, and due to the extremely limited regenerative capacity of spinal cord neurons, the local pathological microenvironment is unfavorable for repair. The surrounding tissues have difficulty compensating effectively, leading to challenges in functional recovery, and current treatment options show limited efficacy. Organoid technology can be combined with stem cell therapy to provide a supportive microenvironment that promotes neural regeneration and functional

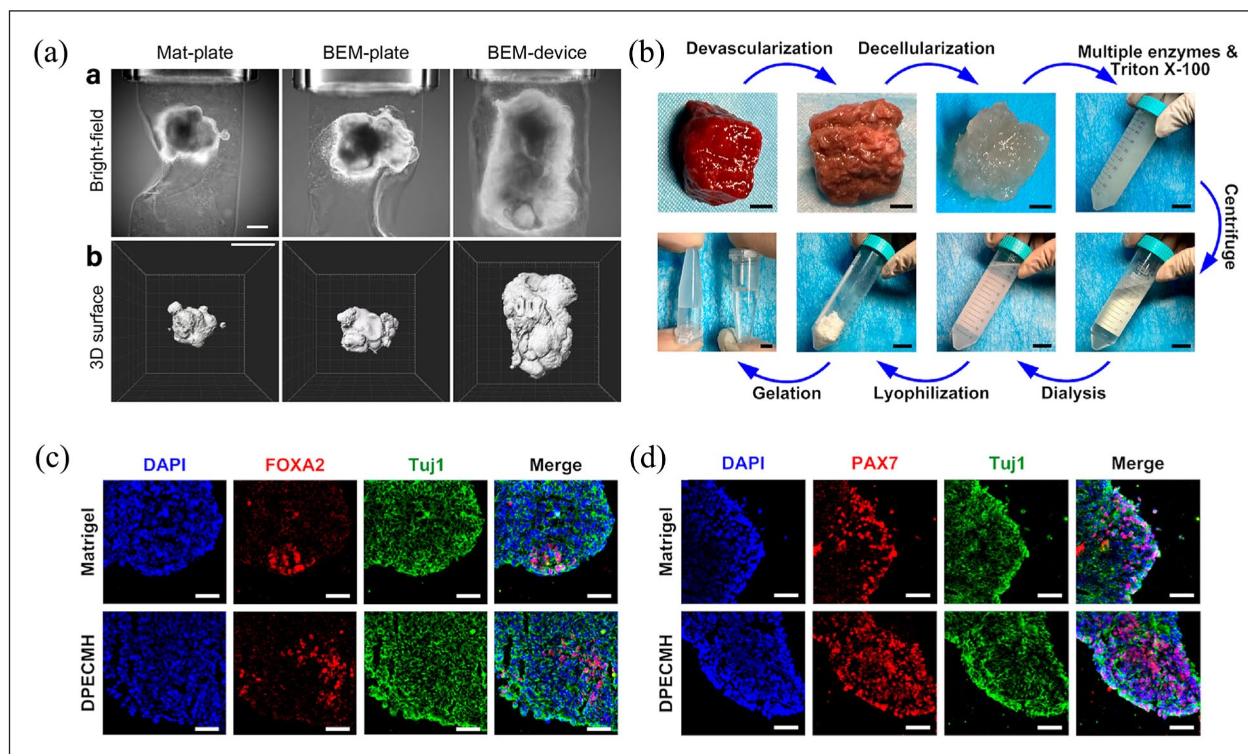


Figure 10. Application of dECM materials in neural organoids and engineered organs culture: (a) light-sheet microscopic bright-field images of brain organoids cultured for 60 days using a microfluidic decellularized human brain tissue-derived brain ECM (BEM) system, (b) Flowchart illustrating the preparation process of DPECMH, (c–d) DPECMH induces the formation of spinal cord organoids exhibiting complex layered structures similar to those of natural spinal cord tissue.
Images adapted with permission from Wang et al.[110] and Cho et al.[238]

restoration. Recent work by Wu et al. utilized decellularized brain ECM hydrogel (DBECMH) derived from rat brain tissue to culture spinal cord organoids from hiPSCs. Compared to Matrigel, organoids cultured in DBECMH exhibited higher expression levels of natural spinal cord segment-specific markers.[236] Wang et al. developed a temperature-sensitive hydrogel using dECM from human placenta (DPECMH), constructing a stable and quantifiable spinal cord organoid system. Spinal cord organoids cultured in DPECMH displayed a richer expression of regional spinal cord markers, such as FOXA2 and PAX7, significantly accelerating differentiation and maturation.[110] The development of brain organoids has also opened new avenues for research into neurodevelopmental disorders and diseases such as microcephaly, schizophrenia, and Alzheimer's disease. Organizing brain-like structures can be achieved by culturing neurons derived from hiPSCs on brain dECM prepared from adult pig brains.[32] Seo et al. introduced a microfibril-aligned 3D culture platform utilizing pig brain dECM, which resulted in hydrogels rich in neurotrophic factors capable of better supporting neuronal growth and maturation, thereby promoting the formation of neural circuits.[237] Additionally, Cho developed a 3D organoid culture platform that simulates brain tissue by integrating dECM from human brain tissues with a bioreactor (microfluidic device). The finely regulated dynamic flow provided by this system significantly reduced neuronal apoptosis and the formation of necrotic areas, enhancing the maturity and quality of the brain organoids.[238] Pancreatic cancer (PC) are characterized by tumor cells that frequently invade neural tissues. Chenyun Song et al. constructed a coculture system of human brain organoids (hBrO) and mouse pancreatic cancer organoids (mPCO) using Matrigel, partially recapitulating the pathophysiological processes of neural invasion in PC. However, a limitation of this system is the lack of an immune cell population and the maturity of the myelination of the neurons within the brain organoids. Utilizing dECM may offer a pathway to create more mature organoid systems.[239]

Researches in application of dECM materials in regenerative medicine are increasing in the past decade. Beyond the aforementioned research advances, the applications of dECM in cardiovascular, bone and cartilage, and male reproductive systems are drawing more and more attention recently. In studies focused on the repair and reconstruction of bone and cartilage, bioactive scaffolds derived from dECM materials not only provide suitable resistance to mechanical stress and physicochemical properties but also exhibit excellent capabilities to promote the regeneration and differentiation of bone

and cartilage cells.[17] For example, Yuan et al. developed an osteo-microenvironment stage-regulative scaffold by combining decellularized bone matrix microparticles and multifunctional magnesium hydroxide nanoparticles with polylactic acid-glycolic acid at an optimized ratio. This scaffold demonstrates enhanced stimulatory effects on bone immunomodulation, angiogenesis, and osteogenesis, allowing better adaptation to different post-transplant stages, including early inflammation, intermediate angiogenesis, and late bone formation.[19] In addressing the increasing prevalence of infertility, especially among young men unable to cryopreserve sperm due to cancer treatments, in vitro sperm culture and maintaining the integrity of immature testicular tissue in vivo are promising solutions.[240] For instance, Gholami et al. optimized decellularization protocols to more effectively remove cells from rat testicular tissue, potentially providing a suitable scaffold for in vitro reconstruction of seminiferous tubule-like structures and functions, aimed at sperm generation.[241]

Heart transplantation is the ultimate treatment for end-stage heart failure, and developing cardiac organoids and functional engineered hearts presents potential alternatives. In a structurally complex organ like the heart, using cardiac dECM scaffolds retains the major protein component and structure of heart ECM and shows superior performance in mechanical testing compared to synthetic scaffolds. Goldfracht et al. combined heart dECM hydrogels, enhanced with chitosan and derived from porcine hearts, with hiPSC-derived cardiomyocytes from patients with genetic heart diseases to construct novel engineered heart tissues. These tissues displayed anisotropic muscular structures, and the embedded cardiomyocytes exhibited more mature characteristics than those in 2D culture, demonstrating various phenotypes of arrhythmias under drug treatment, with enhanced sensitivity to a range of pharmacological agents and programmed electrical stimulation.[242] In treating valvular heart disease, traditional mechanical and bioprosthetic heart valves have limitations. Decellularized pulmonary valve allografts are gaining attention. Vafaee et al. utilized low-concentration SDS to decellularize porcine pulmonary roots, which were then implanted into juvenile sheep's right ventricular outflow tract. After 12 months, these grafts functioned well, resembling their pre-implantation state, with leaflets remaining soft, thin, and pliable. The study also showed effective recellularization of porcine pulmonary root dECM by sheep cells, predominantly mediated by M2 macrophages.[243] Aortic aneurysm and other large vessel diseases often require repair surgery, yet current implants poorly match native tissue, increasing blood flow pulsatility and negatively impacting perfusion supporting related organs. Decellularized natural aorta is an ideal scaffold for aortic implants. Giovanniello et al. employed an optimized decellularization regimen using SDS and DNase to minimize mechanical and structural alterations in tissue, yielding porcine aorta dECM with static and dynamic layered scaffold characteristics closely resembling native human aorta, offering potential as the next generation of aortic implants.[27]

