INTRODUCTION

As result of injuries and diseases, 4.5 million reconstructive surgeries are performed every year in the United States alone with billions associated healthcare expenditure.\(^1\) Chronic wounds, for example, cost US$37 billion to the United States healthcare system every year.\(^2\) Advances in cell-based tissue engineering and regenerative medicine aspire to provide therapeutic treatments for injuries and diseases and to reduce the cost of their treatments. Indeed, numerous studies have shown the potential of cell-based therapies to treat different diseases, including cancer, neurodegenerative disorders, endocrine system-related disorders and cardiovascular diseases and to induce regeneration of soft and hard tissues.\(^3\)-\(^7\) Despite the tremendous progress shown in cell-based therapies, several risks and limitations still need to be addressed,\(^8\),\(^9\) including the loss of most cells after delivery,\(^10\),\(^11\) poor cell engraftment at the site of interest,\(^12\) the need of large amounts of cells and efficient systems to culture them,\(^13\) the inefficacy to bridge large gaps of tissue and

Abstract

Decellularized xenografts are an inherent component of regenerative medicine. Their preserved structure, mechanical integrity and biofunctional composition have well established them in reparative medicine for a diverse range of clinical indications. Nonetheless, their performance is highly influenced by their source (ie species, age, tissue) and processing (ie decellularization, crosslinking, sterilization and preservation), which govern their final characteristics and determine their success or failure for a specific clinical target. In this review, we provide an overview of the different sources and processing methods used in decellularized xenografts fabrication and discuss their effect on the clinical performance of commercially available decellularized xenografts.

KEYWORDS

biomaterials, clinical products, decellularized xenografts, extracellular matrix
the lack of mechanical stability. All these limitations can be potentially addressed by the appropriate selection of a clinical indication specific biomaterial.

An ideal biomaterial for tissue engineering applications should guarantee cytocompatibility, maintain appropriate/desired cellular functions and phenotype for the specific application, induce tissue growth and provide mechanical support until it is absorbed and replaced by natural extracellular matrix (ECM). Synthetic biomaterials can be tailored to obtain desired topographical, mechanical, chemical and morphological properties; however, they do not support cell attachment and bioactivity due to the lack of functional domains/cell recognition sites and often induce foreign-body response and acute inflammation. On the other hand, natural biomaterials present biological compatibility and functionality due to their cell recognition motifs that promote cell adhesion, proliferation and differentiation and advances in chemistry through provision of elegant crosslinking systems offer control over mechanical stability and biodegradation. However, natural biomaterials are of inconsistent composition and high variability as a function of source or batch. Independently on whether the biomaterial is natural or synthetic in origin and despite the significant strides in engineering, currently available scaffold fabrication technologies poorly imitate the in vivo architecture, mechanical properties and compositional complexity of native tissues (Figure 1). Considering that decellularized grafts closely imitate the biophysical, biochemical and biological milieu of the tissue to be replaced, they can overcome all the aforementioned limitations of natural and synthetic scaffolds and ultimately provide functional reparative therapies, as long as issues associated with immune rejection and availability, in the case of allografts, are addressed.

Undeniably, tissue grafts are an inherent part of tissue engineering and regenerative medicine with numerous products being clinically available for a diverse range of clinical indications (Table 1). Herein, we discuss advancements and limitations in xenograft development and how processing steps (eg decellularization, crosslinking, sterilization) affect their properties and differentiate success from failure in their clinical applications.

![Figure 1](image-url) Histology and immunohistochemistry analyses of a collagen-based biomaterial and three xenografts clearly illustrate the superior biofunctionality of the latter, as judged by high levels of compositional and structural biomimicry. Scale bars: 200 µm
2 | PROCESSING OF TISSUE GRAFTS

Each processing step in the developmental cycle of a tissue graft can influence its mechanical, chemical and biological features, determining the success or failure of the implant.²⁵-²⁷ It is therefore an active field of development, as evidenced by numerous registered processing protocols (eg Tutoplast® (RTI Biologics),²⁸ BioCleanse® (RTI Surgical),²⁹ dCELL® Process (Tissue Regenix),³⁰ Tecnoss® (Tecnoss®),³¹-³³ that is also well-regulated (ie FDA provides guidance on medical devices containing materials from animal sources,³¹ any product related on xenotransplantation in humans³³ and specific documentation for registering newly developed materials of animal origin).³³ The general steps necessary to manufacture a tissue graft and the associated quality control checkpoints are sequentially summarized in Figure 2. In this section, we provide an overview of these processing steps.

