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Review

Decellularized Extracellular Matrix: The Role of This Complex Biomaterial in Regeneration

André Guilherme Portela de Paula, Jordana Dinorá de Lima, Thais Sibioni Berti Bastos, Andressa Pacheco Czaikovski, Rebeca Bosso dos Santos Luz, Bruna Sadae Yuasa, Caio Cesar Souza Smanioto, Anny Waloski Robert, and Tárcio Teodoro Braga*

Cite This: *ACS Omega* 2023, 8, 22256−22267

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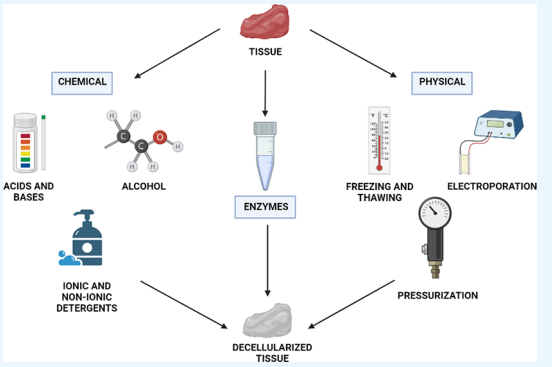
ABSTRACT: Organ transplantation is understood as a technique where an organ from a donor patient is transferred to a recipient patient. This practice gained strength in the 20th century and ensured advances in areas of knowledge such as immunology and tissue engineering. The main problems that comprise the practice of transplants involve the demand for viable organs and immunological aspects related to organ rejection. In this review, we address advances in tissue engineering for reversing the current challenges of transplants, focusing on the possible use of decellularized tissues in tissue engineering. We address the interaction of acellular tissues with immune cells, especially macrophages and stem cells, due to their potential use in regenerative medicine. Our goal is to exhibit data that demonstrate the use of decellularized tissues as alternative biomaterials that can be applied clinically as partial or complete organ substitutes.



1. INTRODUCTION

Organ transplantation is a surgical procedure aimed at substituting nonfunctional organs for functional ones. The technique consists, basically, of transferring a healthy tissue or organ from a donor to a recipient.[1] The transplant can be performed from one individual to another (allografts), from one region to another in the same person (autograft), or using a functional organ or tissue from an animal (xenograft).[2] Transplantation methodology has stimulated advances in numerous areas of knowledge, for example: (i) development of immunosuppressive agents, (ii) improvement of tissue preservation methods, (iii) better understanding of the major histocompatibility complex (MCH), and (iv) innovation in surgical techniques.[3,4] Despite these advances, a major problem after organ transplantation is rejection due to an immune response against the new organ, which stems from genetic disparities involving the human leukocyte antigen (HLA) between the donor and recipient.[5]

In the 20th century, the practice of transplantation gained strength, which allowed a great search to unravel problems such as rejection, which was defined in 1944 by Medawar as a host-versus-graft response (HVG), characterized by an immunological event (sensitization, memory, and tolerance).[6] Years after Medawar's publication, in 1954, the first successful human organ transplantation occurred. The process was performed on two twin brothers, where Ronald donated a kidney to Richard Herrick, who died 8 years after the surgical process due to recurrence of kidney disease, while Ronald died in 2010 after complications from a heart surgery.[7]

Organ transplant rejection occurs by immune responses mainly mediated by mononuclear cells, B and T lymphocytes.[8] The drug regimens are used to suppress the immunity of recipient patients before and after the transplant procedure in order to prevent tissue rejection. However, at the same time, the drugs should be dosed to allow the immune system to maintain sufficient functionality to fight, e.g., infections.[9,10] Currently, immunosuppression involves the use of 3 types of drugs: a calcineurin inhibitor, an antiproliferative agent, and corticosteroids.[11,12] In addition, it is possible to use monoclonal or polyclonal antibody therapy, which aims to prevent cases of acute rejection of the transplanted organ through T lymphocyte depletion.[13,14]

Despite these difficulties, the main factor that limits the practice of transplants is the scarcity of suitable organs, from either the lack of donors or the low quality of available organs (virus-positive donors and recipients; blood group incompatibility; structural condition of the organ).[3,4,15,16] In 2020 over

Received: October 4, 2022
Accepted: January 12, 2023
Published: June 14, 2023

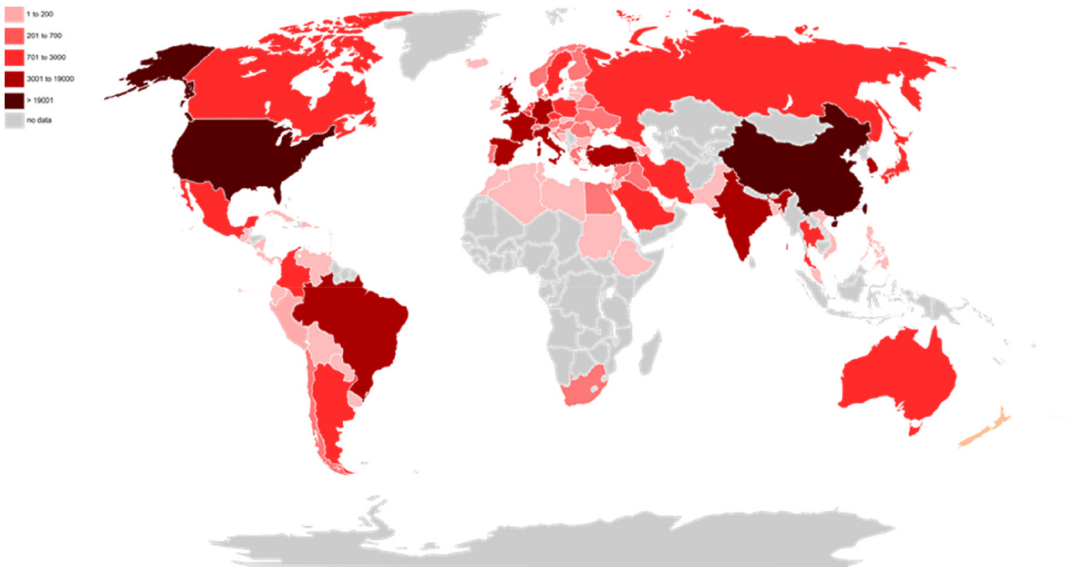


Figure 1. Distribution of transplants performed worldwide in 2020. Reported data of transplants performed in the year 2020 by 85 countries, listed by IRODaT - International Registry in Organ Donation and Transplantation.

