Book Chapter

Decellularized Dermal Matrices: Unleashing the Potential in Tissue Engineering and Regenerative Medicine

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Published **March 16, 2024**

This Book Chapter is a republication of an article published by Viviana P. Ribeiro, et al. at Frontiers in Materials in January 2024. (Rosadas M, Silva IV, Costa JB, Ribeiro VP and Oliveira AL (2024) Decellularized dermal matrices: unleashing the potential in tissue engineering and regenerative medicine. Front. Mater. 10:1285948. doi: 10.3389/fmats.2023.1285948)

How to cite this book chapter: Marta Rosadas, Inês V. Silva, João B. Costa, Viviana P. Ribeiro, Ana L. Oliveira. Decellularized Dermal Matrices: Unleashing the Potential in Tissue Engineering and Regenerative Medicine. In: Prime Archives in Material Science: 5th Edition. Hyderabad, India: Vide Leaf. 2024.

Author Contributions: MR: Writing–original draft, Writing–review and editing. IS: Writing–original draft, Writing–review and editing. JC: Writing–original draft, Writing–review and editing. VR: Supervision, Writing–original draft, Writing–review and editing. AO: Supervision, Writing–original draft, Writing–review and editing.

Funding: The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article. National Funds from Fundação para a Ciência e a Tecnologia (FCT), through the project UID/Multi/50016/2020.

Acknowledgments: Authors acknowledge for the Junior Researcher contract attributed to VR by the BE@T–Bioeconomy for Textiles and Apparel, investment TC-C12-i01—Sustainable Bioeconomy, funded through the Recovery and Resilience Program (PRR). Authors are also grateful for the distinction attributed by FCT to JC with an individual Junior Researcher contract (2022.02781.CEECIND), under the Individual Scientific Employment Stimulus, and to IS for the attribution of a Doctoral Research Grant (2021.05919.BD).

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abstract

Decellularized dermal matrices (dDMs) have emerged as effective biomaterials that can revolutionize regenerative medicine, particularly in the field of wound healing and tissue regeneration. Derived from animal or human skin, dDMs offer great biocompatibility, remarkable biochemistry, and a macromolecular architecture equivalent to the native tissue.

Notably, among the biomimetic extracellular matrix (ECM)-based scaffolds, dDMs stand out due to their inherent dermal microenvironment, holding high value for skin regeneration and reconstructive surgery. The integration of dDMs as a biomaterial base for bioinks in advanced manufacturing technologies opens promising avenues for crafting precise, biomimetic tissue engineering (TE) constructs with optimized recellularization properties. This mini review outlines the main sources, differential decellularization techniques applied to dDMs, and their significance intissue engineering and regenerative medicine. It subsequently delves into the different categories of decellularized materials obtained, their unique physical and biochemical attributes, as well as their applications to promote wound healing and regenerating skin and soft tissues. Additionally, the currently available market products based on dDMs are examined and the main outcomes are compared. Finally, the article highlights current barriers in the field and anticipates the future challenges and applications of dDMs-based therapies.

Keywords

Decellularization; Dermal Matrix; Biomaterials; Biological Scaffolds; Tissue Engineering; Regenerative Medicine

Introduction

The field of tissue engineering and regenerative medicine has progressively advanced over the years, as researchers strive to restore, repair, and replace damaged tissues and organs that go beyond the body's natural self-healing capability. The use of stem cells therapies and/or scaffolding materials to recreate the tissue extracellular matrix (ECM) opens up promising possibilities [1]. Among them, decellularized extracellular matrix (dECM)-based biomaterials have shown increased potential due to their origin in native tissues, which inherently contain numerous structural proteins, glycoproteins, glycosaminoglycans, and cytokines, while maintaining biophysical and topographical cues that can direct cell fate and stimulate its metabolic activity [2,3]. dECMs consist of tissues, from allogeneic or xenogeneic origin, from which the cellular

content has been removed [4,5]. The removal of cells and cellular debris, results in the absence of an immune response after implantation [6,7]. Thus, they present great biocompatibility and biofunctionality which can boost cell adhesion, proliferation, and differentiation for successful tissue repair and regeneration [8]. Considering the significant impact of the ECM on cell behavior, the unique composition of decellularized matrices/scaffolds makes them advantageous when compared to other polymeric-based biomaterials in tissue engineering [1]. The properties of biomimetic dECM-based biomaterials, including bioactivity and preservation of native architecture, result in tissue specificity, which allows the creation of biomaterials that closely resemble a target tissue. This level of cell recognition for replicating specific tissues is challenging to attain with polymeric biomaterials. Consequently, dECM biomaterials have demonstrated effectiveness, particularly when it comes to recellularizing scaffolds [9-11].

While various decellularized scaffolds have been developed for creating artificial tissues and organs, such as heart, lung, kidney, and skin [12], this article specifically focuses on the progress made towards the development of decellularized dermal matrices (dDMs) and their applications in regenerative medicine. DDMs are primarily composed of dermal-specific ECM, which is mainly comprised of collagen type I and III, elastin, fibronectin, and laminin [13]. The dermis is particularly interesting for skin transplantation, as it exhibits skin tissue-specific properties including high physical strength, flexibility and an extensive vasculature avoiding scar tissue formation, tissue granulation and vascular contraction. Moreover, it is very accessible and abundant, being obtained as a by-product from the agro-food industry perfectly aligning with the sustainability principles [14]. Thus, the dDMs stand out among the other types of decellularized tissues due to their availability, versatility, structural integrity, and easy handling, being suitable for a wide range of applications in regenerative medicine.

It is possible to divide the dDMs into two groups based on their source: human or animal [2,15]. While human tissue would be optimal in the interest of avoiding animal diseases spreading and immunogenicity, its availability is very limited [16,17]. Human

tissues and organs can be obtained from cadavers or from surgery wastes. The use of xenogeneic tissues has become a widespread practice in current tissue engineering applications, with a large variety of tissues being used [17]. The most common animal sources are bovine and porcine. Although they compensate for the lack of human tissue, they can carry the risk of diseases, such as bovine spongiform encephalopathy. Furthermore, there are also limitations due to religious beliefs. Nevertheless, dDMs are considered a versatile biomaterial with potential to be explored in its native state, i.e., maintaining the integrity and architecture to be directly implanted in the human body [18]. They enclose the possibility of preserving the channels where blood vessels used to be, now available to host new endothelial cells and potentially generate a new vasculature network inside the intact dermal tissue [2]. Simultaneously, an intact decellularized dermis provides a scaffold by itself and can be applied directly in surgical procedures. This type of product is used in soft tissue reconstruction surgery, such as reconstructive breast and gynecological procedures and hernia repair, or in wound healing, namely, burn wounds, where it is particularly beneficial due to the lack of compatible donors with abundant and healthy tissue [2,3]. Alternatively, the dDMs have been proposed as powder-like ECMs processed into three-dimensional (3D) scaffolds for tissue engineering strategies [17]. These scaffolds can be used alone [19-21], or as hybrid matrices by the combination with synthetic/natural materials aiming to upgrade the mechanical properties, add bioactive components or manipulate the stability of regenerative implants [22-24]. Moreover, recent advancements in the development of dDMs-based living tissue substitutes include recellularization strategies with patient-derived cells, which represented important progresses in clinical practice [25,26].