2.1 | Donor and tissue selection

Porcine and bovine tissues are primarily used in biomedical field, although studies have been carried out using also equine,³⁴,³⁵ ovine,³⁶ caprine,³⁷ kangaroo,³⁸ buffalo³⁹ and ostrich.⁴⁰ The properties of the graft depend not only on the species, but also on the breed, age⁴¹ and tissue section⁴²,⁴³ from where the graft is collected. Among all animal sources, the pig is preferred due to its abundant availability, similar size to human tissues, relative low cost of breeding and extended knowledge of its physiology.⁴⁴-⁴⁸ Bovine tissues, although have shown similarities to human tissues,⁴⁹-⁵¹ in general, their size in adult animals is not appropriate for use in humans and breeding associated expenditure significantly increases the value of goods, creating reimbursement issues. Advancements in molecular biology and genetic edition have allowed for the development of genetically modified animals as source of organs or tissues. Most of these studies are carried out in domestic pigs to prevent the immune rejection of the grafts,⁵² where site-specific nucleases are employed to prevent the presence of the Gal epitope in the donor cells by inactivating the α1,3-galactosyltransferase enzyme.⁵³,⁵⁴ Several studies have demonstrated safety and efficacy in pre-clinical models for skin,⁵²,⁵³ liver,⁵⁴ cornea⁵⁵ and kidney⁵⁶ between genetically modified pigs and non-human primates (in combination with immunosuppressive drugs) and clinical trials are expected in the coming years.

The age of the animal can also influence the characteristics and properties of the tissue graft. For example, the level of crosslinks is age-dependent⁵⁷ and influences, among others, the thermal stability and mechanical properties. In addition, the age influences cell-binding sites,⁵⁸-⁵⁹ impacting on cellular behaviour and phenotype in vitro and in vivo.⁶⁰,⁶¹ For instance, in pig pancreas islets xenotransplantation for the treatment of diabetes, islets from adult pigs present higher resistance to in vivo degradation and higher neovascularization potential due to the presence of a mature ECM.⁴¹ Small intestinal submucosa (SIS) has also been shown to present different mechanical, structural and biological characteristics, as well as M2 macrophage immune response and remodelling potential as a function of the stage of maturity of the pigs.⁶²,⁶³

Screening of the donor is also necessary before harvesting a graft. Although screening of human patients is relatively easy due to availability of medical records,³⁴ this safety net is not necessarily available in animal-derived grafts that harbour high risks of infection of multiple pathogens,⁶⁵,⁶⁶ but not so much of viral contaminants.⁶⁷ The FDA has established guidelines on infectious diseases in xenotransplantation to prevent and control interspecies disease transmission with full instructions and precautions that should be carried out during animal breeding and tissue harvesting.⁶⁸

2.2 | Decellularization, crosslinking, sterilization and preservation

Once the xenograft donor and tissue source have been chosen, its processing follows a sequential order that includes decellularization, crosslinking (optional), sterilization and preservation, using a variety of techniques and agents (Table 2). Decellularization of tissue grafts should have minimal effect on the integrity, microstructure, composition and biological activity of the ECM, while removing all cellular material and reducing antigens that could trigger immune response.⁶⁹ Cellular remnants contain domains that are recognized as foreign matter and trigger immune response⁷⁰,⁷¹ and, although ECM components are highly preserved among species,⁷²-⁷⁶ decellularized ECM components can also elicit immune response⁷² that can induce macrophage polarization to M1 or M2 phenotypes.⁷³ Classically, the presence of cells⁷⁸ and/or cellular material⁷⁹ promotes M1 inflammatory response,⁸⁰ whereas effectively decellularized scaffolds are related to M2 phenotype.⁸¹ However, recent studies suggest that decellularized scaffolds promote a combined M1/M2 macrophage phenotype, involving adaptive immunity,⁸³,⁸⁴ and triggering remodelling. A typical decellularization process involves the lysis of cellular matter with physical means or chemical agents, followed by separation of cellular matter from the ECM with enzymes and finally removal of cell matter and debris with detergents.⁸⁵