Table 1. Current Strategies for Tissue Engineering

STRATEGIES	DESCRIPTION	REFERENCES
Hydrogels	Hydrophilic materials capable of generating 3D structures similar to the extracellular matrix. Their properties include: porous structure, flexibility in the synthesis of the biomaterial, physical properties similar to natural tissues, biocompatibility, and the ability to store nanoparticles and therapeutic biomolecules.	35
3D Bioprinting	Allows the deposit with spatial precision of bioinks, such as hydrogels, cells, and growth factors, mimicking *in vitro* the structure of native tissue and cellular and vascular composition from computational projections or from digitalization of magnetic resonance or computed tomography images.	36, 37
Decellularization	The use of dECM in regenerative medicine aims to minimize adverse reactions to the host, taking advantage of the unique characteristics of this biomaterial: maintaining the biological, mechanical, and structural properties of ECM which ensures the biocompatibility of the graft and the extraction of cells, and cellular debris provide a nonimmunogenic environment and in turn low cytotoxicity.	38

132.193 transplants were performed worldwide (Figure 1); however, in the United States alone there are over 106.088 individuals waiting to receive a new organ, which demonstrates the great inequality between supply and demand for organs.[17]

The number of deaths of patients with late-stage organ failure is dependent on technological innovations, such as the development of artificial tissues. To achieve this, tissue engineering and regenerative medicine strategies for the replacement of nonfunctional organs have been studied over the last years.[18−22]

Tissue engineering uses the knowledge of a natural extracellular matrix (ECM) as a template for the synthesis of biomaterials and scaffolds, aiming to mimic the structure and composition of the original organ.[23] Among the biomaterials, the use of decellularized tissues shows great promise in regenerative medicine. The decellularized organs preserve native ECM composition and structure, allowing recellularization with, e.g., recipient compatible cells, reducing the host immune response.[24−27]

Considering the two main problems of organ transplantation, low organ availability and rejection, our objective in this review is to show some advances in tissue engineering in an attempt to overcome these difficulties. Among the approaches, we will mainly focus on the use of decellularized tissues and how they can be used in therapies. We will also address how these acellular tissues interact with cells of the immune system, specifically macrophages, and with stem cells, both of them with great potential for use in regenerative medicine. Our objective is to demonstrate that the use of decellularized tissues can be an alternative for the generation of scaffolds with potential application for partial or complete organ replacement.

2. TISSUE ENGINEERING STRATEGIES

Tissue engineering (TE) was first defined, in 1993, as "an interdisciplinary field that applies engineering principles to the life sciences in order to develop biological substitutes capable of restoring, maintaining, or improving tissue function".[28] This is a growing area of interest that aims to develop biological substitutes to be used *in vivo* transplant, in order to help solve the problems of high demand and low availability of organs.[29] This strategy involves the use of scaffolds produced from biological and nonbiological materials, which can provide mechanical support in 3D form for cell development and mimic tissue structure[30,31] (Table 1). Studies corroborate that scaffolds favor growth, migration, proliferation, and differentiation and are essential in providing a structure capable of housing human mesenchymal stem cells.[32] It is possible to use scaffolds in transplantation therapy, as in the process of meniscal allograft, and in this process the meniscus can be extracted in its totality or partially, with collagen- or polyurethane-based scaffolds being used in partial extraction.[33] Another study points out the scaffold composed of gelatin and copper capillary alginate, a 3D structure that provides favorable conditions for viable multipotent astrocytic stem cells (neural stem cells) under *in vitro* conditions.[34]

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ACS Omega 2023, 8, 22256−22267