dECM materials for cancer organoids

Despite significant advances in cancer research, cancer continues to pose a severe global health threat. Tissue-engineered tumor models, such as organoids, are getting attention in disease modeling and drug development in cancer research. The ECM in tumors differs considerably from that in normal tissues in terms of deposition, composition, stiffness, and post-translational modifications, indicating a close interplay between ECM and tumors. Therefore, constructing decellularized tumor ECM (dT-ECM) for tissue-engineered tumor models can better simulate the actual tumor microenvironment (TME).[244] Tienderen et al. discovered that compared to tumor-free liver matrices (TFL-M), patient-derived decellularized cholangiocarcinoma matrices (CCA-M) better preserved the CCA-related TME. Organoids cultured with CCA-M exhibited transcriptomic features very similar to patients' tumor tissues in vivo and showed resistance to clinical chemotherapeutic drugs. Furthermore, the growth and proliferation dynamics of organoids differed under the environmental conditions of CCA-M and TFL-M, with CCA-M enabling CCA organoids (CCAO) to better replicate in vivo tumor invasion patterns and ECM-epithelial-mesenchymal transition (EMT), further proving the interaction between tumor cells and their ECM. This finding suggests that ECM may serve as a new therapeutic target for CCA, providing new insights into CCA therapy[245] (Figure 11). Choi et al. developed a lung tissue-derived dECM (LudECM) hydrogel using porcine lung dECM as a 3D-printing bioink, incorporating endothelial cells, lung cancer-associated fibroblasts, and immune cells for recellularization to create a vascularized lung cancer model. Compared to Matrigel and non-vascularized models, the LudECM hydrogel provided signaling stimuli that sustained continuous interactions between cancer cells and stromal cells or cancer cells and stroma within the TME, making lung cancer organoids more representative of real tumor tissues. The organoids in this model better recapitulated pulmonary fibrosis and exhibited significantly increased resistance to targeted anticancer drugs.[246] Digestive system tumors, such as colorectal cancer, are prone to peritoneal metastasis, which significantly lowers survival rates and worsens prognoses. dECM scaffolds derived from patients' tumor peritoneum exhibited higher stiffness than normal peritoneum, indicating that increased stiffness of ECM facilitate colorectal cancer cell invasion. Normal dECM and Matrigel lack signals associated with cancer metastasis. Experimental results showed that

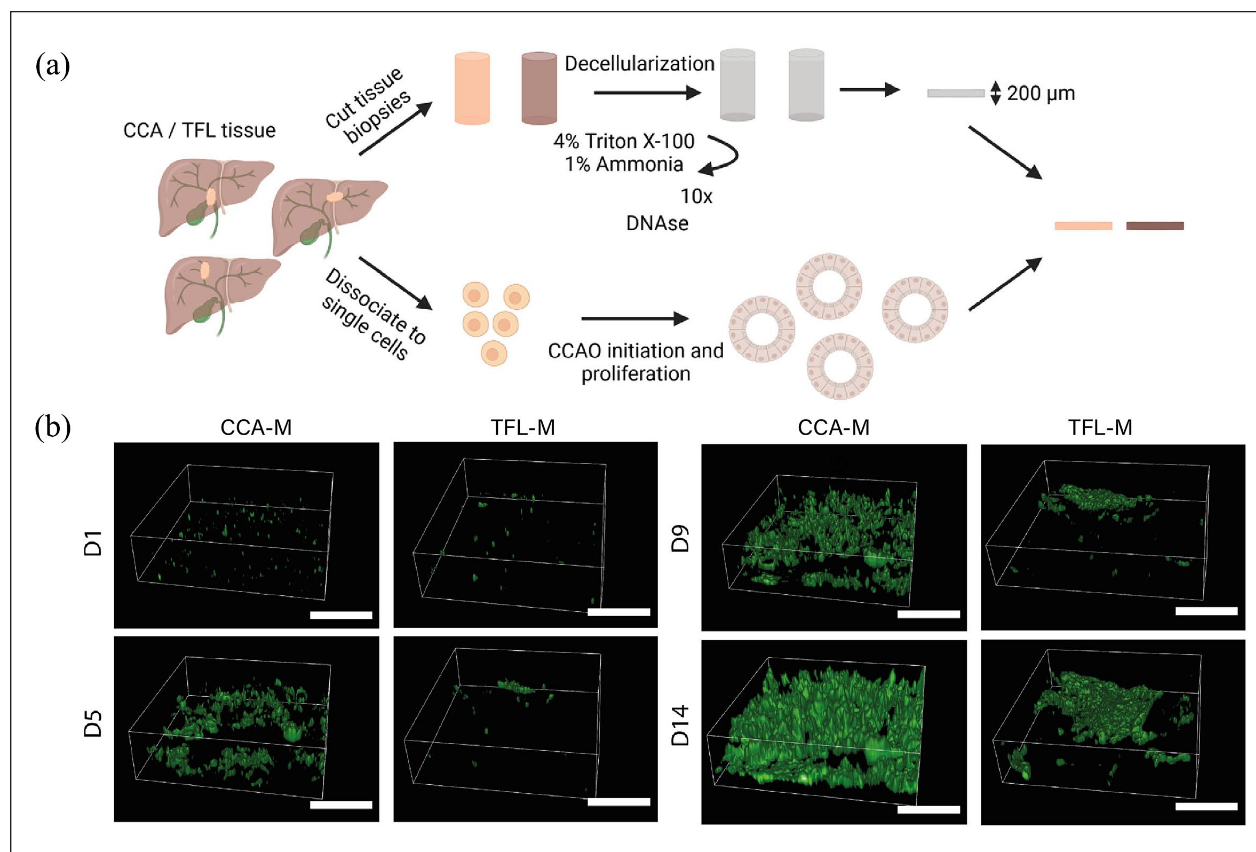


Figure 11. Application of dECM materials in cancer organoids culture: (a) schematic representation of the steps for generating a comprehensive tumor model by culturing organoids using dECM from excised CCA specimens and TFL, (b) representative 3D live cell confocal images demonstrate that CCA-M enhances the growth and migration of CCAO.
Images adapted with permission from Varinelli et al.[247]

colorectal cancer organoids grew earlier on tumor-derived peritoneum dECM than normal peritoneum dECM, indicating that tumor-derived dECM contribute to cancer metastasis.[247]

Current limitations and future directions

dECM is considered as a promising material for constructing organoids and engineered organs, as it helps to make organ models more closely resemble their natural counterparts. However, there are several limitations remains to be addressed. For instance, dECM derived from different tissues and animal sources exhibits variations in composition and structure. To better mimic the complex environment of human native tissues and organs, human-derived dECM materials are ideal; however, the relatively limited availability of human sources restricts their application. In addition to the issues previously mentioned regarding decellularization and postprocessing, the consistency between batches may be affected due to differences in the source and preparation processes of dECM. Moreover, different dECM materials has a unique component and require a specific sterilization method, so that the product can meet the safety requirement of clinical application. Further research is still needed to improve our understanding and application of dECM, especially in selecting ECM sources that more closely mimic human tissues, improving decellularization methods, preserving incorporating more ECM components, reducing immune rejection responses, and promoting in vivo angiogenesis.

dECM materials are considered to undergo a dynamic remodeling process during recellularization process both in vivo and in vitro.[248] In the early stages, they experience matrix remodeling that leads to a fetal-like state, which facilitates the release of bioactive fragments and factors within the dECM. This release enhances cellular activation and chemotaxis, supporting tissue regeneration.[249] As the process progresses, matrix deposition facilitates a transition to a mature state. This transition involves the regulation of various immune and inflammatory cell factors, where moderate deposition supports tissue regeneration and functional recovery, whereas excessive deposition results in fibrotic tissue formation, leading to dysfunction and regenerative failure.[250] Therefore, an ideal dECM material must adapt to this dynamic process, meeting the growth and developmental needs of seeded

cells or organoids to effectively promote tissue regeneration and repair.

Different organ models necessitate distinct substrate components. For instance, laminin-111 is a pivotal component of the intestinal basement membrane, while laminin-11, type IV collagen, and fibronectin are critical for the biliary tract.[251,252] The stiffness and composition of the ECM undergo significant changes in pathological tissue. dECM not only provides adhesion properties but also regulates organ development and organoid formation by offering an appropriate mechanical and physical environment. Diseases such as inflammatory disorders and cancer are often associated with organ and tissue fibrosis. Hyaluronic acid, collagen, and laminin degradation products have been shown to induce inflammation, while pathological changes, such as the marked increase in collagen and laminin content, lead to the replacement of low-density ECM with high-density ECM.[253–255] To better replicate the authentic environment of various organs, it is crucial to optimize the culture conditions of organ models. For example, culturing intestinal organoids in hypoxic conditions and co-culturing with anaerobic bacteria can more closely mimic in vivo conditions.[256] Therefore, when constructing disease models, it is vital to consider the differences in dECM stiffness and composition and to identify methods that better simulate the in vivo environment for cultivating organ models.

The risk of inflammation and immune rejection can be mitigated by utilizing natural matrices, optimizing implantation pathways, or employing various novel decellularization techniques, particularly for xenogeneic or allogeneic donor organs. However, the potential of dECM as an ideal transplantable organ material is challenged by the difficulty of achieving uniform distribution of endothelial cells throughout the organ. This issue leads to thrombosis, loss of vascular patency, and subsequent cell and organ death due to the inability to deliver oxygen and nutrients throughout the organ.[257] Therefore, an intact and continuous endothelial layer is crucial for maintaining normal vascular system functionality. Additionally, to ensure the functional characteristics of organs produced during the recellularization process, it is essential to maximize the diversity, initial density, and specific distribution of various cell types present in the parenchymal space of natural organs.