Crosslinking is a process which ends with an interconnection between molecules. Although it occurs in vivo as a post-translational modification of proteins via enzymatic and non-enzymatic mechanisms, the native crosslinking of tissue grafts may be insufficient and previous decellularization process may compromise ECM’s mechanical properties and stability upon implantation. Therefore, exogenous crosslinking can be used to increase mechanical properties and the reabsorption time in vivo.⁸⁶ However, crosslinking decreases the number of available recognition cues for cell attachment and degradation products can elicit cytotoxicity⁸⁷,⁸⁸ and calcification,⁸⁹ particularly those elicited by chemical agents.⁹⁰,⁹¹ This has motivated research into natural agents,⁹²-⁹⁵ bearing always in mind that the ideal crosslinker should be economical, effective and with minimal side effects.
| Product                              | Manufacturer                        | Species  | Tissue                        |
|-------------------------------------|-------------------------------------|----------|-------------------------------|
| 4BONE™ XBM                         | MIS Implants Technologies Ltd.      | Bovine   | Bone                          |
| Acornea                             | Shenzhen Ainear Cornea Engineering Co, Ltd. | Porcine | Cornea                        |
| Artegraft®                          | Artegraft®                          | Bovine   | Carotid artery                |
| Avalus™                             | Medtronic                           | Bovine   | Pericardium                   |
| Bio-Oss®                            | Geistlich                           | Bovine   | Bone                          |
| Biogide®                            | Geistlich                           | Porcine  | Dermis                        |
| CardioCel®                          | Admedus                             | Bovine   | Pericardium                   |
| Carpentier-Edwards Perimount        | Edwards Lifesciences                | Bovine   | Pericardium                   |
| Collamend™                          | Davol Inc                           | Porcine  | Dermis                        |
| Conexa™                             | Tornier®                            | Porcine  | Dermis                        |
| CorMatrix                           | CorMatrix Cardiovascular            | Porcine  | SIS                           |
| Creos™ Xeno Protect                 | Nobel Biocare                       | Porcine  | n.d.                          |
| CuffPatch™                          | Organogenesis                       | Porcine  | SIS                           |
| Endobon®                            | Zimmer Biomet                       | Bovine   | Bone                          |
| Endof orm™                          | Hollister Woundcare®                | Ovine    | Forestomach                   |
| EZ Derm                             | Möllnlycke Health Care Limited      | Porcine  | Dermis                        |
| Gen-Os                              | OsteoBiol® Tecnos®                  | Equine   | Bone                          |
| Kerecis Omega3 Wound                | Kerecis Ltd                         | Fish (Cod) | Dermis                      |
| Matri Stem™/ Genti x™               | ACell®                              | Porcine  | Bladder                       |
| Matrix Patch®                       | Autotissue                          | Equine   | Pericardium                   |
| Medeo r®                            | DSM                                 | Porcine  | Dermis                        |
| Meso BioMatrix®                     | DSM                                 | Porcine  | Peritoneum                    |
| Miromesh®                           | Miromatrix® Medical Inc             | Porcine  | Liver                         |