Given its wide range of clinical applications, regenerative potential, biomimicry, availability, cost-effectiveness and recellularization potential, dDMs are an extremely compeling biomaterial. The increasing literature about dDMs, highlights the necessity to synthesize the knowledge acquired, as well as identify research gaps. For instance, there are no studies on the correlation between the dermal matrix' sources and its influence on the different tissue engineering and regenerative medicine

applications. Thus, this article reviews the recent refining approaches in decellularizing dermal matrices along with the ultimate recellularization strategies for improved clinical practice. The current and future clinical applications of dDMs in regenerative medicine are overviewed, together with the many commercial dDMs products explored in the market.

Sources and Decellularization Methods of dDMs

The dermis, located between the epidermis and the subcutaneous tissue, is obtained from full-thickness and split-thickness sections of skin from a donor source. The dDMs are characterized by its advantageous dermal ECM microarchitecture being obtained from animal or human sources. Since the 90s, dDMs have been proposed for several tissue engineering and regenerative medicine applications, including skin, soft tissues and mucous membranes' repair [2]. Furthermore, the decellularization protocols that allow to obtain cell- and nuclear-free ECMs, are relatively new lab processes but have been improving in the last years in order to preserve as much as possible the tissue to achieve engineered functional constructs [27]. The different sources, decellularization methods and applications are illustrated in Figure 1.

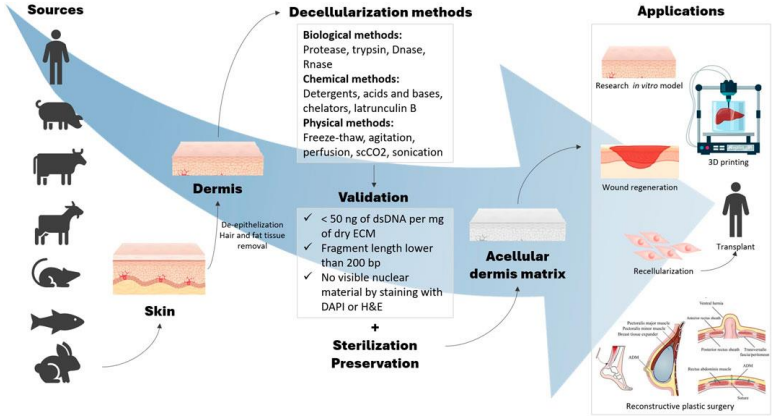

Figure 1: Schematic diagram of the various dermal sources, decellularization methods and ultimate applications of dDMs.

Human and Animal-derived dDMs

In the late 80s the first allogenic composite skin grafts were transplanted in rats as models for treating burn wounds. Positive effects were achieved in terms of inhibiting scar tissue formation reaching a certain level of wound healing [28]. However, the presence of cellular components on the allogenic composite graft caused immune reaction, which triggered the first research studies on decellularized allogenic dermal matrices [29]. The allogenic dDMs (human-derived) are in most cases obtained from human cadavers as they represent an ethically acceptable source for therapeutic application [30,31]. AlloDerm™ stands as a pioneering example of cadaveric dermal allografts in the field of regenerative medicine [32-34]. It revolutionized the approach of tissue grafting by providing a readily available and structurally intact acellular human dermal matrix for several reconstructive and cosmetic procedures [35,36]. The introduction of this product in the market marked significantly the advancements in tissue engineering, offering a versatile solution for wound care and burns [37], breast reconstruction [35], and other medical applications [38]. Dermal tissue obtained from abdominoplasties and mammoplasties are also interesting alternative sources of human skin with promising outcomes in terms of decellularization effectiveness and biocompatibility [39,40]. Nafisi et al. [39], proposed in an experimental study an acellular breast dermal matrix (ABDM) using human breast skin from patients subjected to mammoplasties. The application of this novel ABDM showed promising outcomes for breast reconstruction in a sheep model, providing the total coverage of the tissue and a promising prospective to be used clinically in post-mastectomy breast reconstruction. An alternative approach was proposed by Groth et al. [31], for developing dDMs by using human skin from abdominoplasties. Authors were able to develop different abdominoplasty skin-derived dDMs as novel dressings for wound closure and scar maturation. From the different decellularization protocols tested, all showed promising outcomes in removing the cellular content of the skin to different extents of purity, collagen preservation, and therapeutic properties, which is interesting to better understand the wound healing mechanisms induced after the application of different abdominoplasty skin-derived dDMs. Thus, given the frequency

and relative abundance of resected skin from mammoplasties and abdominoplastic surgeries, these can be considered a viable, safe and sustainable source of dDM for regenerative purposes.

Xenogenic dDMs (animal-derived) are a viable possibility for tissue transplantation being highly available and cost-effective [41]. These are mainly obtained from porcine and bovine sources [42,43], and their differences in terms of collagen fibril architecture and intrinsic mechanical properties can directly influence the application and clinical outcome [44]. The porcine dDMs are usually composed of pure collagen types I and III and elastin, used as a stable matrix that does not need to be artificially crosslinked or subjected to additional chemical treatments [42]. Different researchers have shown that porcine-derived dDMs can positively affect the cell growth of different cell types, e.g., fibroblasts, osteoblasts and endothelial cells, which is crucial for recellularization [45,46]. Thus, porcine-derived dDMs have been proposed for wound dressing [47] and in vascularization strategies [48] for engineering tissues. More recently, the combination of porcine-derived dDMs with bioactive molecules was suggested to increase the antibacterial potential and mechanical properties of these matrices in scaffolding strategies [49]. Bovine-derived dDMs present superior mechanical properties and have motivated their exploitation for certain tissue regeneration strategies, including partial-to full-thickness wound healing [50], diabetic foot ulcers [51], or breast reconstruction [52]. Different authors compared the outcomes in using SurgiMed™ fetal bovine and AlloDerm™ human cadaveric dDMs for implant-based breast reconstruction, showing no significant differences in complication rates after implantation between the two commonly used products [53,54].