Conclusions and outlooks

dECM is designed to remove immunogenic cells while preserving the original tissue architecture and composition. Owing to its inherent structure, enhanced bioactivity, reduced immunogenicity, and favorable biodegradability, dECM has garnered widespread attention in the fields of tissue engineering and biomedical applications. The prevalent decellularization techniques include physical, chemical, and enzymatic methods, which can be employed individually or in combination. Currently, dECM-derived materials have been increasingly applied in the preparation of organoids engineering and other engineered organs. Further research into the specific impact of ECM from different tissues and disease sources on dECM and subsequent bioengineering is needed to enhance the physiological complexity and clinical relevance of organ models.

Despite the challenges in the exploration of dECM for the fabrication of organoids and engineered organs, ongoing research and technological advancements are bringing us closer to the goal of creating viable, transplantable tissue and organs. These developments include the optimization of the decellularization process, refinement of organoid construction methods, scale-up production, and the enhancement of the matrix's biological cues to guide cell behaviors. The continuous improvement of these technologies holds great promise for the future of organoids engineering and engineered organs fabrication.

Acknowledgments

The authors would like to express their sincere gratitude to all copyright holders and organizations who have generously granted permission to reproduce and cite figures and images in this review. We acknowledge the contributions of numerous individuals and institutions whose work has been pivotal in illustrating and supporting the content of our article. We have made every effort to respect and adhere to the copyright policies and permissions granted by each copyright holder.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The research was supported by the National Natural Science Foundation of China (8217081586), and Shenzhen Science and Technology Major Project (KJZD20230923114302006).

ORCID iD

Yunfan He https://orcid.org/0000-0002-5681-4666

References

1. Armandola EA. Tissue regeneration and organ repair: science or science fiction? *MedGenMed* 2003; 5(3): 7–7.
2. Karamanos NK, Theocharis AD, Piperigkou Z, et al. A guide to the composition and functions of the extracellular matrix. *FEBS J* 2021; 288: 6850–6912.
3. Prewitz MC, Seib FP, von Bonin M, et al. Tightly anchored tissue-mimetic matrices as instructive stem cell microenvironments. *Nat Methods* 2013; 10(8): 788–794.

4. Mouw JK, Ou G and Weaver VM. Extracellular matrix assembly: a multiscale deconstruction. *Nat Rev Mol Cell Biol* 2014: 15(12): 771–785.
5. de Soure AM, Fernandes-Platzgummer A, Da Silva CL, et al. Scalable microcarrier-based manufacturing of mesenchymal stem/stromal cells. *J Biotechnol* 2016; 236: 88–109.
6. Kumar SSD and Abrahamse H. Advancement of nanobiomaterials to deliver natural compounds for tissue engineering applications. *Int J Mol Sci* 2020; 21(18): 6752.
7. Liu C, Pei M, Li Q, et al. Decellularized extracellular matrix mediates tissue construction and regeneration. *Front Med* 2022: 16(1): 56–82.
8. Parmaksiz M, Dogan A, Odabas S, et al. Clinical applications of decellularized extracellular matrices for tissue engineering and regenerative medicine. *Biomed Mater* 2016; 11(2): 022003.
9. Oktay K, Taylan E, Kawahara T, et al. Robot-assisted orthotopic and heterotopic ovarian tissue transplantation techniques: surgical advances since our first success in 2000. *Fertil Steril* 2019; 111(3): 604–606.
10. Oktay K, Marin L, Bedoschi G, et al. Ovarian transplantation with robotic surgery and a neovascularizing human extracellular matrix scaffold: a case series in comparison to meta-analytic data. *Fertil Steril* 2022; 117(1): 181–192.
11. Peña OA and Martin P. Cellular and molecular mechanisms of skin wound healing. *Nat Rev Mol Cell Biol* 2024; 25: 599–616.
12. Xu CM, Zhang JM, Wu YF, et al. Research progress on substitutes for autogenous soft tissue grafts in mucogingival surgery. *Hua Xi Kou Qiang Yi Xue Za Zhi* 2019; 37(2): 208–213.
13. Sharma N, El Refaiy A and Sibly TF. Short-term results of rotator cuff repair using GraftJacket as an interpositional tissue-matched thickness graft. *J Orthop* 2018; 15(2): 732–735.
14. Christ T, Grubitzsch H, Claus B, et al. Long-term follow-up after aortic valve replacement with Edwards Prima Plus stentless bioprostheses in patients younger than 60 years of age. *J Thorac Cardiovasc Surg* 2012; 147(1): 264–269.
15. Neethling W, Rea A, Forster G, et al. Performance of the ADAPT-Treated CardioCel® scaffold in pediatric patients with congenital cardiac anomalies: medium to long-term outcomes. *Front Pediatr* 2020; 8: 198.
16. Morton LM and Phillips TJ. Wound healing and treating wounds: differential diagnosis and evaluation of chronic wounds. *J Am Acad Dermatol* 2016; 74(4): 589–605.
17. Ramzan F, Salim A and Khan I. Osteochondral tissue engineering dilemma: scaffolding trends in regenerative medicine. *Stem Cell Rev Rep* 2023; 19(6): 1615–1634.
18. Wang X, Ma Y, Chen J, et al. A novel decellularized matrix of Wnt signaling-activated osteocytes accelerates the repair of critical-sized parietal bone defects with osteoclastogenesis, angiogenesis, and neurogenesis. *Bioact Mater* 2023; 21: 110–128.
19. Yuan Y, Xu Y, Mao Y, et al. Three birds, one stone: an osteo-microenvironment stage-regulative scaffold for bone defect repair through modulating early osteo-immunomodulation, middle neovascularization, and later osteogenesis. *Adv Sci* 2024: 11(6): e2306428.
20. Mokhtarinia K and Masaeli E. Post-decellularized printing of cartilage extracellular matrix: distinction between biomaterial ink and bioink. *Biomater Sci* 2023; 11(7): 2317–2329.
21. Zhu W, Cao L, Song C, et al. Cell-derived decellularized extracellular matrix scaffolds for articular cartilage repair. *The Int J Artif Organs* 2021; 44(4): 269–281.
22. Sevastianov VI, Basok YB, Grigoriev AM, et al. Decellularization of cartilage microparticles: effects of temperature, supercritical carbon dioxide and ultrasound on biochemical, mechanical, and biological properties. *J Biomed Mater Res A* 2023; 111(4): 543–555.
23. Zhou J, Wu N, Zeng J, et al. Chondrogenic differentiation of adipose-derived stromal cells induced by decellularized cartilage matrix/silk fibroin secondary crosslinking hydrogel scaffolds with a three-dimensional microstructure. *Polymers* 2023; 15(8): 1868.
24. Zhu J, Lun W, Feng Q, et al. Mesenchymal stromal cells modulate YAP by verteporfin to mimic cartilage development and construct cartilage organoids based on decellularized matrix scaffolds. *J Mater Chem B* 2023: 11(31): 7442–7453.
25. Tong C, Xia J, Xie B, et al. Immunogenicity analysis of decellularized cardiac scaffolds after transplantation into rats. *Regen Med* 2019; 14(5): 447–464.
26. Wang C, Chen Q, Wang H, et al. Biomechanical scaffolds of decellularized heart valves modified by electrospun polylactic acid. *Appl Biochem Biotech* 2024; 196(7): 4256–4272.
27. Giovanniello F, Asgari M, Breslavsky ID, et al. Development and mechanical characterization of decellularized scaffolds for an active aortic graft. *Acta Biomater* 2023; 160: 59–72.
28. Navaee F, Renaud P, Kleger A, et al. Highly efficient cardiac differentiation and maintenance by thrombin-coagulated fibrin hydrogels enriched with decellularized porcine heart extracellular matrix. *Int J Mol Sci* 2023; 24(3): 2842.
29. Mesquita FCP, Morrissey J, Monnerat G, et al. Decellularized extracellular matrix powder accelerates metabolic maturation at early stages of cardiac differentiation in human induced pluripotent stem cell-derived cardiomyocytes. *Cells Tissues Organs* 2023; 212(1): 32–44.
30. Martins AR, Matias GSS, Batista VF, et al. Wistar rat dermis recellularization. *Res Vet Sci* 2020; 131: 222–231.
31. Magne B, Demers A, Savard É, et al. Speeding up the production of clinical-grade skin substitutes using off-the-shelf decellularized self-assembled dermal matrices. *Acta Biomater* 2023; 167: 249–259.
32. Simsa R, Rothenbücher T, Gürbüz H, et al. Brain organoid formation on decellularized porcine brain ECM hydrogels. *PLoS One* 2021; 16(1): e245685.
33. Turan Sorhun D, Kuşoğlu A and Öztürk E. Developing bovine brain-derived extracellular matrix hydrogels: a screen of decellularization methods for their impact on biochemical and mechanical properties. *ACS Omega* 2023; 8(40): 36933–36947.
34. Arakelian L, Godefroy W, Faivre L, et al. Esophagus Decellularization. In: Kajbafzadeh AM (eds.) *Decellularization methods of tissue and whole organ in tissue engineering. Advances in experimental medicine and biology*. Cham: Springer, vol 1345, pp.7–15.