| mp3®/Gen-Os                         | OsteoBiol® Tecnos®                  | Porcine  | Bone                          |
| OASIS®                             | Cook® Biotech                       | Porcine  | SIS                           |
| PeriGuard®                          | Baxter Healthcare Corporation       | Bovine   | Pericardium                   |
| Permacol™/Zimmer™/ EnduraGen/ Pelvicol® | Tissue Science Laboratories Covidien | Porcine  | Dermis                        |
| Primatrix™                          | TEI Biosciences                     | Bovine   | Foetal dermis                 |
| ProCol®                             | LeMaitre® Vascular                  | Bovine   | Mesenteric vein               |
| Protexa®                            | Tecnos                              | Porcine  | Dermis                        |
| Restore™                            | Depuy Synthes                       | Porcine  | SIS                           |
| SJM Pericardial Patch               | St. Jude Medical Inc                | Bovine   | Pericardium                   |
| Strattice™                          | LifeCell™ Corporation               | Porcine  | Dermis                        |
| Surgimend®                          | Integra LifeSciences                | Bovine   | Foetal dermis                 |
| Decellularization | Crosslinking | Sterilization | Clinical target(s) |
|------------------|-------------|---------------|--------------------|
| High temperature | No          | Gamma         | Bone, dentistry    |
| Chemical (salts and detergents) | No          | Gamma         | Cornea trauma     |
| Chemical | Dialdehyde starch | Propylene oxide in ethyl alcohol | Cardiovascular |
| High temperature | Glutaraldehyde and AOA™ | Liquid chemical sterilization | Chemical |
| n.d. | No          | Gamma         | Bone, dentistry    |
| ADAPT® | Glutaraldehyde | ADAPT®        | Cardiovascular     |
| XenoLogix™ | Glutaraldehyde | Glutaraldehyde | Cardiovascular     |
| Chemical (salts, acids, detergents and hydrogen peroxide) | EDC | ETO | Hernia |
| Chemical (salts and detergents) and enzymatic (Gal epitope) | No | n.d. | Tendon |
| n.d. | No          | ETO           | Cardiovascular     |
| n.d. | No          | n.d.          | Bone, dentistry    |
| n.d. | EDC         | Gamma         | Tendon             |
| High temperature | No          | n.d.          | Bone, dentistry    |
| Chemical (osmotic gradient and detergents) | No | ETO | Wound healing |
| n.d. | Aldehyde    | Aldehyde      | Wound healing      |
| High temperature | No          | Gamma         | Bone, dentistry    |
| Physical | No         | n.d.          | Wound healing      |
| Chemical (PAA, ethanol and dH₂O) | No | E-beam | Wound healing     |
| Chemical (DOA) | No | Chemical | Cardiovascular     |
| OPTRIX™ | No          | ETO           | Hernia, tendon, skin |
| OPTRIX™ | No          | ETO           | Soft tissue        |
| Perfusion. Chemical (detergents) and enzymatic | No | E-beam | Hernia |
| High temperature | No          | Gamma         | Bone, dentistry    |
| Chemical (PAA) | No          | ETO           | Wound healing      |
| Chemical (Basic, ethanol and propylene oxide) | GTA | Ethanol and propylene oxide | Hernia, cardiac surgery |
| Enzymatic | HMDI        | Gamma         | Hernia, wound healing, tendon, soft tissue |
| n.d. | No          | ETO           | Wound healing      |
| Chemical | Glutaraldehyde | Gamma | Cardiovascular     |
| Enzymatic, chemical and physical at low temperature | No | Gamma | Hernia, soft tissue |
| n.d. | No          | E-beam        | Tendon             |
| No | Glutaraldehyde | n.d.         | Cardiovascular     |
| Chemical (detergents) and enzymatic (Gal epitope) | No | E-beam | Hernia, soft tissue |
| Chemical | No          | ETO           | Hernia             |