Given the successful application of animal-derived dDMs in tissue reconstruction, their use has been expanded to different animal species, including fish [55], goat [56], sheep [57], rabbit [58-60], or mouse [61]. Rabbit-derived dermal matrices hold an architecture and collagen-based ECM composition close to that of human dermis, which may be advantageous for avoiding post-transplantation complications. Moreover, the similarities between the mechanical properties of decellularized rabbit skin

and native human skin can be an asset for tissue engineering strategies involving the use of intact dDMs [58,62]. Our group has been working on the first systematic study to effectively isolate and decellularize rabbit dermis while preserving its ECM composition and architecture for direct application in regenerative medicine strategies [60]. In our provisional patent application (submitted in XX 2023), different decellularization methods were applied to assess their efficacy in removing the cellular content within the rabbit dermal matrices and the physicochemical properties were characterized and compared to that of human skin. Moreover, the responsible management of rabbit skin as an industrial by-product represents an advantageous aspect in terms of sustainability and circular economy. In a different context, mouse fetal-derived dDMs were explored for wound healing applications and also showed structural (i.e., collagen density and orientation) and biomechanical (i.e., stiffness) similarities to the normal human adult dermis [61]. Thus, the enormous potential of dDMs is constantly being explored into different regenerative applications. Nonetheless, further research is needed to more effectively assimilate the unique structural characteristics inherent to each dermal source. This customization is essential to align with the precise requirements of the target tissue and scaffolding methodologies.

Innovative Approaches in Dermal Matrices Decellularization

A decellularization process has as main goal to remove the higher amount of cells and cell debris possible, while retaining the surrounding native protein-based ultrastructure. The resulting matrix has to be capable of being sterilized and used as a functional biomaterial, in close contact with the living tissue. Presently, the established benchmarks for determining an appropriate level of cell removal have been outlined by Crapo and Badylak et al [63]. These guidelines stipulate that the maximum permissible presence of DNA residues should not exceed 50 ng of double strained DNA (dsDNA) per mg of dry ECM weight. Moreover, the fragment length is advised to remain below 200 bp. Lastly, the absence of discernible nuclear material in tissue sections must be validated through staining

with 4′,6-diamidino-2-phenylindole (DAPI) or hematoxylin and eosin (H&E) stain. To obtain a decellularized matrix there are plenty of methods described in the literature [5]. However, increasing efforts have been made to improve the efficacy of the processes and the environmental impact of the classic standard methods, by creating innovative technologies capable of removing the cellular content of the tissues to acceptable immunological levels, and while preserving the 3D structure, architecture and matrix components for further recellularization and/or direct implantation strategies [27]. Thus, according to the final purpose of the decellularized matrices and the characteristics of the tissue being processed, different decellularization methods and processing steps can be selected.

Standard Methods

The decellularization protocols typically include three main stages: i) initial tissue pre-processing and pre-treatments, ii) the core decellularization process itself, and iii) post-processing, including sterilization [64].

The foremost stage of tissue processing typically includes the excision of undesirable tissue layers, such us adipose tissue, and application of pre-treatments to enhance the permeability of the tissue and facilitate the decellularization agents' action. For skin-specific decellularization protocols, pre-treatments like de-epithelization and hair follicle removal are performed. De-epithelization consists in the removal of the epidermis and is a crucial step to isolate the dermis and obtain a dDM [65]. Chemical or biological reagents, alongside with mechanical removal, can be used at this stage, ensuring epidermis removal with minimal dermal damages. Hair removal can be done by shaving and subsequent hair follicle removal during the de-epithelization and decellularization treatments [18,66].

Afterwards, the decellularization steps encompasses cell lysis and removal. The traditional methods can be broadly categorized into three groups: biological, chemical, and physical treatments.

Biological interventions hinge upon enzymes, such as proteases, DNases, and RNases, that break cell adhesive proteins and digest residual genetic material. While these enzymes do not

significantly affect the matrix's collagen content, a prolongated exposure can weaken collagen fibers and eliminate laminin, fibronectin, elastin, and glycosaminoglycans (GAGs) [18,66].

Chemical treatments include tissue immersion in solutions containing acid or alkaline agents, alcohol, chelators, or detergents. Acidic solutions, like peracetic acid, and alkaline solutions, such as ammonium hydroxide, sodium sulfide, and calcium hydroxide, can solubilize cellular components and remove DNA by breaking nucleic acids [8]. However, they might also denature ECM components, particularly GAGs, and reduce tissue strength. Peracetic acid has shown to be more effective in disrupting cells while preserving important biomolecules and growth factors, namely, growth factor-β, which is essential for fibroblast and endothelial cell growth [66]. Alcohols can lyse cells through dehydration, aiding the removal of residual DNA and solubilization of lipids [67], but they can also decrease the levels of structural proteins of the ECM. Chelating agents, such as EDTA, disrupt cell adhesion by sequestering divalent cations needed for cell binding, such as calcium and magnesium [68]. Finally, detergents (ionic, non-ionic, or zwitterionic) affect lipid-lipid and lipid-protein interactions, influencing cell membrane integrity. On the other hand, they can have a significant impact on the content of GAGs, laminin, and fibronectin, as well as affect collagen integrity [69]. Triton X-100, benzalkonium chloride, and polyethylene glycol (TBP) stands out for being less harsh on the ECM components [70].

Physical methods utilize temperature, pressure, or force to lyse the cells, and are often combined with other approaches to optimize agent distribution. Additionally, they can be used to facilitate the removal of cell debris and aid in rinsing off chemical or biological agents. Freeze-thaw cycles are applied to generate intracellular ice crystals capable of enhancing cell lysis and detachment [71]. This method can create a more porous structure in the tissue, promoting the diffusion of the decellularization agents. When increasing the tissue size up to whole organs the use perfusion methods become essential for assuring decellularization. These allow for the entrance of chemical/biological agents on the inner sections of tissues for

cell debris removal, using the vasculature channels, while preserving the tissue architecture. Ultimately, the mechanical agitation method is typically applied in smaller and fragile organ sections submerged in decellularization solutions [72]. Regardless of the applied decellularization method, key factors like pH and temperature, are critical in determining the efficiency of the treatment and the level of ECM damage [27]. Moreover, post-decellularization treatments, including wash cycles, sterilization, and shelf-life are critical for a successful implantation without acute reactions and inflammation. Thus, the success of a standard decellularization is always dependent on the combined efficacy of different physical, chemical and biological methods, while creating the minimum impact on the ECM integrity. Although standard methods have proven to be effective in cell removal, they are not optimal in preserving the ECM components.