35. Zeng A, Li H, Liu J, et al. The progress of decellularized scaffold in stomatology. *Tissue Eng Regen Med* 2022; 19(3): 451–461.
36. Aisenbrey EA and Murphy WL Synthetic alternatives to matrigel. *Nat Rev Mater* 2020; 5(7): 539–551.
37. Knipper K, Lyu SI, Quaas A, et al. Cancer-associated fibroblast heterogeneity and its influence on the extracellular matrix and the tumor microenvironment. *Int J Mol Sci* 2023; 24(17): 13482.
38. Kim S, Min S, Choi YS, et al. Tissue extracellular matrix hydrogels as alternatives to Matrigel for culturing gastrointestinal organoids. *Nat Commun* 2022; 13(1): 1692.
39. Ammann CG, Messer RJ, Peterson KE, et al. Lactate dehydrogenase-elevating virus induces systemic lymphocyte activation via TLR7-dependent IFNalpha responses by plasmacytoid dendritic cells. *PLoS One* 2009; 4(7): e6105.
40. Li Y, Yang N, Chen J, et al. Next-generation porcine intestinal organoids: an apical-out organoid model for swine enteric virus infection and immune response investigations. *J Virol* 2020; 94(21): 10–1128.
41. Meran L, Massie I, Campinoti S, et al. Engineering transplantable jejunal mucosal grafts using patient-derived organoids from children with intestinal failure. *Nat Med* 2020; 26: 1593–1601.
42. Ray K. Next-generation intestinal organoids. *Nat Rev Gastroenterol Hepatol* 2020; 17(11): 649–650.
43. Meran L, Tullie L, Eaton S, et al. Bioengineering human intestinal mucosal grafts using patient-derived organoids, fibroblasts and scaffolds. *Nat Protoc* 2023; 18(1): 108–135.
44. Willemse J, van Tienderen G, van Hengel E, et al. Hydrogels derived from decellularized liver tissue support the growth and differentiation of cholangiocyte organoids. *Biomaterials* 2022; 284: 121473.
45. Krüger M, Samsom RA, Oosterhoff LA, et al. High level of polarized engraftment of porcine intrahepatic cholangiocyte organoids in decellularized liver scaffolds. *J Cell Mol Med* 2022; 26(19): 4949–4958.
46. Wilson HV. A new method by which sponges may be artificially reared. *Science* 1907; 25(649): 912–915.
47. Evans M. Origin of mouse embryonal carcinoma cells and the possibility of their direct isolation into tissue culture. *J Reprod Fertil* 1981; 62(2): 625–631.
48. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci USA* 1981; 78(12): 7634–7638.
49. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; 282: 1145–1147.
50. Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 2009; 459(7244): 262–265.
51. Hofbauer P, Jahnel SM, Papai N, et al. Cardioids reveal self-organizing principles of human cardiogenesis. *Cell* 2021; 184(12): 3299–3317.
52. Hendriks D, Artegiani B, Hu H, et al. Establishment of human fetal hepatocyte organoids and CRISPR-Cas9-based gene knockin and knockout in organoid cultures from human liver. *Nat Protoc* 2021; 16(1): 182–217.
53. Turco MY, Gardner L, Kay RG, et al. Trophoblast organoids as a model for maternal-fetal interactions during human placentation. *Nature* 2018; 564(7735): 263–267.
54. Kopper O, De Witte CJ, Lõhmussaar K, et al. An organoid platform for ovarian cancer captures intra- and interpatient heterogeneity. *Nat Med* 2019; 25(5): 838–849.
55. Murphy AR, Campo H, Kim JJ. Strategies for modelling endometrial diseases. *Nat Rev Endocrinol* 2022; 18(12): 727–743.
56. Edmonds ME and Woodruff TK. Testicular organoid formation is a property of immature somatic cells, which self-assemble and exhibit long-term hormone-responsive endocrine function. *Biofabrication* 2020; 12(4): 045002.
57. Holtfreter J. Experimental studies on the development of the pronephros. *Rev Can Biol* 1943; 3: 220–250.
58. Weiss P and Taylor AC. Reconstitution of complete organs from single-cell suspensions of chick embryos in advanced stages of differentiation. *Proc Natl Acad Sci USA* 1960; 46(9): 1177–1185.
59. Michael L. *Cellular membranes in development*. Amsterdam: Elsevier.
60. Li ML, Aggeler J, Farson DA, et al. Influence of a reconstituted basement membrane and its components on casein gene expression and secretion in mouse mammary epithelial cells. *Proc Natl Acad Sci USA* 1987; 84(1):1 36–40.
61. Shannon JM, Mason RJ and Jennings SD. Functional differentiation of alveolar type II epithelial cells in vitro: effects of cell shape, cell-matrix interactions and cell-cell interactions. *Biochim Biophys Acta Mol Cell Res* 1987; 931(2): 143–156.
62. Eiraku M, Watanabe K, Matsuo-Takasaki M, et al. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell* 2008; 3(5): 519–532.
63. Eiraku M, Takata N, Ishibashi H, et al. Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* 2011; 472(7341): 51–56.
64. Nakano T, Ando S, Takata N, et al. Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell* 2012; 10(6): 771–785.
65. Spence JR, Mayhew CN, Rankin SA, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature* 2011; 470(7332): 105–109.
66. Lancaster MA, Renner M, Martin CA, et al. Cerebral organoids model human brain development and microcephaly. *Nature* 2013; 501(7467): 373–379.
67. Xia Y, Nivet E, Sancho-Martinez I, et al. Directed differentiation of human pluripotent cells to ureteric bud kidney progenitor-like cells. *Nat Cell Biol* 2013; 15(12): 1507–1515.
68. Takasato M, Er PX, Becroft M, et al. Directing human embryonic stem cell differentiation towards a renal lineage generates a self-organizing kidney. *Nat Cell Biol* 2014; 16(1): 118–126.
69. Taguchi A, Kaku Y, Ohmori T, et al. Redefining the in vivo origin of metanephric nephron progenitors enables generation of complex kidney structures from pluripotent stem cells. *Cell Stem Cell* 2014; 14(1): 53–67.
70. Boj SF, Hwang CI, Baker LA, et al. Organoid models of human and mouse ductal pancreatic cancer. *Cell* 2015; 160(1): 324–338.