(Continues)
Sterilization aims to reach a sterility assurance level (SAL), referring to the likelihood of bioburden present after the sterilization; where a recommended level for devices in contact with blood is SAL $10^{-6}$.96 The use of xenografts carries the risk of pathogen transmission between species, which makes necessary the application of effective sterilization methods at the final stages of their processing. The ideal sterilization technique must be safe, easy to use and effective. Steam, chemicals and high temperatures should be employed with caution as they have the potential to disrupt and denature the ECM structure, making them unsuitable for the sterilization of tissue grafts. Gamma or E-beam ionizing radiation, ethylene oxide (ETO) or peracetic acid (PAA) are in general preferred for tissue graft sterilization, as ISO standards for medical devices are already established.97 Nonetheless, they also can compromise the properties of tissue grafts.98-100 Therefore, other methods such as supercritical carbon dioxide ($\text{ScCO}_2$)101 are being explored as alternatives, with promising results to-date.102-104

**TABLE 1** (Continued)

| Product                        | Manufacturer                  | Species   | Tissue  |
|--------------------------------|-------------------------------|-----------|---------|
| Surgisis®/Biodesign®/AxoGuard® | Cook® Medical                 | Porcine   | SIS     |
| SynerGraft® 100                | CryoLife                      | Bovine    | Ureter  |
| Tarsys®                       | IOP Inc                       | Porcine   | SIS     |
| TissueMend™                   | Stryker®/TEI Biosciences      | Bovine    | Foetal  |
| Toronto SPV® Valve            | St. Jude Medical Inc          | Porcine   | Heart   |
| Tutobone®                     | Tutogen Medical               | Bovine    | Bone    |
| Tutopatch®/Tutomesh®          | RTI Biologics®                | Bovine    | Pericard |
| UNITE™ Biomatrix/OrthADAPT™   | Synovis                        | Equine    | Pericard |
| Veritas                        | Baxter Healthcare Corporation | Bovine    | Pericard |
| Vivendi                        | Labcor                        | Bovine    | Pericard |
| XCM Biologic®                 | Ethicon                       | Porcine   | Dermis  |
| Xenomatrix                     | Davol Inc                     | Porcine   | Dermis  |
| Xenoderm                       | MBP                            | Porcine   | Dermis  |

Abbreviation: n.d., not disclosed.
| Decellularization | Crosslinking | Sterilization | Clinical target(s) |
|-------------------|-------------|---------------|--------------------|
| Chemical (PAA and hypotonic rinses) | No | ETO | Hernia, nerve |
| Hypotonic and enzymatic (nucleases) | No | n.d. | Cardiovascular |
| Chemical | No | ETO | Tendon |
| Chemical (Hypotonic, solvents, H$_2$O$_2$) | No | Glutaraldehyde | Cardiovascular |
| Tutoplast® | No | Gamma | Hernia, wound healing, soft tissue |
| n.d. | UltiFix Process | Ethylene dichloride | Wound healing, Tendon |
| Chemical (Acid) | No | E-beam | Hernia, soft tissue |
| Chemical | Poly-glycol agent | Hydrogen peroxide | Cardiovascular |
| OPTRIX™ | No | ETO | Hernia, tendon, wound healing |
| AquaPure™ | No | E-beam | Hernia |
| n.d. | HMDI | Gamma | Skin, wound healing |

The effects that the preservation and the duration of storage have on a tissue graft are commonly overlooked and not specified in the protocols; however, they can affect the structure and therefore the properties of a decellularized graft. The most extended techniques for the preservation of acellular tissue grafts are freeze drying and cryopreservation. Freeze drying results in stable materials that can be further sterilized with physical irradiation methods or ETO. However, during the process of crystal nucleation occurs, which may damage the ECM structure, thus, parameters such as temperature and cooling speed should be closely monitored and appropriately optimized. Lyoprotectants that protect the tissue from the crystals growth can be used, although they may also affect the ECM structure and its biomechanical properties.

Cryopreservation is a cooling process in wet state in the presence of cryoprotectants. Cryopreservation has been shown to preserve the functionality of tissue grafts, but cryoprotectants may induce a cytotoxic side effect.

In the processing of a tissue graft, once each step has been finalized, the assessment of its efficacy and effects on the material have to be assessed. Efficacy of decellularization is generally assessed through histology (Figure 1) and DNA quantification, where 50 DNA ng/mg dry tissue is considered a safe threshold. The degree of crosslinking can be calculated by quantifying the free amines or denaturation temperature. Counting of colony-forming units (CFU)/ml after sterilization can be employed to calculate the reduction on the number of viable microorganisms. Also, effects of the processing steps on the ECM structure and the mechanical properties of the grafts must be analyzed, followed by classic in vitro and in vivo compatibility assays and specific assays for the specific future application of the tissue graft.

### 3 | Xenografts in Clinical Indications

Many xenografts are commercially available for various clinical indications. Table 3 summarizes in vitro, in vivo and clinical data that have been obtained to-date with commercially available xenografts. In this section, we discuss advances and shortfalls of xenografts per clinical indication.