Refining Approaches

Newer approaches are emerging to improve tissues' decellularization focusing on gentler, biofriendly, residueless, targeted, and specific methods and protocols [73]. For instance, there is an ongoing exploration for detergent-free methods due to the concerns about their adverse effects on the ECM and difficulty to wash them, despite their great effectivity in removing cells [64]. In this sense, Bera et al. [74], created a detergent-free minimalistic approach for goat dermis decellularization. Their protocol was based on the utilization of hypotonic/hypertonic sodium chloride solutions and was compared with three established methods, trypsin/Triton X-100, trypsin/SDS/Triton X-100, and trypsin/NaOH. The authors' protocol showed to be equally successful in removing the cellular content, although it suffered a decrease in the collagen content. This method was able to better preserve the GAGs as compared the traditionally established methods and supported excellent cell attachment, proliferation, stretching, and migration. Farrokhi et al. [75], also explored a different detergent-free murine dermis decellularization protocol based on the utilization of latrunculin B. This natural compound derived from marine sponges is known for its ability to disrupt actin cytoskeletal of cells and can be used for decellularization

purposes [63,76]. The authors compared their protocol with detergent-based methods and concluded that their method was the only one able to effectively decellularize the tissue while preserving GAGs, elastin content, and maintain better biomechanical properties. Although this reagent can be a good option to substitute detergents, its removal from the tissue must be efficient, due to the inherent toxicity of latrunculin B. For this reason, the author's protocol involves a significant number of washing step, which appeared to be successful based on their good results of cytocompatibility and biocompatibility.

A highly promising technique is the decellularization through supercritical CO_2 ($scCO_2$). This fluid is characterized by high diffusivity, and low density, viscosity and surface tension, leading to a high mass transfer capability and a potent solvent strength [64,77]. Although the precise mechanism of $scCO_2$ decellularization remains uncertain, the most relevant hypothesis is by supercritical extraction with a contribution from the high pressure which is able to induce cell bursting. $ScCO_2$ has affinity for lipids, but in the cell membrane and in nucleic acid there are polar molecules that can be corrupted by the use of co-solvents (e.g., ethanol) enhancing the fluid's solvating power [47,64]. This method has important advantages, i.e., low EMC damages, is non-toxic, environmentally friendly, does not leave residues, odors and is efficient in eliminating chemical residues [47,78]. Wang et al. [77], have already produced a porcine acellular dermal matrix using $scCO_2$ as decellularization method, resulting in a non-toxic and biocompatible material capable of accelerating wound healing in a full-thickness *in vivo* model. Another study conducted by Giang et al. [78], proposed the decellularization of human dermis using $scCO_2$ with ethanol as co-solvent. Even though they did not reach the acellular conditions establish by Crapo and Badylak et al. [63], of less than 50 ng of dsDNA per mg of dry sample, they were able to remove the majority of the cells. The resultant matrix demonstrated to have excellent biomechanical properties, showing similarities to the native skin, and a very promising content of growth factors and anti-inflammatory cytokines [78]. These results confirm the potential of this technology to remove cells without substantially alter the ECM's bioactivity and biofunctionality.

Bioreactors are a useful tool that can enhance the reproducibility, automatization, and scale-up of decellularization protocols. Perfusion and immersion-agitation decellularization bioreactors are the prime contenders, existing a variety of parameters that can be adjusted to optimize the process [27].

Perfusion-based bioreactors have been highly explored in the literature and are mainly used to decellularize whole organs. An example of a successful perfusion bioreactor was constructed by Poornejad et al. [79], for porcine kidney decellularization. This equipment allowed the optimization of the exposure time to harsh detergents, namely, SDS, to maximize its potential for tissue decellularization while minimize its side effects. This method resulted in a 30,5% increase in the preservation of GAGs and 22% increase in the preservation of collagen in comparison to the control method.

Regarding the immersion-agitation bioreactors, these are easier to use, cost-effective, and simpler, allowing to decellularize several samples at once. Nevertheless, they can be more susceptible to shear forces and collisions. Carbonaro et al. [80], presented a novel 3D printed sample holder for agitation-based decellularization of multiple specimens, designed to increase the homogeneity, reproducibility, and efficiency of the decellularization process in such bioreactors. The sample holder loaded with the tissue samples was immersed in the decellularization reagent solution within a beaker and placed on a magnetic stirrer that spinned the whole apparatus. Velocity parameters were able to be controlled and standardized using human skin samples for comparing the procedure with and without the sample holder. This method was able to reduce the protocol time from 36 h to 24 h, obtaining a highly preserved and homogeneous ECM.

Currently, there are already a few decellularization bioreactors being commercialized, namely, Harvard Apparatus: ORCA and the Ebers Tubular Chamber Bioreactor [27]. When considering bioreactors for dermal decellularization, there remains a need for deeper exploration to bridge the gap between laboratory-scale setups and scalable platforms.

Innovative Scaffolding Strategies and Clinical Applications of dDMs

The utilization of dDMs as scaffolding structures holds an enormous potential in the tissue engineering and regenerative medicine field, capitalizing the unique properties of the acellular dermis as a biomaterial for tissue repair and regeneration. Moreover, there is a range of possibilities in its use as an intact matrix or as a powder further processed into a scaffold through different and innovative technologies [31,81]. Standard or more innovative scaffolding strategies can be applied to process dDMs to use it as a biomaterial or for further enhancement of biomechanical and bioactivity performance [47,82]. The current literature has been exploring the clinical utility of dDMs across a broad range of applications [2,43,83], examining their successful integration into medical practices and their potential to revolutionize patient care. Table 1 summarizes some examples of dDMs found in the literature, their dermis source, decellularization methodologies, scaffolding strategies and tissue regeneration applications.

Table 1: List of examples of dDMs developed found in the literature, their source, decellularization method, scaffolding strategy, and application.