71. Broutier L, Andersson-Rolf A, Hindley CJ, et al. Culture and establishment of self-renewing human and mouse adult liver and pancreas 3D organoids and their genetic manipulation. *Nat Protoc* 2016; 11(9): 1724–1743.
72. Drost J, Karthaus WR, Gao D, et al. Organoid culture systems for prostate epithelial and cancer tissue. *Nat Protoc* 2016; 11(2): 347–358.
73. Tan Q, Choi KM, Sicard D, et al. Human airway organoid engineering as a step toward lung regeneration and disease modeling. *Biomaterials* 2016; 113: 118–132.
74. Koehler KR, Nie J, Longworth-Mills E, et al. Generation of inner ear organoids containing functional hair cells from human pluripotent stem cells. *Nat Biotechnol* 2017; 35(6): 583–589.
75. Sachs N, De Ligt J, Kopper O, et al. A living biobank of breast cancer organoids captures disease heterogeneity. *Cell* 2018; 172(1): 373–386.
76. Wimmer RA, Leopoldi A, Aichinger M, et al. Human blood vessel organoids as a model of diabetic vasculopathy. *Nature* 2019; 565(7740): 505–510.
77. Brassard JA and Lutolf MP. Engineering stem cell self-organization to build better organoids. *Cell Stem Cell* 2019; 24(6): 860–876.
78. Hofer M and Lutolf MP. Engineering organoids. *Nat Rev Mater* 2021; 6(5): 402–420.
79. Chen W, Liu X, Chen Q, et al. Angiogenic and osteogenic regeneration in rats via calcium phosphate scaffold and endothelial cell co-culture with human bone marrow mesenchymal stem cells (MSCs), human umbilical cord MSCs, human induced pluripotent stem cell-derived MSCs and human embryonic stem cell-derived MSCs. *J Tissue Eng Regen Med* 2018; 12(1): 191–203.
80. Wu Q, Yang B, Cao C, et al. Therapeutic antibody directed osteogenic differentiation of induced pluripotent stem cell derived MSCs. *Acta Biomater* 2018; 74: 222–235.
81. Hunt NC, Hallam D, Karimi A, et al. 3D culture of human pluripotent stem cells in RGD-alginate hydrogel improves retinal tissue development. *Acta Biomater* 2016; 49: 329–343.
82. Libby AR, Briers D, Haghighi I, et al. Automated design of pluripotent stem cell self-organization. *Cell Syst* 2019; 9(5): 483–495.
83. Vargas-Valderrama A, Messina A, Mitjavila-Garcia MT, et al. The endothelium, a key actor in organ development and hPSC-derived organoid vascularization. *J Biomed Sci* 2020; 27: 1–13.
84. Zheng X, Han D, Liu W, et al. Human iPSC-derived midbrain organoids functionally integrate into striatum circuits and restore motor function in a mouse model of Parkinson's disease. *Theranostics* 2023; 13(8): 2673.
85. Kim W, Gwon Y, Park S, et al. Therapeutic strategies of three-dimensional stem cell spheroids and organoids for tissue repair and regeneration. *Bioact Mater* 2023; 19: 50–74.
86. Qian X, Jacob F, Song MM, et al. Generation of human brain region-specific organoids using a miniaturized spinning bioreactor. *Nat Protoc* 2018; 13(3): 565–580.
87. Phelan MA, Gianforcaro AL, Gerstenhaber JA, et al. An air bubble-isolating rotating wall vessel bioreactor for improved spheroid/organoid formation. *Tissue Eng Part C Methods* 2019; 25(8): 479–488.
88. Cohen PJ, Luquet E, Pletenka J, et al. Engineering 3D micro-compartments for highly efficient and scale-independent expansion of human pluripotent stem cells in bioreactors. *Biomaterials* 2023; 295: 122033.
89. Su M, Ruan L, Dong X, et al. Current state of knowledge on intelligent-response biological and other macromolecular hydrogels in biomedical engineering: a review. *Int J Biol Macromol* 2022; 227: 472–492.
90. Grigoryan B, Paulsen SJ, Corbett DC, et al. Multivascular networks and functional intravascular topologies within biocompatible hydrogels. *Science* 2019; 364(6439): 458–464.
91. Yang P, Ju Y, Hu Y, et al. Emerging 3D bioprinting applications in plastic surgery. *Biomater Res* 2023; 27(1): 1.
92. Cordero-Espinoza L, Dowbaj AM, Kohler TN, et al. Dynamic cell contacts between periportal mesenchyme and ductal epithelium act as a rheostat for liver cell proliferation. *Cell Stem Cell* 2021; 28(11): 1907–1921.
93. Yi SA, Zhang Y, Rathnam C, et al. Bioengineering approaches for the advanced organoid research. *Adv Mater* 2021; 33(45): 2007949.
94. Gjorevski N, Nikolaev M, Brown TE, et al. Tissue geometry drives deterministic organoid patterning. *Science* 2022; 375(6576): eaaw9021.
95. Hwangbo H, Chae S, Kim W, et al. Tumor-on-a-chip models combined with mini-tissues or organoids for engineering tumor tissues. *Theranostics* 2024; 14(1): 33.
96. Kozlowski MT, Crook CJ and Ku HT. Towards organoid culture without Matrigel. *Commun Biol* 2021; 4(1): 1387.
97. Hinderer S, Layland SL and Schenke-Layland K. ECM and ECM-like materials - biomaterials for applications in regenerative medicine and cancer therapy. *Adv Drug Deliver Rev* 2016; 97: 260–269.
98. Safari F, Fani N, Eglin D, et al. Human umbilical cord-derived scaffolds for cartilage tissue engineering. *J Biomed Mater Res A* 2019; 107(8): 1793–1802.
99. Hoshiba T. Cultured cell-derived decellularized matrices: a review towards the next decade. *J Mater Chemi B* 2017; 5: 4322–4331.
100. Hoshiba T. Decellularized extracellular matrix for cancer research. *Materials* 2019; 12(8): 1311.
101. Stahl EC, Bonvillain RW, Skillen CD, et al. Evaluation of the host immune response to decellularized lung scaffolds derived from α-Gal knockout pigs in a non-human primate model. *Biomaterials* 2018; 187: 93–104.
102. Asthana A, Chaimov D, Tamburrini R, et al. Decellularized human pancreatic extracellular matrix-based physiomimetic microenvironment for human islet culture. *Acta Biomater* 2023; 171: 261–272.
103. Wang H, Li S, Dai Q, et al. Culture on a native bone marrow-derived extracellular matrix restores the pancreatic islet basement membrane, preserves islet function, and attenuates islet immunogenicity. *FASEB J* 2020; 34(6): 8044–8056.
104. Fang H, Xu J, Ma H, et al. Functional materials of 3D bioprinting for wound dressings and skin tissue engineering applications: a review. *Int J Bioprint* 2023; 9(5): 757.
105. Kirsner RS, Bohn G, Driver VR, et al. Human acellular dermal wound matrix: evidence and experience. *Int Wound J* 2013; 12(6): 646–654.
106. Modi A, Haque A, Deore V, et al. Interposition GraftJacket allografts for irreparable rotator cuff tears. *Bone Joint J* 2022; 104(1): 91–96.

107. Shakouri-Motlagh A, Khanabdali R, Heath DE, et al. The application of decellularized human term fetal membranes in tissue engineering and regenerative medicine (TERM). *Placenta* 2017; 59: 124–130.
108. Francis MP, Breathwaite E, Bulysheva AA, et al. Human placenta hydrogel reduces scarring in a rat model of cardiac ischemia and enhances cardiomyocyte and stem cell cultures. *Acta Biomater* 2016; 52: 92–104.
109. Murchison AC, Odanga JJ, Treadwell ML, et al. Human placenta-derived ECM supports tri-lineage differentiation of human induced pluripotent stem cells. *Int J Stem Cells* 2020; 13(3): 432–438.
110. Wang Z, Liu R, Liu Y, et al. Human placenta decellularized extracellular matrix hydrogel promotes the generation of human spinal cord organoids with dorsoventral organization from human induced pluripotent stem cells. *ACS Biomater Sci Eng* 2024; 10(5): 3218–3231.
111. Shakouri-Motlagh A, O'Connor AJ, Brennecke SP, et al. Native and solubilized decellularized extracellular matrix: a critical assessment of their potential for improving the expansion of mesenchymal stem cells. *Acta Biomater* 2017; 55: 1–12.
112. Gonzalez-Fernandez T, Tenorio AJ, Saiz AM, Jr, et al. Engineered cell-secreted extracellular matrix modulates cell spheroid mechanosensing and amplifies their response to inductive cues for the formation of mineralized tissues. *Adv Healthc Mater* 2021; 11(10): 2102337.
113. Wan HY, Shin RL, Chen JC, et al. Dextran sulfate-amplified extracellular matrix deposition promotes osteogenic differentiation of mesenchymal stem cells. *Acta Biomater* 2021; 140: 163–177.
114. Choi SH, Lee K, Han H, et al. Prochondrogenic effect of decellularized extracellular matrix secreted from human induced pluripotent stem cell-derived chondrocytes. *Acta Biomater* 2023; 167: 234–248.
115. Kusuma GD, Brennecke SP, O'Connor AJ, et al. Decellularized extracellular matrices produced from immortal cell lines derived from different parts of the placenta support primary mesenchymal stem cell expansion. *PLoS One* 2017; 12(2): e0171488.
116. Kim BS, Das S, Jang J, et al. Decellularized extracellular matrix-based bioinks for engineering tissue- and organ-specific microenvironments. *Chem Rev* 2020; 120(19): 10608–10661.
117. Citro A, Neroni A, Pignatelli C, et al. Directed self-assembly of a xenogeneic vascularized endocrine pancreas for type 1 diabetes. *Nat Commun* 2023; 14: 878.
118. Alaby Pinheiro Faccioli L, Suhett Dias G, Hoff V, et al. Optimizing the decellularized porcine liver scaffold protocol. *Cells Tissues Organs* 2022; 211: 385–394.
119. Ansari T, Southgate A, Obiri-Yeboa I, et al. Development and characterization of a porcine liver scaffold. *Stem Cells Dev* 2020; 29: 314–326.
120. Zhang CY, Fu CP, Li XY, et al. Three-dimensional bioprinting of decellularized extracellular matrix-based bioinks for tissue engineering. *Molecules* 2022; 27: 3442.
121. Prasertsung I, Kanokpanont S, Bunaprasert T, et al. Development of acellular dermis from porcine skin using periodic pressurized technique. *J Biomed Mater Res B Appl Biomater* 2008: 210–219.
122. Mansour RN, Karimizade A, Enderami SE, et al. The effect of source animal age, decellularization protocol, and sterilization method on bovine acellular dermal matrix as a scaffold for wound healing and skin regeneration. *Artif Organs* 2023; 47: 302–316.
123. Poornejad N, Frost TS, Scott DR, et al. Freezing/thawing without cryoprotectant damages native but not decellularized porcine renal tissue. *Organogenesis* 2015; 11: 30–45.
124. Nonaka PN, Campillo N, Uriarte JJ, et al. Effects of freezing/thawing on the mechanical properties of decellularized lungs. *J Biomed Mater Res A* 2014; 102(2): 413–419.
125. Moffat D, Ye K and Jin S. Decellularization for the retention of tissue niches. *J Tissue Eng* 2022; 13: 1768591455.
126. Crapo PM, Gilbert TW and Badylak SF. An overview of tissue and whole organ decellularization processes. *Biomaterials* 2011; 32: 3233–3243.
127. Seo Y, Jung Y and Kim SH Decellularized heart ECM hydrogel using supercritical carbon dioxide for improved angiogenesis. *Acta Biomater* 2018; 67: 270–281.
128. de Wit RJJ, van Dis DJ, Bertrand ME, et al. Scaffold-based tissue engineering: supercritical carbon dioxide as an alternative method for decellularization and sterilization of dense materials. *Acta Biomater* 2023; 155: 323–332.
129. Duarte MM, Silva IV, Eisenhut AR, et al. Contributions of supercritical fluid technology for advancing decellularization and postprocessing of viable biological materials. *Mater Horiz* 2022; 9: 864–891.
130. Koo MA, Jeong H, Hong SH, et al. Preconditioning process for dermal tissue decellularization using electroporation with sonication. *Regen Biomater* 2022; 9: b71.
131. Bae JY, Park SY, Shin YH, et al. Preparation of human decellularized peripheral nerve allograft using amphoteric detergent and nuclease. *Neural Regen Res* 2021; 16: 1890–1896.
132. Singh G, Satpathi S, Gopala Reddy BV, et al. Impact of various detergent-based immersion and perfusion decellularization strategies on the novel caprine pancreas derived extracellular matrix scaffold. *Front Bioeng Biotechnol* 2023; 11: 1253804.
133. Zubarevich A, Osswald A, Amanov L, et al. Development and evaluation of a novel combined perfusion decellularization heart-lung model for tissue engineering of bioartificial heart-lung scaffolds. *Artif Organs* 2023; 47: 481–489.
134. Hashimoto Y, Yamashita A, Negishi J, et al. 4-Arm PEG-functionalized decellularized pericardium for effective prevention of postoperative adhesion in cardiac surgery. *ACS Biomater Sci Eng* 2021; 8: 261–272.
135. Zhang JS, Wang ZB, Lai ZZ, et al. Polyethylene glycol crosslinked decellularized single liver lobe scaffolds with vascular endothelial growth factor promotes angiogenesis in vivo. *HBPD INT* 2023; 22: 622–631.
136. Liu K, He Y, Yao Y, et al. Methoxy polyethylene glycol modification promotes adipogenesis by inducing the production of regulatory T cells in xenogeneic acellular adipose matrix. *Materials Today Bio* 2021; 12: 100161.
137. Wong V, Gada S, Singh M, et al. The development of small-caliber vascular grafts using human umbilical artery: an evaluation of methods. *Tissue Eng Part C Methods* 2023; 29: 1–10.