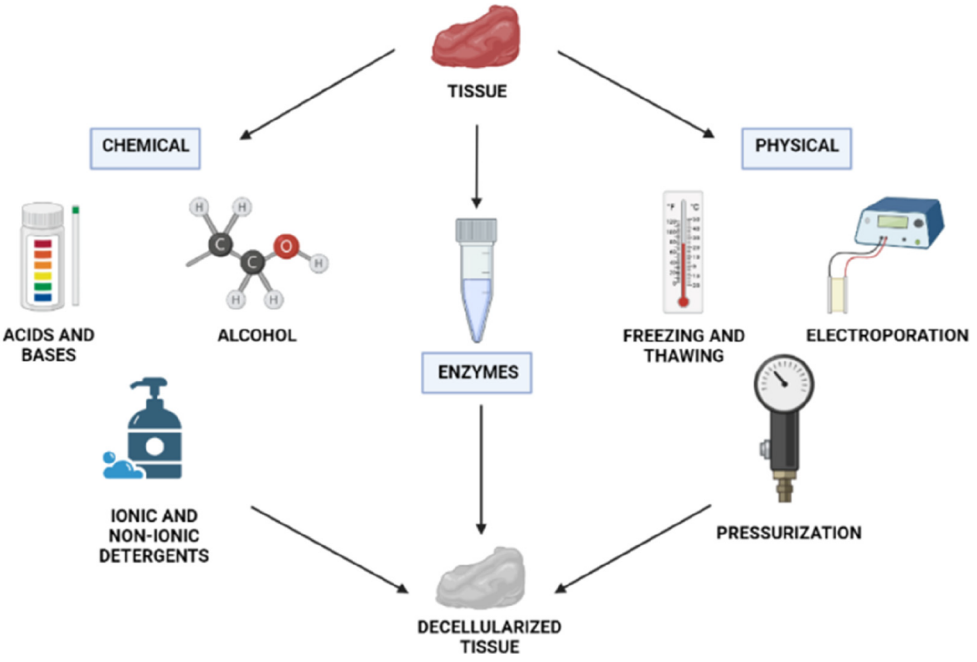

Figure 2. Overview of decellularization strategies. There are numerous techniques for obtaining decellularized tissue. The final objective of the process is to destroy the cells and extract the cellular debris generated, keeping the ECM intact.

There are many types of biomaterials that can be used to produce scaffolds for tissue engineering. The most commonly used definition of biomaterial was conceived during the 1991 Chester Consensus Conference, described as a substance or combination of substances of natural or synthetic origin used for indeterminate periods of time that can partially or totally replace a tissue, maintaining its function, with the aim of generating quality of life for the individual.[39] Biomaterials are classified as natural or synthetic. In general, biomaterials of natural origin are composed of polypeptides, polysaccharides, nucleic acids, and hydroxyapatites and presented advantages over synthetic biomaterials, such as (i) selective cell adhesion, (ii) similarities in the mechanical properties of tissues *in vivo*, and (iii) biodegrability. Synthetic biomaterials are mostly polymers that are relatively inexpensive and with evident elastic characteristics, contributing to the increase of biocompatibility and the control of degradation of these structures.[40]

Several techniques are used to obtain biomaterials: one of them is the use of hydrogels. The 3D structures generated by hydrogels, which mimic the ECM, must meet the basic requirements of biocompatibility and mechanical and physical characteristics appropriate for each tissue, which provide a suitable environment for cell adhesion, which contributes to tissue regeneration.[41] Hydrogels are used in the therapy of many diseases, mostly in regenerative transport of molecules and cells, and these applications are better addressed elsewhere.[42−44]

3D bioprinting, which consists of printing biomaterials consisting of living cells or active biomolecules, is a method that involves the deposition of layers of bioink, resulting in 3D structures.[45] The technique has been a breakthrough in tissue engineering and regenerative medicine, generating supports capable of maintaining cells in appropriate microenvironmental conditions.[46,47] Macro-architectural properties are the main characteristics of 3D bioprinting, which allow cell distribution within the constructs; however, these structures have little control over the microarchitecture of the tissue, and it is difficult to control cell orientation.[48]

So, the main objective of TE is to create a scaffold that mimics the composition and structure of the tissue or organ of interest, is biocompatible, has low immunogenictiy, and is capable of maintaining cells or stimulating host cells to repopulate the scaffold, making it a functional tissue. Synthetic scaffolds have some limitations, such as the difficulty in creating the complex vascular network needed to keep cells viable and in reproducing the mechanical properties of the native organ, and because the synthetic matrix components are discrepant from the natural matrix, these materials become potentially immunogenic.[49−51] Therefore, considering the complexity of the organs, in terms of both structure and composition, an interesting alternative would be to use the organ itself as a scaffold. This can be achieved through decellularization.

2.1. Decellularization. Decellularization is a method that aims to remove all cellular and nuclear material of tissue while maintaining the ECM with preserved composition, biological activity, and mechanical integrity.[52] A tissue is considered decellularized when (i) it possesses double-stranded DNA in a concentration less than 50 ng/mg of dry weight tissue, (ii) it possesses fragments of DNA only less than 200 base pairs, and (iii) in the absence of visible nuclear material in the stains of DAPI and hematoxilin and eosin. The use of nucleases assists in the removal of residual genetic material, which results in complete decellularization, avoiding immune reactions in the host.[53,54]

Decellularization aims to keep the 3D structure and vasculature of the entire organ intact for future applications, including recellularization of the tissue for use in transplants.[55] Vascularized decellularized tissues are a breakthrough in tissue engineering because they present unique characteristics. These tissues are biocompatible and have a high capacity of endothelialization, which ensures better tissue cell repopulation, and the conformational structure of the tissue is

https://doi.org/10.1021/acsomega.2c06216
ACS Omega 2023, 8, 22256−22267

respected. The biomaterial is composed of components of the natural extracellular matrix (collagen, elastin, glycosaminoglycans, and macromolecules) that generate a microenvironment suitable for cellular activity. Collagen and elastin fibers are responsible for providing elasticity to the tissue, ensuring resistance to tissue damage by pulsating blood flow. The use of dECM aims in regenerative medicine to minimize adverse reactions to the recipient, by maintaining the biological, mechanical, and structural properties of the ECM, and the extraction of cells and cellular debris provides a non-immunogenic environment of low cytotoxicity, ensuring the biocompatibility of the graft.[56]

Decellularization can be performed with detergents, enzymes, chelating agents, physical agents, and a combination of physical and chemical agents.[57−60] The substances most commonly used in the decellularization process are ionic detergents since these molecules are efficient in breaking cell membranes and lipids.[61] The main techniques and reagents for obtaining decellularized tissue are shown in Figure 2, and the other ways of obtaining a decellularized extracellular matrix (dECM) are laid out in the review by Zhang et al.[62]

After decellularization, a cell-free scaffold is obtained, with the structure and composition of the native ECM of the organ or tissue of interest. This decellularized scaffold can be used as a whole or, for example, in the form of hydrogels, which lose 3D conformation but maintain the composition of the ECM.[63,64]

Studies have advanced in obtaining decellularized tissues capable of replacing those already damaged,[65] focused on the replacement of muscle tissues of the upper limbs. The use of decellularized tissues that aid in the differentiation of pluripotent stem cells into functional cells is also performed.[66]

It is expected that this cell-free scaffold will generate a lower immune response if applied to patients. In addition, it allows the inclusion of cells compatible with the receptor, contributing to a reduction in the immune response and, consequently, in the rejection of the new tissue. Considering the complexity of the interaction of immune system cells and other cell types used for recellularization of dECM, we will address this in more depth, with more specific examples, in the following topics.