#### 3.1 | Soft tissue

Porcine dermis is extensively used in hernia and abdominal wall repair. Although crosslinking provides desirable properties for hernia repair (e.g., longer durability, more stable mechanical properties in early stages of healing), crosslinked xenografts are associated with scattered results, complications (e.g., mechanical failure, disintegration, infection) and gather the highest number FDA reports. Therefore, crosslinked xenografts in hernia repair require further improvement of the crosslinking techniques, implementing detoxification steps or alternative crosslinking approaches. This can be substantiated considering that non-crosslinked porcine dermal matrices have shown better integration and mechanical properties after implantation and acceptable results in clinics, which match the lower immune response observed in vitro and in vivo. Similar results have also been obtained in cases of infections, where non-crosslinked dermis has shown superior outcomes to crosslinked porcine dermal xenografts. It is worth noting that although the use of xenografts is generally accepted as safe in contaminated fields, this is still a matter of debate in the field. Porcine SIS is
| Method/agent | Mode of action | Examples of tissues | Drawbacks |
|--------------|----------------|---------------------|-----------|
| **Decellularization** | | | |
| **Application methods** | | | |
| Immersion/mechanical agitation | Incubation at determined conditions of time and agitation Thin tissues need short times at high agitation, thick tissues require long periods at middle agitation | SIS, urinary bladder, tendon, dermis, pericardium, amniotic membrane, urinary bladder | Most employed method High amount of reactive is needed |
| Perfusion | Distribution of decellularizing agents through tissue's vasculature Constant or gradual pressure Indicated for whole, highly vascularized organs | Heart, lung, trachea, liver, kidney, SIS, skeletal muscle and skin | The optimal protocol is still elusive Shear forces are likely to damage the basement membrane of tissue's vasculature Not efficient in dense ECM tissues |
| **Physical methods** | | | |
| Freezing/thawing | Crystals formed during the decrease of temperature break down cell's cytosol and membrane | Tendon, cartilage, skin, lung, artery, intervertebral disc, spleen, nerve, SIS, cornea | Can damage tissue graft ECM and structure, affecting its mechanical properties |
| High hydrostatic pressure | Pressurization above freezing point temperature of water disrupts cells without ice crystals formation | Aortic valves and vessels, skin | Requires extensive washing with DNase |
| Microwave radiation | Enhances the effect of chemical decellularizing agents | Tendon, aorta | Not-uniform distribution of electromagnetic microwaves results in heterogeneous decellularization |
| Sonication | Ultrasounds facilitate the penetration of decellularizing agents and destroy cell's membranes and nuclei | Larynx, trachea, myocardium | It affects tissue's ECM and structure Attention must be paid to pH, temperature and conductivity |
| Supercritical carbon dioxide | High-transport and inert gas that promotes cell removal without interacting the ECM | Aorta, adipose tissue, tendon | It affects GAG and growth factor content |
| **Chemical agents** | | | |
| Alkaline (sodium hydroxide, ammonia, calcium oxide) and acid agents (deoxycholic acid, peracetic acid, acetic acid) | Solubilization of cytosol and disruption of nucleic acids | Pericardium, cornea, kidney, bladder, amniotic membrane, artery, urinary bladder, tendon-bone interface, tendon, liver | It affects the integrity of ECM components |
| Detergents | Solubilization of cell membranes and breakage of interactions between DNA, protein and lipids | | |
| Non-ionic (Triton X-100) | Attack the interaction of lipids with other lipids and proteins | Aorta, aortic valve, liver, umbilical vein, or annulus fibrosus | Lower capacity than other methods to remove cellular material |
| Ionic (SDS, Triton X-200) | Include cationic or anionic group Effective at solubilizing membrane proteins | Liver, annulus fibrosus, nerve | Denaturing agents that negatively affect ECM content |
| Zwitterionic (CHAPS, SB10) | Detergents that combine properties of both ionic and non-ionic detergents | Cardiac vessels, heart, lung | Negative effect in ECM |
| Method/agent                                | Mode of action                                                                 | Examples of tissues                                    | Drawbacks                                               |
|--------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------|---------------------------------------------------------|
| Hypotonic and hypertonic solutions (NaCl 1.5 mol/L, NaCl 3 mol/L) | It lyses cells through osmotic pressure It cannot remove cell remnants         | SIS, 280 cornea, 281 aortic valve, 272 cartilage       | Ineffective on their own to remove cell debris          |
| Alcohols and solvents (Isopropanol, ethanol, tributyl phosphate) | Cell lysis by dehydration, precipitation of remaining DNA and solubilization of lipids | Adipose tissue, 283 cornea, 284 temporomandibular joint disc, 285 tendon, 289 cartilage | It affects mechanical properties and ECM structure      |
| Chelators (EDTA, EGTA)                      | Isolate calcium and magnesium ions, necessary for fibronectin and collagen cell binding | Liver, 286 trachea, 287                                | Not effective on their own                              |