dDM source	Decellularization method	Scaffolding strategy	Application	References
Human cadaver	Chemical treatment (Ionic and nonionic detergents, chelators, buffers)	Intact acellular cadaveric dermis (AlloDerm™)	Burn wounds	Ayaz et al. (2021)
Human cadaver	Physical treatment (electric dermatome for epidermis removal); Chemical treatment (ionic compounds); Enzymatic treatment (Trypsin)	Intact acellular dermal matrix	Covering wounds associated with tendons exposure	Melandri et al. (2020)
Human cadaver	Chemical treatment (Hypo and hypertonic solutions, ionic and nonionic detergents)	Intact acellular dermal matrix	Augment tissue regeneration	Milan et al. (2020)
Human cadaver	Supercritical carbon dioxide	Intact acellular dermal matrix	Skin allograft	Giang et al. (2022)
Human breast	Mechanical treatment to remove fat tissue; Chemical treatment (Ionic and nonionic detergents)	Intact acellular dermal graft	Implant-based Breast Reconstruction	Nafisi et al. (2017)
Human abdomen	Chemical treatment (Ionic and nonionic detergents)	Intact acellular dermal matrix	Deep wound treatment	Groth et al. (2021)
Human abdomen	Chemical treatment (Ionic and nonionic detergents)	Intact decellularized dermal matrix	Cardiac repair and regeneration	Belviso et al. (2020)
Human abdomen/Porcine	Chemical treatment (Nonionic detergents, chelators, disinfectants); Enzymatic treatment (Trypsin)	3D printing	*In vitro* Skin disease model—type 2 diabetes	Kim et al. (2021)
Porcine	Supercritical carbon dioxide	$scCO_2$ derived collagen matrix	Diabetic wound healing	Chou et al. (2020)
Porcine	Supercritical carbon dioxide	$scCO_2$ derived collagen matrix	Wound healing and regeneration	Wang et al. (2020a)
Porcine	Chemical treatment (Nonionic detergents); Enzymatic treatment (Trypsin)	3D Printing	Skin substitute/regeneration	Won et al. (2019)
Porcine	Chemical treatment (Nonionic detergents, chelators); Enzymatic treatment (Trypsin, DNase)	3D Printing	Skin substitute and *in vitro* skin model	Kim et al. (2018)
Porcine	Chemical treatment (Nonionic detergents, organic solvents, chelators, disinfecting agents)	3D printing	Soft Tissue Engineering	Lee et al. (2020)
Fetal bovine and calf	Mechanical treatment to remove the epidermis and hypodermis; Chemical treatment (organic solvents, disinfecting agents); Enzymatic treatment (trypsin)	Intact acellular dermal matrices	Full-thickness wound healing	Mansour et al. (2023)
Fish	Chemical treatment (ionic compounds, nonionic detergents, acids and bases, disinfecting agents)	Intact acellular dermis matrix	Wound healing	Li et al. (2021)
Fetal mouse	Enzymatic treatment (Dispase); Chemical treatment (nonionic detergents, organic compounds)	Intact acellular dermal matrices	Scarless wound healing	Han et al. (2016)
Murine	Chemical treatment (Hypo and hypertonic solutions); Enzymatic treatment (Dispase II, Latrunculin B)	Intact acellular dermis matrix	Wound healing	Farrokhi et al. (2018)
Goat	Chemical treatment (Hypo and hypertonic solutions, bases); Enzymatic treatment (Trypsin-EDTA)	3D printing	Tissue engineering and regeneration applications	Bera et al. (2022)

Decellularized Dermis as an Intact Matrix for Tissue Regeneration

The dDMs are characterized by the advantageous ECM microstructure in terms of three-dimensionality, fibrous architecture and mechanical properties. For this reason it has been highly explored as an intact full-matrix applied to repair skin and other soft tissue defects. In fact, its potential has been recognized as a regenerative tissue matrix with different sources of exploitation (human and animal) and decellularization strategies capable of promoting an homogenous cell removal and maintain intact the ECM structural properties [27]. Such capacity is detrimental to guarantee the quality of the dDMs to be directly used as a scaffold in tissue engineering and regenerative medicine strategies [43]. Tissue engineered skin scaffolds are intended to stimulate tissue healing, re-epithelialization and neovascularization. In this regard, the choice of an appropriate scaffold architecture is important. From the different manufacturing techniques used for scaffolds' processing, freeze-drying and chemical cross-linking methods are some of the most used, being the latter capable of providing enhanced mechanical strength to the processed biomaterials [66]. However, freeze-dried scaffolds not always represent the desired tissue architecture, while some of the crosslinked methods can negatively influence clinical results [73]. For instance, Melman el at compared the biocompatibility of five different biologic scaffolds, between them 3 were examples of commercially available crosslinked and non-crosslinked dDMs (AlloDerm, Permacol, and Strattice), after being used in a porcine model of ventral hernia repair [84]. The author's data suggested that crosslinking has a negative influence in cellular infiltration, ECM deposition, scaffold degradation, and neovascularization, while the integrity and strength of the repair site were not significantly impacted by the crosslinking process.

Non-crosslinked materials are in principle more prone to interact with the biological environment and stimulate cells for ECM deposition and neovascularization, which makes them a better choice to be incorporated into the tissue [84]. After decellularization, dermal tissue scaffolds hold unique properties

as they contain all the ECM components of the dermis and retain an intact dermal structure to become integrated into the native tissue and thus increasing biocompatibility for an accelerated regenerative process [85,86]. Several other decellularized dermal full-matrices are commercially available, some derived from human cadavers, e.g., GraftJacket [87] and AlloMax [88], others produced from porcine dermis (e.g., Strattice), or bovine dermis (e.g., MatriDerm) [89], which confirms their potential and clinical utility for several surgical specialties. As example, three distinct human-based acellular dermal matrices were proposed for implant-based breast reconstruction, showing distinct and yet appropriate incorporation into the host tissue and a favorable environment for cell infiltration and collagen deposition within the dECMs [90]. In a different approach, human-derived acellular dermal matrices were conjugated with split thickness skin grafts as an additional thick layer for promoting support and wound healing in I-stage exposed tendons in the foot [91]. The acellular full-matrices provided support and improved the mechanical and functional properties of the split thickness skin grafts for a more efficient coverage of the exposed tendons. In a recent study [92], an acellular fish skin was chemically modified using different rations of Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) in order to improve the mechanical properties, denaturation and degradability of the intact fish dermis as a biological scaffold for tissue engineering applications. Authors showed that it is possible to chemically modify the acellular matrices and still maintain intact their dermal structure/architecture for improved regenerative purposes.

Taken together, these studies demonstrate that the decellularized dermis has inherent advantageous ECM microstructure that can be preserved or minimally affected for specific tissue healing and regenerative approaches. This is demonstrated by the numerous commercially available products with remarkable clinical achievements (Table 2). Nevertheless, additional investigations remain imperative to refine decellularization techniques, aiming to generate acellular dermal matrices with intact and open microstructure suitable for recellularization in different tissue engineering contexts.

Table 2: List of dDM-based products, source, processing methodologies, storage temperature, shelf-life and intended applications.