3. INTERACTION OF A DECELLULARIZED EXTRACELLULAR MATRIX AND CELLS

The ECM is a complex structure composed of different types of collagens, glycosaminoglycans, proteoglycans, elastin, fibronectin, and laminin.[67] The ECM, in addition to providing support to cells, is capable of stimulating cell proliferation, migration, differentiation, and other processes, not only due to its composition but also, e.g., due to its three-dimensional structure and rigidity.[68]

The ECM has also fundamental participation in immune events, such as monocyte chemoattraction and polarization.[69] Biochemically, some studies suggest that ECM molecules, for example, type I collagen, seem to influence macrophage activation, differentiation, and secretion of metalloproteinase 9 *in vitro*. Another experiment shows that some molecules like vitronectin, laminin, and matrigel, as well as the structural geometry of macrophages, increase the expression of arginase-1 (Arg1) *in vitro*, a marker of anti-inflammatory macrophages. Thus, ECM composition directly influences macrophage phenotyping.[70,71] Similarly, molecules such as heparan-sulfate are responsible for binding to cytokines, e.g. IL-2, allowing them to induce an innate response.[72] In addition, Gvaramia et al.[73] showed that the dECM can bind IL-4 *in vitro*, stimulating the polarization of human monocytes to an anti-inflammatory profile and consequently decreasing the inflammatory response. Besides interacting with molecules in the intercellular space, some products of the ECM that show partial proteolysis, the matrikines, can also regulate the cellular behavior.[74] The following subsections will show examples of how immune system cells, more specifically macrophages, and stem cells interact with the dECM.

3.1. Macrophage Profiles and Transplant Rejection.

Macrophage polarization is one key event in the rejection of transplanted organs. Macrophage polarization refers to changes in gene expression, surface markers, and factors secreted by these cells upon stimulation by different cytokines and microenvironment-related factors. The polarization of monocytes recruited from the circulation results in different profiles of macrophages in the tissues, with a greater pro-inflammatory or pro-resolving tendency.[75]

It is common to consider a binary distinction between populations of these cells: the M1, also referred to as classically activated macrophages, acting to promote inflammatory processes, and the M2, also called alternatively activated, with an anti-inflammatory or pro-resolving character.[75] Commonly, these different macrophage phenotypes are classified based on the molecules involved in polarization induction, the classically polarized ones being obtainable *in vitro* through stimulation with lipopolysaccharide (LPS) and interferon γ (IFNγ), a situation that seeks to mimic a microenvironment related to Th1 lymphocytes *in vivo*. The alternatively polarized ones are related to stimulation by IL-4, referring to a microenvironment with a greater influence on Th2 lymphocytes.[75,76] Regarding the action of these cells, pro-inflammatory ones demonstrate greater activation of signaling pathways involving STAT1 and NF-kB and the production of molecules such as induced nitric oxide synthase (iNOS). Anti-inflammatory macrophages, on the other hand, would be more active in the context of inflammation resolution, tissue repair, and fibrosis involving PPAR receptor pathways and the STAT6 factor and higher expression of arginase and IL-10, for example.[75]

However, this dichotomy does not seem to faithfully reflect macrophage diversity and plasticity. First, the so-called classically and alternatively activated macrophages *in vitro* do not seem to correspond exactly to the cells analyzed *in vivo*.[76] Moreover, different subtypes of macrophages related to the M2 phenotype have already been described, such as M2a, M2b, and M2c,[77] as well as another macrophage polarization phenotype, M4.[78] Because of that, the idea of a spectrum of macrophage activation has been gaining strength, in which solely the subtype M1 and M2 phenotypes would be simplifications.[75,79] Despite that, the simplistic division between pro- and anti-inflammatory macrophages is still widely used in the literature and will be adopted in the following sections.

Considering the transplant environment and the mechanisms surrounding transplant tolerance, there are several molecules that can polarize macrophages. In early graft tolerance, a lack of IL-33 could be responsible for increased iNOS$^+$ macrophages and early graft rejection.[80] Considering long-term rejection, analysis of kidney biopsies described that anti-inflammatory macrophage infiltration correlates with a progressively reduced glomerular filtration rate.[81] Furthermore,

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ACS Omega 2023, 8, 22256−22267