| Enzymatic agents                           |                                                                                  |                                                        |                                                        |
| Proteases                                  | Cleaving of specific substrate and recognition motifs of proteins              |                                                        |                                                        |
| Trypsin                                    | Cleaves cell adhesion proteins on the carboxyl side of the amino acids arginine and lysine | Kidney, 245 skin, 26 aortic valve, 272 annulus fibrous | Prolonged exposures result in ECM damage               |
| Dispases                                   | Cleave collagen type IV and fibronectin Employed to remove epithelium layers    | Cornea, 254 skin                                       | It disrupts ECM components of the basement membrane    |
| Collagenase                                | Cleave specific sites of different collagen types                              | Aortic valves, 290                                     | It causes severe damage to on ECM                       |
| Nucleases                                  | Hydrolyse the bonds of ribonucleic and deoxyribonucleic acid chains; endonucleases cleave the interior bonds and exonucleases target the terminal ones | Cornea, 288 muscle, 291                               | Ineffective on their own                               |
| α-galactosidase                            | Cleaves GAGs present in the ECM (reduction of Gal epitope)                    | Pericardium, 292, 293 anterior cruciate ligament        | Reduction of GAGs                                      |
| Lipase                                     | Hydrolysis of lipids present in the tissue                                    | Amniotic membrane, 295                                | Ineffective on its own                                 |
| Crosslinking                               |                                                                                  |                                                        |                                                        |
| Physical crosslinking                      |                                                                                  |                                                        |                                                        |
| UV radiation                               | Free radicals produced by irradiation for bonds from aromatic amino acid residuals | Bovine pericardium, 296                                | Physical crosslinkers are not effective in tissue grafts and are rarely employed |
| Dehydrothermal                            | Removal of bound water and formation of ester and amide bonds intramolecularly | Skin, 297                                              |                                                        |
| Chemical agents                            |                                                                                  |                                                        |                                                        |
| Glutaraldehyde (GTA)                       | Reacts with primary amines generating intra and intermolecular bonds, reaction between an amino group and an aldehyde group from lysine or hydroxyllysine or through reaction between two contigous aldehydes | Oesophagus, 298 liver, 299 amniotic membrane, 300 pericardium, 90 aorta, 301, 302 aortic valve, 301 | Cytotoxic effects, acute immune reaction upon implantation and calcification Detoxification with glycine is advised |
| 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) | Activation of carboxylic groups from aspartic and glutamic acid residues followed by a reaction with primary amines from lysine, forming an amide bond | SIS, 303 pericardium, 304, 305 skin, 306 heart valves, 307 | Lower crosslinking efficiency than GTA Inflammation effects and calcification, but at a lower extent than GTA |
| Method/agent | Mode of action | Examples of tissues | Drawbacks |
|--------------|---------------|---------------------|-----------|
| Epoxy compounds (HDMI, polyglycidyl ether) | Epoxy compounds form bonds between amino, carboxyl and hydroxyl groups | Skin,\(^{308,309}\) aorta,\(^{330}\) pericardium\(^{92}\) | Lower efficiency than GTA and EDC related to cytotoxicity and inflammation |
| Natural agents | | | |
| Genipin | Polyphenols carbonyl functional groups react with primary free amines or through the formation of a nitrogen-triazine and a further aromatic monomer | Pericardium,\(^{95}\) trachea,\(^{311}\) cartilage,\(^{312}\) annulus fibrosus,\(^{313}\) liver,\(^{99}\) | Lower efficiency than chemical agents High cost |
| Other polyphenols (Procyanidins, epigallocatechin gallate) | | Cartilage,\(^{312}\) arteries,\(^{314}\) heart valves\(^{315}\) | Early stage of research Low crosslinking efficiency |
| Sterilization | | | |
| Physical sterilization | | | |
| Gamma irradiation | Ionizing radiation (gamma and electron beam) damages the nucleic acids of the pathogens directly or indirectly through radiolysis of water and production of hydroxyl radicals | Tendon,\(^{316}\) cartilage,\(^{100}\) lungs\(^{317}\) | High doses negatively affect ECM structure and mechanical properties |