Product	Source	Decellularization	Sterilization	Storage	Shelf-life	Application
ArthroFLEX® (LifeNet Healths)[1]	Allograft	MatrACELL®	Gamma irradiation (<20 kGy)	RT (in glycerol)	3 years	Soft-tissue repair (rotator cuff and achilles, tendons)
ArthroFLEX® SCR (LifeNet Healths)[2]	Allograft	MatrACELL®	Gamma irradiation (<20 kGy)	RT (in glycerol)	3 years	Superior Capsular Reconstruction
ArthroFLEX® BioWasher (LifeNet Healths)[3]	Allograft	MatrACELL®	Gamma irradiation (<20 kGy)	RT (in glycerol)	3 years	Suture reinforcement in soft tissue repairs (e.g., rotator cuff)
OraCELLs LifeNet Healths)[4]	Allograft	MatrACELL®	Gamma irradiation (<20 kGy)	RT (in glycerol)	n/d	Periodontal defects Ridge preservation Soft tissue correction
AlloMax® (CR Bard/Davol Inc., Cranston, RI, United States)[5]	Allograft	n/d	n/d	n/d	n/d	n/d
AlloPatch® (MTF Biologics, Edison, NJ, United States)[6]	Allograft	n/d	Aseptically produced	RT	3 years	Replace integumental tissue or soft tissue repair
AlloPatch® Pliable, Allograft Dermal Matrix (MTF Biologics, Edison, NJ, United States)[7]	Allograft	n/d	Aseptically produced	RT	3 years	Chronic or acute wound repair
Cryopreserved Split Thickness Skin (MTF Biologics, Edison, NJ, United States)[8]	Allograft	n/d	n/d	n/d	n/d	Acute burn wounds
FlexHD® Pliable PRE (MTF Biologics, Edison, NJ, United States)[9]	Allograft	n/d	n/d	n/d	n/d	Pre-pectoral breast reconstruction
FlexHD® STRUCTURAL, Acellular Hydrated Dermis (MTF Biologics, Edison, NJ, United States)[10]	Allograft	n/d	n/d	n/d	n/d	Complex abdominal wall reconstruction, including Grade III and Grade IV contaminated hernias
SomaGen® Meshed, Allograft Dermal Matrix (MTF Biologics, Edison, NJ, United States)[11]	Allograft	n/d	Aseptically produced	RT	n/d	Wound repair
AlloDerm® (LifeCell Corp., Bridgewater, NJ, United States)[12]	Allograft	n/d	n/d	RT	n/d	Root coverage Gingival augmentation Soft tissue augmentation around implants
Alloskin™ AC Acellular Dermal Matrix (AlloSource, Centennial, CO, United States)[13]	Allograft	n/d	Electron beam irradiation	RT	2 years	Acute and chronic wound healing
AlloSkin™ RT (AlloSource, Centennial, CO, United States)[14]	Allograft	n/d	Electron beam irradiation	RT	2 years	Acute and chronic wound repair

AlloMend™ (AlloSource, Centennial, CO, United States)[15]	Allograft	DermaTrue	Electron beam irradiation	RT	2 years	• Breast reconstruction1 • Pelvic organ prolapse2 • Superior capsular reconstruction3 • Rotator cuff repair4 • Tendon augmentation5 • Fat pad replacement6 • Hernia repair2 • Abdominal wall reconstruction2
AlloMend™ DUO (AlloSource, Centennial, CO, United States)[16]	Allograft	DermaTrue	Electron beam irradiation	RT	n/d	Soft tissue reconstruction
AlloMend™ ULTRA THICK (AlloSource, Centennial, CO, United States)[17]	Allograft	DermaTrue	Electron beam irradiation	RT	2 years	Soft tissue reconstruction
AlloMend™ EXTRA LARGE (AlloSource, Centennial, CO, United States)[18]	Allograft	DermaTrue	Electron beam irradiation	RT	2 years	Soft tissue reconstruction
Coll-e-derm™ (Parametrics Medical, Leander, TX, United States)[19]	Allograft	OPEN DERMAL MATRIX™	Gamma irradiation (<20 kGy	RT	5 years	Wound healing (e.g., rotator cuff repair, lateral ankle stabilization, and hip labral repairs)
DermACELL AWM® (LifeNet Health® , Virginia Beach, VA, United States)[20]	Allograft	MatrACELL®	Gamma irradiation	RT (in glycerol)	1.5–5 years (depend on thickness and size)	Chronic wound healing
Dermapure® (Tissue Regenix Group, San Antonio, TX, United States)[21]	Allograft	dCELL®	n/d	RT	n/d	Replace integumental tissue or soft tissue repair
DermaSpan™ Acellular Dermal Matrix (Biomet Orthopedics, Warsaw, IN, United States)[22]	Allograft	n/d	Sterile (proprietary process)	RT	n/d	Wound regeneration
FlowerDerm™ (Flower Orthopedics, Horsham, PA, United States)[23]	Allograft	n/d	Sterile	RT	2 years	wound regeneration. Diabetic Foot Ulcer, Venous Foot Ulcer)
GammaGraft™ (LifeSciences, Inc., Pittsburgh, PA, United States)[24]	Allograft	n/d	Sterile (Promethean's proprietary technology)	RT	n/d	Burn and chronic wound repair
GraftJacket™ RTM (Wright Medical Group N.V., Memphis, TN, United States)[25]	Allograft	n/d	Electron beam irradiation	RT	2 years	Bone, tendon and ligament repair
hMatrix® ADM (Bacterin International, Inc., Belgrade, MT, United States)[26]	Allograft	n/d	Sterile (proprietary technology)	frozen	5 years	Deep wound repair
SimpliDerm® (Aziyo Biologics, Silver Spring, MD, United States)[27]	Allograft	n/d	Sterile	n/d	n/d	Wound repair
Matrix HD® Allograft (RTI Surgical, Alachua, FL, United States)[28]	Allograft	n/d	Tutoplast™ Tissue Sterilization process	RT	5 years	Soft tissue reinforcement, protection, and covering

SureDerm (Hans Biomed)[29]	Allograft	n/d	Sterile	RT	3 years	• Patch Augmentation • Breast Reconstruction • Rhinoplasty • Achilles, Tendon & Ligament Reconstruction • Soft-tissue Defect • Wound and burn patient treatment
PriMatrix® Dermal Repair Scaffold (Integra LifeSciences Corp., Plainsboro, NJ, United States)[30]	Xenograft (fetal bovine)	n/d	Sterile	RT	5 years	• Partial and full thickness wounds • Pressure, diabetic, and venous ulcers • Second-degree burns • Surgical wounds • Trauma wounds • Tunneled/undermined wounds • Draining wounds
SurgiMend® (Integra Life Sciences, Princeton, NJ, United States)[31]	Xenograft (fetal bovine)	n/d	Ethylene oxide	RT	n/d	• Plastic and reconstructive surgery • Muscle flap reinforcement • Hernia repair including abdominal, inguinal, femoral, diaphragmatic, scrotal, umbilical, and incisional hernia
Fortiva (RTI Surgical, Florida, United States)[32]	Xenograft (porcine)	n/d	Tutoplast® Tissue Sterilization Process (low gamma irradiation)	RT	n/d	• Soft tissue reinforcement • Surgical repair of damaged or ruptured soft tissue membranes
Permacolt (Medtronci, Minneapolis, United States)[33]	Xenograft (porcine)	n/d	n/d	RT	n/d	• Ventral hernia repair • Abdominal wall reconstruction
Stratticet RTM (AbbVie company.)[34]	Xenograft (porcine)	n/d	n/d	n/d	n/d	Soft tissue repair
Endoform™ dermal template (Hollister Wound Care, Libertyville, IL, United States)[35]	Xenograft (ovine)	n/d	Sterile	15°C-40°C (dry area)	n/d	• Partial and full-thickness wounds repair • Pressure, venour and diabetic ulcers repair • Chronic vascular ulcers repair • Tunneled/undermined wounds repair • Surgical wounds repair • Traumatic wounds repair • Draining wounds repair
EZ Derm® (Mölnlycke Healthcare, Norcross, GA, United States)[36]	Xenograft (porcine)	n/d	n/d	RT	n/d	• Partial skin loss injuries repair • Temporary cover