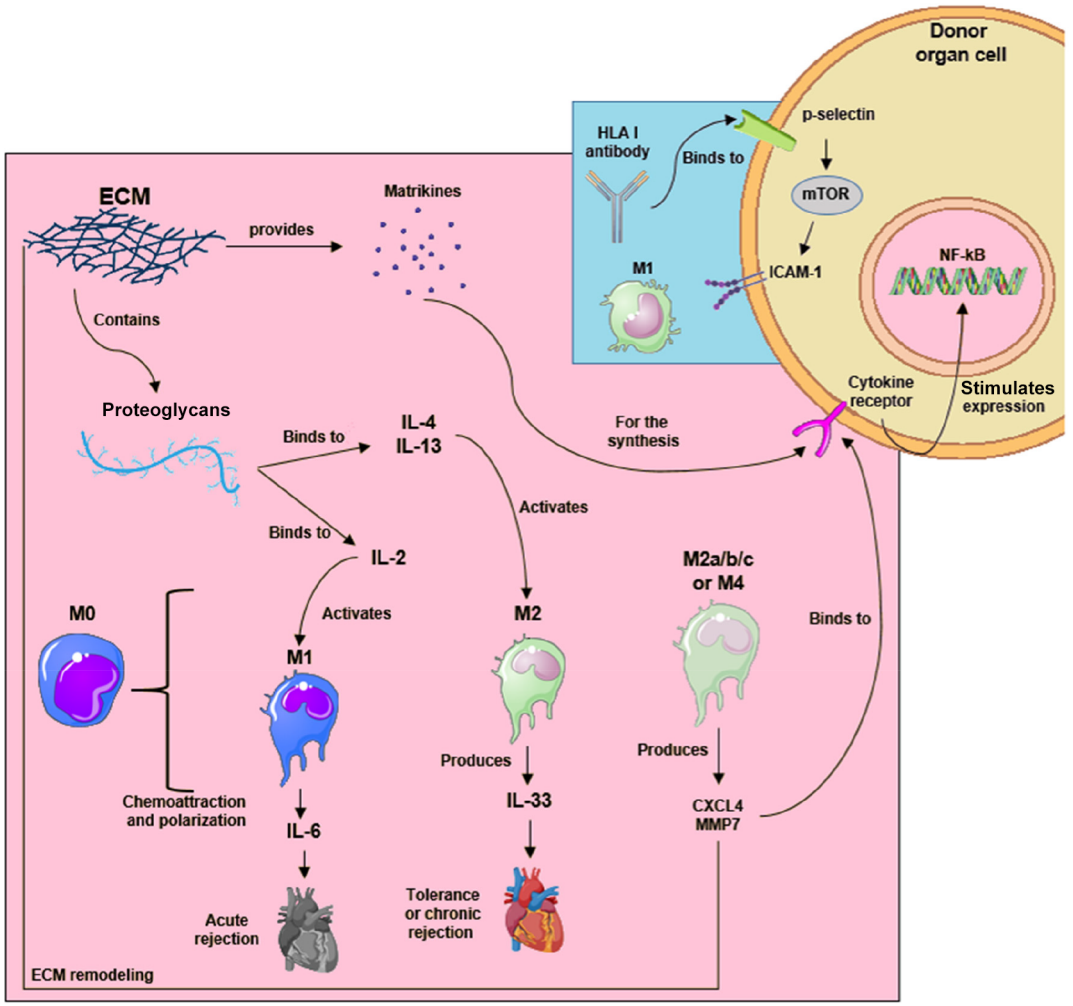

Figure 3. Innate and adaptive responses to transplantation. ECM participation on macrophage activation in transplantation outcomes. Recognition of HLA antibodies in adaptive transplant rejection. The images were obtained from Servier Medical Art (http://smart.servier.com/), Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

a cytokine environment related to pro-inflammatory macrophages is related to the context of acute graft rejection, while anti-inflammatory macrophages appear to play an important role in the process of fibrosis and chronic rejection.[82] These and other results point to macrophages, their polarization possibilities, and interactions with the ECM as key elements in the context of transplant tolerance and rejection (Figure 3), requiring further detailed studies to optimize the techniques of this procedure.

It was described that the use of porcine brain-derived dECM, in a murine spinal cord injury model, could modify macrophage polarization, which was commonly pro-inflammatory to macrophages toward an anti-inflammatory macrophage profile.[83] Similarly, using a tooth-tissue-derived dECM combined with a drug, rosiglitazone, to improve teeth root regeneration demonstrated a downregulation in the expression of the $NOS2^{+}$ pro-inflammatory macrophage by activating the PPAR-γ-NF-κB pathway.[84] On the other hand, Chakraborty et al.[85] demonstrated that decellularized goat corneas implanted in rabbits were able to evoke an immune response with a predominance of M1 macrophages due to exposure of epitopes of ECM structural molecules during the decellularization process. The main epitope pointed out as the cause of immune response was the α-1,3-galactosyltransferase (α-gal). This epitope is also observed in porcine decellularized tissues and is of great importance since humans may develop antibodies against it.[86]

The use of cross-linking of the dECM with chondroitin sulfate—a substance naturally found in cartilage—was able to reverse the inflammatory response caused by the dECM.[85] All these findings point out that, despite being promising, the xenotransplantation of the dECM can lead to different immune responses and that the dECM association with drugs and other natural substances could attenuate a possible inflammatory response caused by the dECM and therefore promote an effective dECM treatment.

Given emphasis on the use of dECMs as scaffolds in the context of organ bioengineering, Petrosyan et al.[87] showed that the renal dECM from healthy mice as well as from animals with Alport syndrome—in which there is progressive renal fibrosis—stimulates macrophage polarization to an anti-inflammatory macrophage in *in vitro* experiments, whereas a synthetic ECM tends to stimulate the polarization of these cells to a pro-inflammatory macrophage profile.[87]

Not only does the dECM contribute to an anti-inflammatory environment but also an anti-inflammatory environment facilitates the formation of the ECM. In this regard, Witherel et al.[88] demonstrated that in *in vitro* experiments a "mixed"