138. Bera AK, Sriya Y and Pati F. Formulation of dermal tissue matrix bioink by a facile decellularization method and process optimization for 3D bioprinting toward translation research. *Macromol Biosci* 2022; 22: e2200109.
139. Das P, Mishra R, Devi B, et al. Decellularized xenogenic cartilage extracellular matrix (ECM) scaffolds for the reconstruction of osteochondral defects in rabbits. *J Mater Chem B* 2021; 9: 4873–4894.
140. Tao M, Ao T, Mao X, et al. Sterilization and disinfection methods for decellularized matrix materials: review, consideration and proposal. *Bioact Mater* 2021; 6: 2927–2945.
141. Li W, Wang F, Barnett C, et al. A comparative study on fabrication techniques of gelable bone matrix derived from porcine tibia. *J Biomed Mater Res B Appl Biomater* 2021; 109: 2131–2141.
142. Zhao F, Cheng J, Zhang J, et al. Comparison of three different acidic solutions in tendon decellularized extracellular matrix bio-ink fabrication for 3D cell printing. *Acta Biomater* 2021; 131: 262–275.
143. Fidalgo C, Iop L, Sciro M, et al. A sterilization method for decellularized xenogeneic cardiovascular scaffolds. *Acta Biomater* 2018; 67: 282–294.
144. Bednarski DM, Lantz EE, Bobst CE, et al. Sterilization of epidermal growth factor with supercritical carbon dioxide and peracetic acid; analysis of changes at the amino acid and protein level. *Biochim Biophys Acta Proteins Proteomics* 2020; 1868: 140334.
145. Lin S, He Y, Tao M, et al. Fabrication and evaluation of an optimized xenogenic decellularized costal cartilage graft: preclinical studies of a novel biocompatible prosthesis for rhinoplasty. *Regen Biomater* 2021; 8: b52.
146. Bakhtiar H, Rajabi S, Pezeshki-Modaress M, et al. Optimizing methods for bovine dental pulp decellularization. *J. Endodont* 2021; 47: 62–68.
147. Singh A, Singh SK, Kumar V, et al. Derivation and characterization of novel cytocompatible decellularized tissue scaffold for myoblast growth and differentiation. *Cells* 2023; 13(1): 41.
148. Kuravi KV, Sorrells LT, Nellis JR, et al. Allergic response to medical products in patients with alpha-gal syndrome. *J Thorac Cardiovasc Surg* 2022; 164: e411–e424.
149. Wilson SL, Sidney LE, Dunphy SE, et al. Keeping an eye on decellularized corneas: a review of methods, characterization and applications. *J Funct Biomater* 2013; 4(3): 114–161.
150. Sauter J, Degenhardt H, Tuebel J, et al. Effect of different decellularization protocols on reendothelialization with human cells for a perfused renal bioscaffold of the rat. *BMC Biotechnol* 2023; 23(1): 8.
151. Fooladi S, Faramarz S, Dabiri S, et al. An efficient strategy to recellularization of a rat aorta scaffold: an optimized decellularization, detergent removal, and Apelin-13 immobilization. *Biomater Res* 2022; 26(1): 46.
152. Duisit J, Amiel H, Wüthrich T, et al. Perfusion-decellularization of human ear grafts enables ECM-based scaffolds for auricular vascularized composite tissue engineering. *Acta Biomater* 2018; 73: 339–354.
153. Gerchman Y, Cohen-Yaniv V, Betzalel Y, et al. The involvement of superoxide radicals in medium pressure UV derived inactivation. *Water Res* 2019; 161: 119–125.
154. Walker S, Dittfeld C, Jakob A, et al. Sterilization and cross-linking combined with ultraviolet irradiation and low-energy electron irradiation procedure: new perspectives for bovine pericardial implants in cardiac surgery. *Thorac Cardiovasc Surg* 2022; 70: 33–42.
155. Sun Y, Lovric V, Wang T, et al. Effects of SCCO, gamma irradiation, and sodium dodecyl sulfate treatments on the initial properties of tendon allografts. *Int J Mol Sci* 2020; 21(5): 1565.
156. de Sousa Iwamoto LA, Duailibi MT, Iwamoto GY, et al. Evaluation of ethylene oxide, gamma radiation, dry heat and autoclave sterilization processes on extracellular matrix of biomaterial dental scaffolds. *Sci Rep* 2022; 12: 4299.
157. Mendes GC, Brandão TR and Silva CL. Ethylene oxide sterilization of medical devices: a review. *Am J Infect Control* 2007; 35: 574–581.
158. Zhou C, Zhou L, Liu J, et al. Kidney extracellular matrix hydrogel enhances therapeutic potential of adipose-derived mesenchymal stem cells for renal ischemia reperfusion injury. *Acta Biomater* 2020; 115: 250–263.
159. Fercana GR, Yerneni S, Billaud M, et al. Perivascular extracellular matrix hydrogels mimic native matrix microarchitecture and promote angiogenesis via basic fibroblast growth factor. *Biomaterials* 2017; 123: 142–154.
160. Keshvari MA, Afshar A, Daneshi S, et al. Decellularization of kidney tissue: comparison of sodium lauryl ether sulfate and sodium dodecyl sulfate for allotransplantation in rat. *Cell Tissue Res* 2021; 386: 365–378.
161. Zhang H, Wang Y, Zheng Z, et al. Strategies for improving the 3D printability of decellularized extracellular matrix bioink. *Theranostics* 2023; 13: 2562–2587.
162. Stocco E, Barbon S, Mammana M, et al. Preclinical and clinical orthotopic transplantation of decellularized/engineered tracheal scaffolds: a systematic literature review. *J Tissue Eng* 2023; 14: 1778642130.
163. Alabi BR, LaRanger R and Shay JW. Decellularized mice colons as models to study the contribution of the extracellular matrix to cell behavior and colon cancer progression. *Acta Biomater* 2019; 100: 213–222.
164. Sensi F, D'angelo E, Biccari A, et al. Establishment of a human 3D pancreatic adenocarcinoma model based on a patient-derived extracellular matrix scaffold. *Transl Res* 2023; 253: 57–67.
165. Sung SY, Lin YW, Wu CC, et al. Supercritical carbon dioxide-decellularized arteries exhibit physiologic-like vessel regeneration following xenotransplantation in rats. *Biomater Sci* 2023; 11(7): 2566–2580.
166. Kasravi M, Ahmadi A, Babajani A, et al. Immunogenicity of decellularized extracellular matrix scaffolds: a bottleneck in tissue engineering and regenerative medicine. *Biomater Res* 2023; 27(1): 10.
167. Saldin LT, Cramer MC, Velankar SS, et al. Extracellular matrix hydrogels from decellularized tissues: structure and function. *Acta Biomater* 2017; 49: 1–15.
168. Wang T, Huang Q, Rao Z, et al. Injectable decellularized extracellular matrix hydrogel promotes salivary gland regeneration via endogenous stem cell recruitment and suppression of fibrogenesis. *Acta Biomater* 2023; 169: 256–272.
169. Alavarse AC, Frachini ECG, Da Silva RLCG, et al. Crosslinkers for polysaccharides and proteins: synthesis