Kerecis™ Omega3 Wound (Kerecis, Arlington, VA, United States)[37]	Xenograft (piscine)	n/d	Sterile	RT	3 years	• Diabetic ulcers • Chronic vascular ulcers • Venous ulcers • Trauma wounds • Acute surgical wounds • Surgical wounds • Imminent failure of split thickness skin graft • Post-Injection Necrosis
XenMatrixt BD (Becton, Dickinson and Company, New Jersey, United States)[38]	Xenograft (porcine)	AquaPure™	E-beam sterilization	n/d	n/d	Soft tissue reinforcement and repair

Decellularized Dermal-based Biomaterials for Tissue Regeneration

The dDMs can be a source of powdered matrix for further processing into specific scaffold architectures for different applications [3]. The advantage is the possibility of integrating dDMs in advanced manufacturing approaches, such as 3D bioprinting, for the development of functional bioinks [93].

3D bioprinting is an advanced technology that entails the fabrication of intricate three-dimensional structures through the sequential deposition of bioinks, facilitating cell viability, tissue integration, and functional restoration for enhanced regenerative therapies [94,95]. The precise arrangement of cells, growth factors, and biomaterials enables the generation of functional tissues possessing specific desired properties. The selection of the bioink is a critical consideration in this process, as it is heavily reliant on the target tissue or organ [93]. Scaffolds can be printed using a combination of dDMs, biomaterials, biomolecules, and cells, providing a versatile and tailored approach for tissue regeneration [94].

Porcine skin has emerged as the primary source for producing dDMs-based bionks intended for application in skin regeneration. The dDMs-based bioinks demonstrate a substantial content of essential components, effectively providing cues which are essential for cellular activities, despite of the harshness of the prior decellularization and drying processes, employed to mitigate immune responses [96]. Moreover, the preservation of

crucial biological cues and the inclusion of pivotal cell adhesion proteins, notably fibronectin, collagen, and laminin, within decellularized dECMs assume an indispensable role in fostering a suitable environment for cell attachment, proliferation, and the facilitation of tissue regeneration [71]. This biological signaling assumes supreme significance when dECMs are conjoined in bioinks with alternative biomaterials lacking these specific biological attributes. The work of Won et al. [19], illustrates well this potential, by developing a 3D bioprinting where human dermal fibroblasts (HDFs) were incorporated into a bioink based on dDMs from porcine skin to produce 3D layer-by-layer constructs. The presence of dDMs in the bioink facilitated the viability of HDFs post-printing and provided an optimal microenvironment that was demonstrated by heightened gene expression related to skin morphology. These findings suggest that the inclusion of dDMs in the bioink enhances the bioprinted constructs' capacity to support HDFs and fosters their functional behavior.

Furthermore, dDMs have demonstrated promising outcomes when combined with other biomaterials. For instance, Jin et al. [23], successfully generated a functional full-thickness skin model to act as a functional skin substitute by integrating dDMs with gelatin methacrylamide (GelMA). GelMA, that possess adjustable mechanical characteristics, was utilized as a structural bioink to enhance the suboptimal printability and mechanical attributes exhibited by dDMs. Employing a 3D bioprinting methodology, the researchers not only facilitated cell viability, proliferation, and epidermis reconstruction *in vitro* but also observed significant wound healing and re-epithelization *in vivo*. The findings from these studies represent a noteworthy advancement in the field of functional skin substitutes, underscoring the potential of dDMs-based bioinks in tissue regeneration.

The integration of 3D bioprinting with dDMs has facilitated the development of pertinent *in vitro* skin models, driven by several factors: 1) the escalating regulatory demands and prohibition of animal experimentation for substance testing; 2) the inherent inaccuracies in predicting human responses due to genetic

disparities between humans and animals; and 3) the growing emphasis on personalized medicine approaches, where therapies are tailored based on an individual's genetics, gender, age, anatomy or other relevant characteristic [97-99]. In response to these challenges, there has been a concerted effort to engineer *in vitro* human skin models, aiming to address these limitations and provide more physiologically relevant platforms for testing and studying skin-related processes and treatments [99]. In a recent study, Kim et al. [81], used dDMs-based bioinks to engineer a diseased human skin equivalent with the aim of replicate the pathophysiological hallmarks associated with type 2 diabetes in an *in vitro* setting. This innovative model successfully captured the cellular and functional abnormalities observed in diabetic skin, presenting a valuable and physiologically relevant platform for investigating disease progression, discerning potential therapeutic targets, and evaluating candidate drugs in a controlled and patient-specific manner. Thus, the utilization of more mimetic *in vitro* models holds a considerable promise for advancing the knowledge of several pathologies and developing targeted interventions for this condition.

Notwithstanding these notable accomplishments, the clinical translation of dDMs-based bioinks from xenogeneic sources requests diligent attention to several concerns and challenges. Of particular significance are the ethical considerations and regulatory challenges arising from the use of xenogenic tissue, which remains a prominent apprehension. The regulatory approval process for xenogeneic tissue-based products is often characterized by heightened complexity and prolonged duration, primarily attributable to meticulous scrutiny necessitated by safety and ethical considerations. Consequently, these exigent evaluations may introduce delays in the developmental timeline and subsequent availability of such products [100]. Furthermore, the composition of xenogeneic tissues may exhibit substantial disparities in relation to human tissues, rendering the precise alignment of xenogeneic matrix properties with those of the intended human tissue a considerable challenge. As an alternative, researchers have explored the implementation of human dDMs-based biomaterials in 3D bioprinting endeavors [20,101]. By incorporating human-derived dDMs, these

approaches seek to circumvent the challenges associated with xenogenic sources and hold promise for overcoming ethical, regulatory and matrix composition obstacles in tissue regeneration applications. Jorgensen et al. [101], demonstrated noteworthy advancements in the field of 3D bioprinting by showcasing enhanced biological, physical, and printability properties of fibrinogen hydrogel through supplementation with human dDMs. Their study highlights the potential of dDMs as a valuable component for optimizing bioink formulations in 3D bioprinting applications, thereby contributing to the development of improved tissue-engineered constructs.