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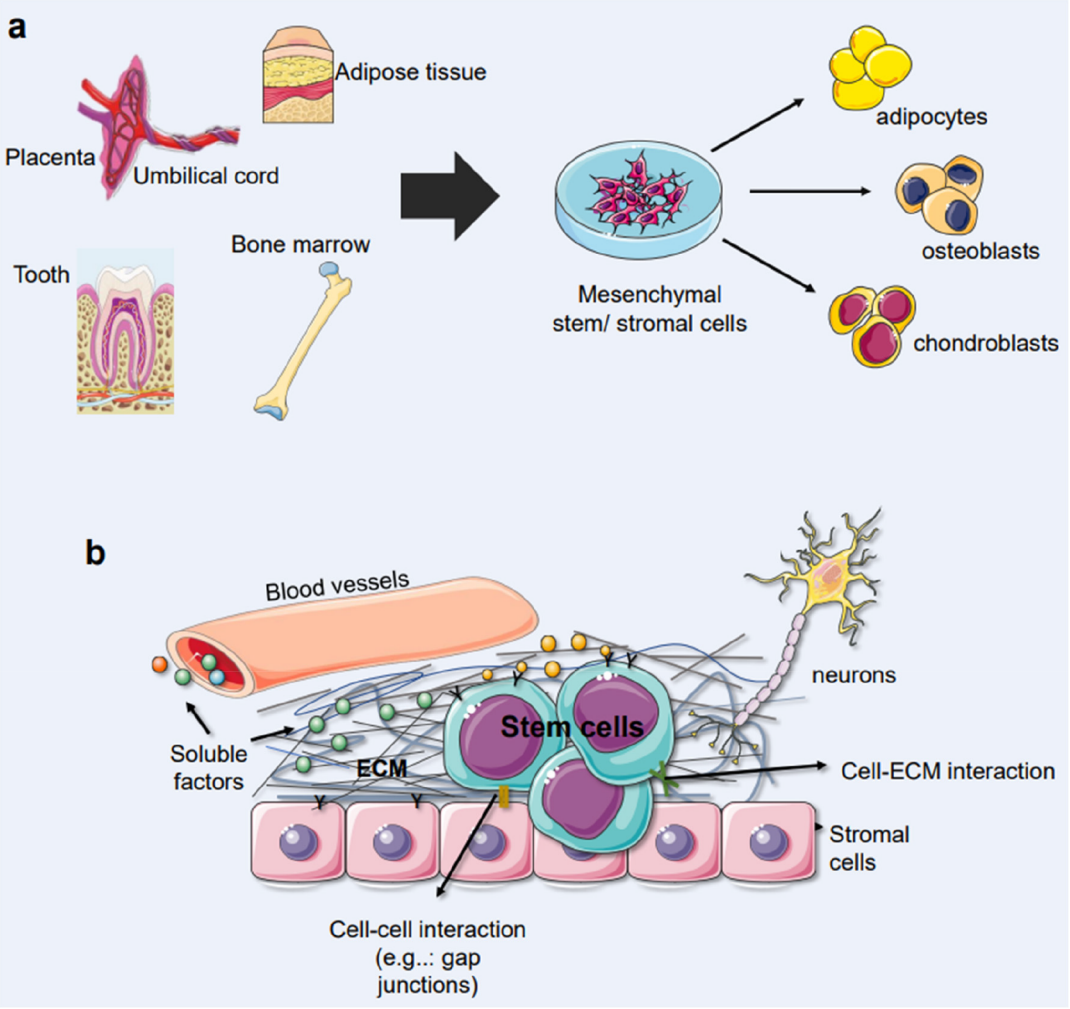

Figure 4. Mesenchymal stem/stromal cells and their niche. (a) Mesenchymal stem/stromal cells can be isolated from different tissues and have the potential to differentiate into adipocytes, osteoblasts, and chondroblasts. (b) Stem cell niche composition. The images were obtained from Servier Medical Art (http://smart.servier.com/), Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

pro-inflammatory/anti-inflammatory macrophage phenotype, originating from stimulation with factors related to both phenotypes, was related to the deposition of an ECM with lower fiber alignment when compared to the "pure" M2 phenotype, suggesting a lower pro-fibrotic character. Furthermore, in a murine model, subcutaneous implantation of hydrogels containing IL-4 and IL-13 stimulated macrophage polarization toward M2 compared to hydrogels without cytokines, which elicited predominantly M1 response. The presence of M2 was associated with greater production of glycosaminoglycan-rich ECM and less fiber alignment, consistent with a less fibrotic ECM.[88]

In another point of view, we could highlight how macrophages influence the ECM. Thus, Sapudom et al.[89] suggest that the most commonly activated anti-inflammatory M2a macrophages[90] act on the differentiation of fibroblasts involving the TGF-β1 pathway and promoting a period with increased cell migration and remodeling of the ECM, while anti-inflammatory M2c macrophages stimulate the process of dedifferentiation of myofibroblasts into fibroblasts, a process related to the termination of the resolution process. The results also imply that the presence of a predominantly M2a environment for a long period may be related to the fibrotic process and that IL-10 is related to the defining stage of the resolution process.[89] The anti-inflammatory macrophage (M2a) undergoes activation in the presence of IL-4/IL-13. In particular, IL-4 contributes to the increased expression of CD36, responsible for binding to oxidized low density lipoproteins, and the increased presence of these receptor proteins is involved in the elimination of debris, helping to end the inflammatory response. The anti-inflammatory macrophages (M2c) are activated by glucocorticoids, IL-10, and TGF-β. Glucocorticoids are involved in macrophage down adhesion, dissemination, phagocytosis, and apoptosis. IL-10 is a Th1 cell inhibitor, which binds to the IL-10 receptor; when the receptor is autophosphorylated, there is activation of the transcription factor STAT3, responsible for regulating the expression of pro-inflammatory cytokines.[90]

3.2. Stem Cell–dECM Interaction. Tissue engineering could involve, in addition to different types of biomaterials, the association with cell types that may further favor the regeneration of injured tissue. One of the most researched cell types in this area is the stem cell. Stem cells were first described in 1961, by Drs. James A. Till and Ernest A. McCulloch at the University of Toronto in Canada, based on the study and observation that rare cells present in the bone marrow of mice were capable of differentiating into other cell

https://doi.org/10.1021/acsomega.2c06216
ACS Omega 2023, 8, 22256−22267

types.[91] Stem cells are an unspecialized cell type with the ability to self-renew and differentiate into various cell types.[92]

Regarding the use of stem cells in clinical studies, the most used cell types so far are mesenchymal stem/stromal cells (MSCs). These cells can be isolated from different adult tissues (allowing autologous transplantation) such as bone marrow, adipose tissue, umbilical cord cells, menstrual blood, and others;[93,94] that is, they can be virtually present in all tissues of the adult organism (Figure 4a). These cells could self-renew and can generate cells of mesodermal origin, such as adipocytes, osteocytes, and chondrocytes. In normal conditions, the MSC and other adult stem cells present in tissues are present in specific niches.[95] They help maintain tissue homeostasis and regenerate small lesions[94] (Figure 4b).