conditions, mechanisms, and crosslinking efficiency, a review. *Int J Biol Macromol* 2022; 202: 558–596.
170. Lewies A, Botes L, van den Heever JJ, et al. Monomeric glutaraldehyde fixation and amino acid detoxification of decellularized bovine pericardium for production of biocompatible tissue with tissue-guided regenerative potential. *Heliyon* 2023; 9: e19712.
171. Browe DC, Mahon OR, Díaz-Payno PJ, et al. Glyoxal cross-linking of solubilized extracellular matrix to produce highly porous, elastic, and chondro-permissive scaffolds for orthopedic tissue engineering. *J Biomed Mater Res A* 2019; 107: 2222–2234.
172. Oryan A, Kamali A, Moshiri A, et al. Chemical crosslinking of biopolymeric scaffolds: current knowledge and future directions of crosslinked engineered bone scaffolds. *Int J Biol Macromol* 2018; 107: 678–688.
173. Musilkova J, Filova E, Pala J, et al. Human decellularized and crosslinked pericardium coated with bioactive molecular assemblies. *Biomed Mater* 2019; 15: 15008.
174. Huang X, Zheng C, Ding K, et al. Dual-crosslinked bioprosthetic heart valves prepared by glutaraldehyde crosslinked pericardium and poly-2-hydroxyethyl methacrylate exhibited improved antithrombogenicity and anticalcification properties. *Acta Biomater* 2022; 154: 244–258.
175. Wang J, Kong L, Gafur A, et al. Photooxidation crosslinking to recover residual stress in decellularized blood vessel. *Regen Biomater* 2021; 8: a58.
176. Zhu L, Yuhan J, Yu H, et al. Decellularized extracellular matrix for remodeling bioengineering organoid's microenvironment. *Small* 2023; 19(25): 2207752.
177. Kanetaka K and Eguchi S. Regenerative medicine for the upper gastrointestinal tract. *Regen Ther* 2020; 15: 129–137.
178. Huch M, Dorrell C, Boj SF, et al. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. *Nature* 2013; 494: 247–250.
179. Giobbe GG, Crowley C, Luni C, et al. Extracellular matrix hydrogel derived from decellularized tissues enables endodermal organoid culture. *Nat Commun* 2019; 10: 5658.
180. Pironi L, Cuerda C, Jeppesen PB, et al. ESPEN guideline on chronic intestinal failure in adults - update 2023. *Clin Nutr* 2023; 42: 1940–2021.
181. Pironi L. Definition, classification, and causes of short bowel syndrome. *Nutr Clin Pract* 2023; 38: S9–S16.
182. Urbani L, Camilli C, Phylactopoulos DE, et al. Multistage bioengineering of a layered oesophagus with in vitro expanded muscle and epithelial adult progenitors. *Nat Commun* 2018; 9: 4286.
183. Ibi Y and Nishinakamura R. Kidney bioengineering for transplantation. *Transplantation* 2023; 107: 1883–1894.
184. Sobreiro-Almeida R, Quinteira R and Neves NM. Renal regeneration: the role of extracellular matrix and current ECM-based tissue engineered strategies. *Adv Healthc Mater* 2021; 10: e2100160.
185. Nishimura Y. Current status and future prospects of decellularized kidney tissue. *J Artif Organs* 2023; 26: 171–175.
186. Sant S, Wang D, Abidi M, et al. Mechanical characterization of native and sugar-modified decellularized kidneys. *J Mech Behav Biomed Mater* 2021; 114: 104220.
187. Ofenbauer A, Sebinger DD, Prewitz M, et al. Dewaxed ECM: a simple method for analyzing cell behaviour on decellularized extracellular matrices. *J Tissue Eng Regen Med* 2015; 9: 1046–1055.
188. Padalhin AR, Park CM and Lee BT. Streamlined system for conducting in vitro studies using decellularized kidney scaffolds. *Tissue Eng Part C Methods* 2018; 24: 42–55.
189. Zambon JP, Ko IK, Abolbashari M, et al. Comparative analysis of two porcine kidney decellularization methods for maintenance of functional vascular architectures. *Acta Biomater* 2018; 75: 226–234.
190. Kim JW, Nam SA, Yi J, et al. Kidney decellularized extracellular matrix enhanced the vascularization and maturation of human kidney organoids. *Adv Sci* 2022; 9: e2103526.
191. Lih E, Park W, Park KW, et al. A bioinspired scaffold with anti-inflammatory magnesium hydroxide and decellularized extracellular matrix for renal tissue regeneration. *ACS Cent Sci* 2019; 5(3): 458–467.
192. Chen FM and Liu X. Advancing biomaterials of human origin for tissue engineering. *Prog Polym Sci* 2016; 53: 86–168.
193. Vanslambrouck JM, Wilson SB, Tan KS, et al. Enhanced metanephric specification to functional proximal tubule enables toxicity screening and infectious disease modelling in kidney organoids. *Nat Commun* 2022; 13: 5943.
194. Vanslambrouck JM, Tan KS, Mah S, et al. Generation of proximal tubule-enhanced kidney organoids from human pluripotent stem cells. *Nat Protoc* 2023; 18: 3229–3252.
195. Guo B, Zhou Q, Chen J, et al. Orthotopic transplantation of functional bioengineered livers in rats. *ACS Biomater Sci Eng* 2023; 9: 1940–1951.
196. Septiana WL, Ayudyasari W, Gunardi H, et al. Liver organoids cocultured on decellularized native liver scaffolds as a bridging therapy improves survival from liver failure in rabbits. *In Vitro Cell Dev Biol Anim* 2023; 59(10): 747–763.
197. Dias ML, Paranhos BA and Goldenberg RC. Liver scaffolds obtained by decellularization: a transplant perspective in liver bioengineering. *J Tissue Eng* 2022; 13: 20417314221105305.
198. Arki MK, Moeinabadi-Bidgoli K, Hossein-Khannazer N, et al. Amniotic membrane and its derivatives: novel therapeutic modalities in liver disorders. *Cells* 2023; 12(16): 2114.
199. Wang Y, Nicolas CT, Chen HS, et al. Recent advances in decellularization and recellularization for tissue-engineered liver grafts. *Cells Tissues Organs* 2017; 203: 125–136.
200. Chen J, Ma S, Yang H, et al. Generation and metabolomic characterization of functional ductal organoids with biliary tree networks in decellularized liver scaffolds. *Bioact Mater* 2023; 26: 452–464.
201. Tomofuji K, Fukumitsu K, Kondo J, et al. Liver ductal organoids reconstruct intrahepatic biliary trees in decellularized liver grafts. *Biomaterials* 2022; 287: 121614.
202. Willemse J, van der Laan LJW, de Jonge J, et al. Design by nature: emerging applications of native liver extracellular matrix for cholangiocyte organoid-based regenerative medicine. *Bioengineering* 2022; 9: 110.