The potential of dDMs as a powder-based biomaterial has been underscored by their capacity to preserve essential components of the ECM while being able to perform as a bioink in 3D bioprinting applications. This characteristic renders dDMs particularly attractive for creating biomimetic structures to the integration of stem cells and bioactive materials, as well as providing suitable biomechanical environment that facilitates tissue regeneration. Taken together, these attributes position dDMs as a compelling candidate for advancing the field of 3D bioprinting and hold great promise for fostering innovative approaches in tissue regeneration. However, it is crucial to emphasize the diminished quantity of scientific research in this field and the importance of having further pre-clinical evidence to solidify the role of dDMs as a promising candidate for regenerative medicine applications.

Lab-to-Clinic Translation and Commercially Available Products

The use of allograft and xenograft skin substitutes has been widely accepted in the clinics due to their preserved dermal architecture, high collagen, and elastin content. There are some examples of commercially available scaffolds from dDMs currently available (Table 2). These products are sold all over the world for use in surgery procedures like breast reconstruction or in the repair of soft tissues such as tendon and gingival tears. However, the majority of their target applications are for acute and chronic wound regeneration. These matrices can be typically acquired from a variety of sources.

The majority of commercially available tissues come from humans (i.e., allografts), while a smaller number come from other animals (i.e., xenografts), primarily porcine and then bovine. The difference in tissue origin can be associated with different clinical outcomes for the same condition. Commercial human dDMs (n = 312) were found to be more effective in healing patients with diabetic foot ulcers, with shorter mean healing times and a higher likelihood of complete healing than when compared to a standard operating care. These findings suggest that human dDMs can improve diabetic foot ulcer outcomes, reduce healthcare burden by accelerating healing, and reduce treatment duration [102]. In complicated ventral hernias, bovine and porcine-based dDMs had somewhat higher recurrence rates than typical synthetic mesh repairs [103]. Another study compared patients who received porcine or bovine dDMs reinforcement and found that the bovine dDM was associated with fewer wound problems and recurrences [104]. This could be related to the acute inflammation associated with the porcine dDM. However, further research is needed to fully understand and evaluate the risks and benefits of using xenografts as a more cost-effective alternative to human dDMs [105].

Piscean sources have also been explored, since it presents a cost-effective alternative with some architectural similarities to human skin [106]. Moreover, it has been reported properties of antiviral, antibacterial [107,108], inflammation regulation [109] and pain management [110,111] associated with the presence of mega-3 polyunsaturated fatty acids combined with the reduced risk of viral and prion transmission. Kerecis™ Omega3 Wound, fish skin dDM, presents preliminary clinical results that indicate improved wound healing for patients previously treated with conventional wound treatment (vacuum therapy) [112].

The processing methods used to create these dDMs can impact their overall quality and performance as skin substitutes. Additionally, the choice of processing methodology may also affect the immunogenicity and biocompatibility of the dDMs, further influencing its clinical outcomes. Therefore,

understanding the differences in processing methodologies is crucial when selecting the most suitable dDMs for specific applications in the medical field. These tissues undergo sterilization processes such as gamma or e-beam irradiation to ensure their safety for use in patients. Additionally, some of these decellularized grafts are produced aseptically to minimize the risk of contamination. Moreover, market-ready dDMs also vary in the decellularization protocol applied; for instance, MatrACELL-processed comprises a patent-protected decellularization method that includes the use of N-Lauroyl sarcosinate, recombinant endonuclease, and antibiotics [113]. These differences can lead to different outcomes in clinical trials. A prospective cohort study evaluated the outcomes of implant-based breast reconstruction using dDMs [114,115]. Study participants received one of four dDMs brands and were compared to the control group. Results revealed that patients who received FlexHD and AlloMax had significantly higher rate of complications in explantation, reoperation, and infections 2 years after surgery when compared to patients who received SurgiMend, AlloDerm, or no dDM. Additional data supports differences in the safety profiles of dDMs brands, even among the dDMs from the same origin, that could be related to processing methodology; however, additional clinical data are required to assess benefits and risks [116-119].

This highlights the importance of conducting further research to gather comprehensive data on the long-term outcomes and potential complications associated with different dDMs brands. By conducting further research and gathering this comprehensive data, healthcare professionals will have the necessary information to make informed decisions. This will also provide valuable data for researchers to further improve or produce new dermal matrices, ensuring the development of safer and more effective products.

Conclusion and Perspectives

The field of tissue engineering and regenerative medicine has witnessed continuous progress as researchers strive to address tissue and organ damage that exceeds the body’s natural healing

capacity. While stem cell therapies present relevant clinical outcomes for the treatment of several challenging diseases and injuries, the rehabilitation and rebuilding of extensive damaged or diseased tissues or organs calls for the adoption of sophisticated tissue engineering approaches and innovative biomaterials. These technologies are designed to effectively mimic the intricate native tissue architecture and provide the necessary mechanical, biochemical, and topographical cues for successful integration and functional restoration. dECM-based biomaterials, derived from allogeneic or xenogeneic tissues, offer this possibility by providing an immunogenically safe extracellular environment. While various decellularized scaffolds have been developed, dDMs are amongst the most used due to its processing easiness and application versatility, being used in surgical procedures for several regenerative applications.
Commercially available options demonstrate the success of native dermal dECM materials, which show high host cells' infiltration being employed in burn wound treatment, soft tissue defects, prosthetic coverage, and even pelvis or abdominal wall reconstruction. Human, porcine, bovine, fish, rabbit, and mouse-derived acellular dermal matrices have all shown promise in wound healing applications, maintaining stable dermal architecture for scarless repair and regeneration requirements. The potential of dDMs extends beyond direct application, as they can be transformed into powdered material for specialized scaffold architectures using advanced manufacturing techniques such as 3D bioprinting. This has allowed for the creation of better replicating skin models, potentially decreasing reliance on animal testing and opening avenues for personalized medicine applications.

Collectively, the herein presented examples underline the advantageous composition and microstructure of decellularized dermis for tissue healing and regeneration. Newer approaches such as detergent-free methods and the use of $scCO_2$ are emerging to improve tissues' decellularization. This also includes the use of bioreactors to enhance the reproducibility, automatization, and scale-up of these decellularization protocols, including whole organ decellularization. Sterilization assurance is also an important aspect to take into consideration mostly

when considering of-the-shelf products based on dDMs and the fact that most of the standard sterilization processes will damage ECM-based products. The use of $scCO_2$ for the simultaneous decellularization and sterilization of biological tissue is expanding this technology to a new world of possibilities in ECM processing. To validate decellularization some metrics have been commonly accepted as basis to verify the absence of cells and cell nuclei, and to assess the DNA reduction. However, no unified and quantitative standard criteria have yet been officially established to evaluate ECM decellularization and post-processing. Therefore, further multidisciplinary investigation is essential to establish the metrics and generate meaningful comparative results that can clearly point out the best processes towards better preserved and safer ECMs. This is mandatory to facilitate the translation of the most promising technologies from lab to the clinics.

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