MSCs have already been used to treat different types of diseases and injuries, including infarct, spinal cord injury, diabetes, and others, demonstrating beneficial effects.[96,41] For example, mice that received an injection of MSCs showed preservation of some cardiac functions, due to a reduction of scar stiffness, attenuation of postinfarction remodeling, and improved cardiac muscle compliance, when compared to animals that received a vehicle.[97] However, over the years of research, it was observed that the potential of MSCs went beyond differentiating into tissue cells and that their effects were caused much more by the paracrine factors secreted by them. These factors can reduce apoptosis, induce proliferation, promote angiogenesis,[98−100] and regulate the immune response, e.g., suppressing the activity of B lymphocytes, natural killer (NK) cells, and dendritic cells.[101−103]

Currently, about 650 clinical trials are involved in the use of the regenerative potential of MSCs, all phase 2 for diseases such as osteoarthritis, neuropathies, lung disorders, spinal cord injury, heart disease, Crohn's disease, and diabetes mellitus.[104] In a study related to osteogenesis imperfecta, infants underwent transplantation of stem cells extracted from bone marrow; these cells acted to increase bone mineral density; and a reduction in the number of bone fractures was observed.[105]

Another type of stem cell that has potential use in tissue engineering is pluripotent stem cells (PSCs): embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). These cells have a greater capacity for self-renewal than MSCs, as they can differentiate into all cell types of the adult organism. Currently, different protocols allow the differentiation of these cells into cardiac cells,[106,107] hepatocytes,[108,109] neurons,[110,111] and retinal epithelial cells,[112] etc. Considering the ethical issues and rejection problems of ESCs, human iPSCs have gained prominence since they are obtained from the reprogramming of adult cells, allowing autologous transplantation and reducing ethical issues. All these associated characteristics reveal the potential use of stem cells in regenerative medicine and tissue engineering.

It is known that the ECM can influence the behavior of stem cells, and this relationship is being extensively studied.[113,114,95,115] So, specific combinations of different ECMs could lead to specific cellular behaviors. In this context, the use of decellularized matrices of specific organs and tissues may contain the set of factors and molecules of ECM that favor the achievement of specific phenotypes. As it is a field of great interest, the dECM has been used for the treatment of several diseases and as a way to improve the differentiation or phenotype of cells *in vitro*. The relationship between various cell types, including stem cells, with different decellularized matrices from different organs and by different methodologies was recently reviewed by Cramer and Badylak,[116] Agmon and Christman,[117] and Hillebrandt et al.[118] Here we will show some recent examples of the interaction of different dECMs with stem cells, attempts at dECM recellularization, and some therapeutic possibilities.

Regarding cardiac regeneration, many studies have developed association strategies between dECsM and different stem cell types.[119] Cardiac-tissue-derived dECM has already been used in 3D cultures to increase the maturation of cardiomyocytes generated from iPSC,[120] to generate bioinks for 3D printing of patches associated with cardiac progenitor stem cells and MSCs, and to improve regeneration after cardiac injury.[121] Cardiac-tissue-derived dECM was also associated with MSC for muscle regeneration in cases of volumetric muscle loss,[122] by promoting the differentiation of iPSC cells and cardiopoietic human amniotic fluid cells[123] among others. A relevant point is choosing the best ECM region to be decellularized, if not the whole organ, as the ECM derived from different regions of the heart influenced, e.g., adipose-derived MSCs and human pulmonary microvascular endothelial cells in specific ways.[124] Furthermore, there have been several attempts to recellularize the whole organ, with undifferentiated PSCs, partially spontaneously committed PSCs, and PSCs differentiated to cardiac progenitors or cardiomyocytes.[125−130]

One critical point in recellularization is the vascularization of the organ. Ciampi et al.[131] showed that human iPSC-derived endothelial cells were efficient in recellularizing the vessels of decellularized rat kidney scaffolds, being able to cover more than 80% of the vasculature (glomerular and peritubular capillaries and small vessels).[131]

Recently, the formation of iPSC-derived islet organoids coated with a decellularized rat pancreatic ECM was demonstrated. The results showed that the organoids were able to respond to insulin and glucagon, in addition to being composed of endocrine cell types, including α, β, δ, and pancreatic polypeptide cells.[132] Complementary analyses showed that one of the main ECM components related to the effects was type 4 collagen.[132] It was also recently demonstrated that the decellularized retina of mice and pigs repopulated with hiPSC-derived retinal pigment epithelial cells or ocular progenitor cells was able to guide these cells to a specific organization, indicating the specificity of the ECM.[133]

Another possible use of the dECM is in liver regeneration. Some studies have shown that pretreatment of dECMs with a conditioned medium derived from a liver cell line (HepG2) contributes to better recellularization with iPSC-derived MSCs, liver cells (HepG2), and endothelial cells.[134] Furthermore, it is not necessarily only hepatic ECMs that can have beneficial effects on hepatocyte culture. Kehtari et al.[135] showed that decellularized Wharton's jelly was able to promote hiPSC differentiation to hepatocytes.[135] A new strategy aimed at tissue engineering application for liver regeneration was to generate a bioink from the liver ECM associated with hiPSC-derived hepatocytes. These cells remained viable, in addition to presenting greater area and better functionality.[136]