203. Dimou Z, Michalopoulos E, Katsimpoulas M, et al. Evaluation of a decellularization protocol for the development of a decellularized tracheal scaffold. *Anticancer Res* 2019; 39: 145–150.
204. Güney TG, Herranz AM, Mumby S, et al. Epithelial-stromal cell interactions and extracellular matrix mechanics drive the formation of airway-mimetic tubular morphology in lung organoids. *iScience* 2021; 24: 103061.
205. Nichols JE, La Francesca S, Niles JA, et al. Production and transplantation of bioengineered lung into a large-animal model. *Sci Transl Med* 2018; 10(452): eaao3926.
206. Vazquez-Armendariz AI and Tata PR. Recent advances in lung organoid development and applications in disease modeling. *J Clin Invest* 2023; 133(22): e170500.
207. Hawkins F, Kramer P, Jacob A, et al. Prospective isolation of NKX2-1-expressing human lung progenitors derived from pluripotent stem cells. *J Clin Invest* 2017; 127: 2277–2294.
208. Rodrigues Toste de Carvalho AL, Liu HY, Chen YW, et al. The in vitro multilineage differentiation and maturation of lung and airway cells from human pluripotent stem cell-derived lung progenitors in 3D. *Nat Protoc* 2021; 16: 1802–1829.
209. Valdoz JC, Franks NA, Cribbs CG, et al. Soluble ECM promotes organotypic formation in lung alveolar model. *Biomaterials* 2022; 283: 121464.
210. Ng WH, Varghese B and Ren X. Understanding and engineering the pulmonary vasculature. In: Ng WH, Varghese B and Ren X (ed.) *Engineering translational models of lung homeostasis and disease*. Cham: Springer International Publishing, 2023, pp.247–264.
211. Raredon MSB, Engler AJ, Yuan Y, et al. Microvascular fluid flow in ex vivo and engineered lungs. *J Appl Physiol* 2021; 131: 1444–1459.
212. Asthana A, Tamburrini R, Chaimov D, et al. Comprehensive characterization of the human pancreatic proteome for bioengineering applications. *Biomaterials* 2021; 270: 120613.
213. Hashemi J, Barati G and Bibak B. Decellularized matrix bioscaffolds: implementation of native microenvironment in pancreatic tissue engineering. *Pancreas* 2021; 50: 942–951.
214. Lim LY, Ding SSL, Muthukumaran P, et al. Tissue engineering of decellularized pancreas scaffolds for regenerative medicine in diabetes. *Acta Biomater* 2023; 157: 49–66.
215. Goh SK, Bertera S, Richardson T, et al. Repopulation of decellularized organ scaffolds with human pluripotent stem cell-derived pancreatic progenitor cells. *Biomed Mater* 2023; 18(2): 025018.
216. Heaton ES, Hu M, Liu T, et al. Extracellular matrix-derived peptide stimulates the generation of endocrine progenitors and islet organoids from iPSCs. *J Tissue Eng* 2023; 14: 20417314231185858.
217. Glorieux L, Vandooren L, Derclaye S, et al. In-depth analysis of the pancreatic extracellular matrix during development for next-generation tissue engineering. *Int J Mol Sci* 2023; 24(12): 10268.
218. Uday Chandrika K, Tripathi R, Kameshwari Y, et al. Refunctionalization of decellularized organ scaffold of pancreas by recellularization: whole organ regeneration into functional pancreas. *Tissue Eng Regen Med* 2021; 18: 99–112.
219. Karimi S, Khorsandi LS and Ai J. Fabrication of bioartificial pancreas using decellularized rat testicular tissue. *Acta Histochem* 2022; 124: 151928.
220. Zhang L, Qiu H, Wang D, et al. Enhanced vascularization and biocompatibility of rat pancreatic decellularized scaffolds loaded with platelet-rich plasma. *J Biomater Appl* 2020; 35: 313–330.
221. Singh G, Senapati S, Satpathi S, et al. Establishment of decellularized extracellular matrix scaffold derived from caprine pancreas as a novel alternative template over porcine pancreatic scaffold for prospective biomedical application. *FASEB J* 2022; 36: e22574.
222. Tremmel DM, Sackett SD, Feeney AK, et al. A human pancreatic ECM hydrogel optimized for 3-D modeling of the islet microenvironment. *Sci Rep* 2022; 12(1): 7188.
223. Sevastianov VI, Ponomareva AS, Baranova NV, et al. Decellularization of human pancreatic fragments with pronounced signs of structural changes. *Int J Mol Sci* 2022; 24(1): 119.
224. Berkova Z, Zacharovova K, Patikova A, et al. Decellularized pancreatic tail as matrix for pancreatic islet transplantation into the greater omentum in rats. *J Funct Biomater* 2022; 13(4): 171.
225. Del Rio A, Negro F, Piersanti V, et al. Uterus transplant update: innovative fertility solutions and the widening horizons of bioengineering. *Eur Rev Med Pharmaco Sci* 2021; 25(9): 3405–3410.
226. Almeida GHD, Iglesia RP, Araujo MS, et al. Uterine tissue engineering: where we stand and the challenges ahead. *Tissue Eng Part B Rev* 2022; 28(4): 861–890.
227. Brännström M, Racowsky C, Richards EG, et al. Absolute uterine infertility a cornelian dilemma: uterine transplantation or surrogacy? *Fertil Steril* 2023; 119(6): 918–929.
228. Daryabari SS, Fendereski K, Ghorbani F, et al. Whole-organ decellularization of the human uterus and in vivo application of the bio-scaffolds in animal models. *J Assist Reprod Gen* 2022; 39: 1237–1247.
229. Masoomikarimi M, Salehi M, Noorbakhsh F, et al. A combination of physical and chemical treatments is more effective in the preparation of acellular uterine scaffolds. *Cell J* 2023; 25: 25–34.
230. Yi X, Liu F, Gao K, et al. Reconstructable uterus-derived materials for uterus recovery toward efficient live births. *Adv Mater* 2022; 34: e2106510.
231. Li X, Wang Y, Ma R, et al. Reconstruction of functional uterine tissues through recellularizing the decellularized rat uterine scaffolds by MSCs in vivo and in vitro. *Biomed Mater* 2021; 16(3): 35023.
232. Sehic E, Thorén E, Gudmundsdottir I, et al. Mesenchymal stem cells establish a pro-regenerative immune milieu after decellularized rat uterus tissue transplantation. *J Tissue Eng* 2022; 13: 1768609162.

233. Gómez-Álvarez M, Bueno-Fernandez C, Rodríguez-Eguren A, et al. Hybrid endometrial-derived hydrogels: human organoid culture models and in vivo perspectives. *Adv Healthc Mater* 2024; 13: e2303838.
234. Jamaluddin MFB, Ghosh A, Ingle A, et al. Bovine and human endometrium-derived hydrogels support organoid culture from healthy and cancerous tissues. *Proc Natl Acad Sci USA* 2022; 119: e2086927177.
235. Kofman S, Mohan N, Sun X, et al. Human mini brains and spinal cords in a dish: modeling strategies, current challenges, and prospective advances. *J Tissue Eng* 2022; 13: 20417314221113391.
236. Wu W, Liu Y, Liu R, et al. Decellularized brain extracellular matrix hydrogel aids the formation of human spinal-cord organoids recapitulating the complex three-dimensional organization. *ACS Biomater Sci Eng* 2024; 10(5): 3203–3217.
237. Seo Y, Jeong S, Chung JJ, et al. Development of an anisotropically organized brain dECM hydrogel-based 3D neuronal culture platform for recapitulating the brain microenvironment in vivo. *ACS Biomater Sci Eng* 2020; 6(1): 610–620.
238. Cho AN, Jin Y, An Y, et al. Microfluidic device with brain extracellular matrix promotes structural and functional maturation of human brain organoids. *Nat Commun* 2021; 12: 4730.
239. Song C, Chen X, Ma J, et al. Construction of a pancreatic cancer nerve invasion system using brain and pancreatic cancer organoids. *J Tissue Eng* 2023; 14: 20417314221147113.
240. Gholami K, Solhjoo S and Aghamir SM. Application of tissue-specific extracellular matrix in tissue engineering: focus on male fertility preservation. *Reprod Sci* 2022; 29(11): 3091–3099.
241. Kiani M, Movahedin M, Halvaei I, et al. Formation of organoid-like structures in the decellularized rat testis. *Iran J Basic Med Sci* 2022; 24(11): 1523.
242. Goldfracht I, Efraim Y, Shinnawi R. Engineered heart tissue models from hiPSC-derived cardiomyocytes and cardiac ECM for disease modeling and drug testing applications. *Acta Biomater* 2019; 92: 145–159.
243. Vafaee T, Walker F, Thomas D, et al. Repopulation of decellularised porcine pulmonary valves in the right ventricular outflow tract of sheep: role of macrophages. *J Tissue Eng* 2022; 13: 20417314221102680.
244. García-Gareta E, Pérez MÁ and García-Aznar JM. Decellularization of tumours: a new frontier in tissue engineering. *J Tissue Eng* 2022; 13: 20417314221091682.
245. van Tienderen GS, Rosmark O, Lieshout R, et al. Extracellular matrix drives tumor organoids toward desmoplastic matrix deposition and mesenchymal transition. *Acta Biomater* 2023; 158: 115–131.
246. Choi YM, Lee H, Ann M, et al. 3D bioprinted vascularized lung cancer organoid models with underlying disease capable of more precise drug evaluation. *Biofabrication* 2023; 15(3): 034104.
247. Varinelli L, Guaglio M, Brich S, et al. Decellularized extracellular matrix as scaffold for cancer organoid cultures of colorectal peritoneal metastases. *J Mol Cell Biol* 2023; 14(11): mjac064.
248. Xu M, Su T, Jin X, et al. Inflammation-mediated matrix remodeling of extracellular matrix-mimicking biomaterials in tissue engineering and regenerative medicine. *Acta Biomater* 2022; 151: 106–117.
249. Gaggar A and Weathington N. Bioactive extracellular matrix fragments in lung health and disease. *J Clin Invest* 2016; 126(9): 3176–3184.
250. Witherel CE, Abebayehu D, Barker TH, et al. Macrophage and fibroblast interactions in biomaterial-mediated fibrosis. *Adv Healthc Mater* 2019; 8(4): 1801451.
251. Rezakhani S, Gjorevski N and Lutolf MP. Extracellular matrix requirements for gastrointestinal organoid cultures. *Biomaterials* 2021; 276: 121020.
252. Yavitt FM, Kirkpatrick BE, Blatchley MR, et al. 4D materials with photoadaptable properties instruct and enhance intestinal organoid development. *ACS Biomater Sci Eng* 2022; 8(11): 4634–4638.
253. Lin SN, Musso A, Wang J, et al. Human intestinal myofibroblasts deposited collagen VI enhances adhesiveness for T cells - a novel mechanism for maintenance of intestinal inflammation. *Matrix Biol* 2022; 113: 1–21.
254. Valatas V, Kitamura K, Ward SG, et al. Editorial: stromal and immune cell interactions in intestinal inflammation and fibrosis. *Front Immunol* 2023; 14: 1152140.
255. Sharma V, Letson J and Furuta S. Fibrous stroma: driver and passenger in cancer development. *Sci Signal* 2022; 15(724): eabg3449.
256. Kakni P, Jutten B, Teixeira Oliveira Carvalho D, et al. Hypoxia-tolerant apical-out intestinal organoids to model host-microbiome interactions. *J Tissue Eng* 2023; 14: 20417314221149208.
257. Lap Nguyen V and Obara H. Numerical assessment of recellularization conditions to vessel occlusion. *Biomech Model Mechanobiol* 2023; 22(3): 1035–1047.