Applications of the dECM have also been described for neural and adipose tissue engineering. A recent strategy used for spinal cord injury regeneration was using the decellularized optic nerve loaded with a neurotrophin-3-overexpressing oligodendrocyte precursor cell, mimicking the white matter-like tissue and which, when applied to a white matter defect

https://doi.org/10.1021/acsomega.2c06216
ACS Omega 2023, 8, 22256−22267

model, showed improvement in the animal's condition.[137] For adipose tissue engineering, for example, an injectable hydrogel-associating muscle-adhesive protein with poly(*N*-isopropyl acrylamide) and decellularized adipose tissue powder was described. This hydrogel with rat-adipose-derived stem cells was able to induce adipogenic differentiation.[138]

Other approaches involved the use of ECMs derived from cell cultures rather than specific tissues or organs. For example, in 2014, it was reported that dECM obtained from cultures of MSC, MSC-derived osteoblasts, and two types of smooth muscle cells had different effects on uninduced MSCs. While the first maintained the stemness and improved proliferation and motility, the others promoted the commitment to osteoblast and different smooth muscle cells, respectively.[139] More recently, another work used the dECM from MSC and showed that it was nonimmunogenic and able to improve the proliferation and trilineage differentiation (adipocytes, osteoblasts, and chondrocytes) of MSCs.[113]

Briefly, we note that the use of the dECM helps to mimic the microenvironment of the tissue of interest, contributing to the differentiation and maturation of stem cells *in vitro*, which can be used not only for future therapies but also in drug testing assays, for example. In addition, the studies showed that scaffolds, at least those of smaller proportions or in hydrogel form, can be recellularized with different stem cells and, when implanted, assist in the regeneration of damaged tissue. In other words, the examples showed here demonstrate the potential of dECM, not only for *in vivo* applications, alone or associated with cells, but for also *in vitro* assays.

4. CONCLUDING REMARKS

The present review made explicit the search of tissue engineering for new biomaterials for partial or complete replacement of "diseased" tissues, with the intention of decreasing the high demand for organs for transplantation. Besides the search for organs, keeping them healthy is another concern because in most synthetic tissues biomaterials are responsible for causing immunogenicity, characterized by the imbalance between pro- and anti-inflammatory immune cells, requiring the use of antirejection drugs to prevent tissue loss.

In the current landscape, tissue engineering is looking for a biomaterial that can work on both fronts, and one option may be the use of decellularized tissues. These biomaterials are pointed out by studies as tissues that do not generate immunogenicity because their composition is practically identical among human beings, besides using the advantages of the extracellular matrix (recruitment and fixation of cells). Another important aspect is the use of stem cells from the patient himself, as mesenchymal stem cells, which end up in the tissue being differentiated by differentiation factors secreted by the matrix, besides the secretion of factors with immunomodulatory properties (reduction in immunogenicity) and stimulation of local cells to differentiate themselves. The use of more advanced technologies, such as the 3D printer, is being used to obtain synthetic structures and organs. Studies indicate that the use of extracellular matrix and human cells for the synthesis of bioprinting have resulted in the printing of entire organs.

Currently the commercialization of decellularized tissues is already a reality;[51] however, even with the numerous advances and benefits that have been exposed in relation to the use of a decellularized extracellular matrix, there are still many challenges in whole organ bioengineering. For this technology to become viable in clinical transplant practice, some conditions are necessary, such as standardization of decellularization protocols for different organs and large-scale production of different cell types since the number of cells for recellularization of more complex organs can be high, in addition to being necessary to ensure that the entire tissue is completely recellularized, with all the cell types required for the correct functioning of the organ.[49,50]

Similarly, ethical aspects should be taken into consideration since the biomaterials are mostly obtained by financing from large private biotechnology companies, which can make the practice controversial since the donated organs would generate for-profit products.[140] Finally, it is also necessary to define the best model for evaluating the interaction between the recipient and the transplanted organ, ensuring patient safety.[50]

AUTHOR INFORMATION

Corresponding Author

Tárcio Teodoro Braga − *Department of Pathology, Federal University of Parana, Curitiba, Parana 80060-000, Brazil; Graduate Program in Biosciences and Biotechnology, Institute Carlos Chagas, Fiocruz, Parana 81310-020, Brazil;* orcid.org/0000-0002-2989-1076; Email: tarcio.braga@ufpr.br

Authors

André Guilherme Portela de Paula − *Department of Pathology, Federal University of Parana, Curitiba, Parana 80060-000, Brazil*

Jordana Dinorá de Lima − *Department of Pathology, Federal University of Parana, Curitiba, Parana 80060-000, Brazil*

Thais Sibioni Berti Bastos − *Department of Pathology, Federal University of Parana, Curitiba, Parana 80060-000, Brazil*

Andressa Pacheco Czaikovski − *Department of Pathology, Federal University of Parana, Curitiba, Parana 80060-000, Brazil*

Rebeca Bosso dos Santos Luz − *Department of Pathology, Federal University of Parana, Curitiba, Parana 80060-000, Brazil*

Bruna Sadae Yuasa − *Department of Pathology, Federal University of Parana, Curitiba, Parana 80060-000, Brazil*

Caio Cesar Souza Smanioto − *Department of Pathology, Federal University of Parana, Curitiba, Parana 80060-000, Brazil*

Anny Waloski Robert − *Stem Cells Basic Biology Laboratory, Carlos Chagas Institute − FIOCRUZ/PR, Curitiba, Parana 81350-010, Brazil*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c06216

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Capes for the support.

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