| Electron beam | | Tendon,\(^{318,319}\) ligament,\(^{320}\) bone\(^{321}\) | Low penetration capacity in dense ECM tissues It affects ECM structure and mechanical properties |
| Supercritical carbon dioxide (ScCO\(_2\)) | High penetration and transport capacity, while inert, serves as a mean to wash off pathogens without interacting with ECM | Meniscus,\(^{102}\) tendon,\(^{322}\) heart,\(^{103}\) lungs\(^{104}\) | Low efficacy on its own, but great potential in combination with other chemical agents Early stage of research It affects ECM structure |
| Chemical sterilization | | | |
| Ethylene oxide (ETO) | Microbicidal, fungicidal and antiviral activity based on the alkylation of nucleic acids and enzymes | Bladder,\(^{323}\) cartilage,\(^{100}\) liver,\(^{270}\) skin\(^{324}\) | Lower penetrability than physical agents Requires complex equipment |
| Peracetic acid (PAA) | Powerful oxidizing agent which is bactericidal and fungicidal at low dilutions | Cartilage,\(^{100}\) liver,\(^{270}\) urethra,\(^{325}\) nerve,\(^{326}\) tendon,\(^{327}\) trachea,\(^{328}\) SIS\(^{329}\) | Cytotoxic without appropriate rinsing It affects cell viability It partially crosslinks ECM |
| Gas plasma | Charged gas which contains the same proportions of anions and cations, interacting with the metabolites of microorganisms by oxidation/reduction processes | Bone,\(^{330,331}\) cornea\(^{113}\) | Early stage of research It affects ECM structure |
| Preservation | | | |
| Freeze drying | Sublimation process that results in dry and stable grafts | Arteries,\(^{105}\) heart valves,\(^{107}\) nerve\(^{106}\) | Formed nucleation crystals affect ECM structure and mechanical properties Lyoprotectants can be used, but compromise cytocompatibility |
| Cryopreservation | Cooling process in wet state in the presence of a cryoprotectants solution | Tendon,\(^{332}\) aorta,\(^{308}\) heart valves\(^{109}\) | Long preservation times negatively affect ECM structure and mechanical properties Cryoprotectants can elicit cytotoxicity |
| Xenograft product       | In vitro studies | In vivo studies | Clinical studies | Main findings                                                                                                                                                                                                                                                                                                                                 |
|------------------------|------------------|----------------|------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Artegraft®              | —                | —              | [219,220]        | Clinical data: As haemodialysis access or lower extremity bypass, initial positive results regarding primary and secondary patency values until 5 y, comparable to synthetic ePTFE                                                                                                           |
| Avalus™                 | —                | —              | [224]            | Clinical data: Safety for aortic valve replacement in 686 patients reported in a prospective non-randomized multicentre study, although bleeding rates increased in the long-term follow-up                                                                                                                                  |
| Bio-Oss®                | [197]            | —              | [200,201]        | In vitro: Promoted secretion of VEGF by periodontal ligament cells, but in a lower extent than other xenogeneic bone grafts from porcine and equine origin. Clinical data: Increased osteogenesis and width of the alveolar process alone or in combination with autogenous tissue, with a high (96%) survival of dental implants.                                      |
| CardioCel®              | [333,334]        | [211,335,336] | [211-213]        | In vitro: Closest mechanical properties to young aortic valves leaflets in a comparative study including other crosslinked materials. High cytocompatibility and capability to promote the adhesion and proliferation of mesenchymal stem cells (MSCs). In vivo: Effective delivery of MSCs in a mice infarcted model, promoting regeneration and integration of the tissue. A lamb aortic valve replacement and pericardial patch showed integration, remodelling and absence of calcification after 7 mo. Contrary, a vascular patch in pigs showed severe calcification after 1 y. Clinical data: Short-term small studies have shown good performance and integration of the material in repaired aortic valves of paediatric patients. Contrary, 2 y of follow-up study of 101 infant patients with congenital heart diseases showed some cases of aorta thickening, although no calcification. |
| Carpenter-Edwards Perimount | —                | —              | [223]            | Clinical data: Low incidence of valve-related complications and deterioration in a retrospective study on 2,659 patients (70.7 ± 10.4 y) after 20 y of follow-up                                                                                                                                                                                                 |
| Collamend™              | [120,130,337]    | [121,338-340]  | —                | In vitro: Presented higher mechanical properties and resistance to enzymatic degradation than other tissue sources and non-crosslinked matrices. Elicited an inflammatory response as per cytokine production by macrophages in vitro. In vivo: Subcutaneous models showed poor cell invasion, remodelling and neovascularization, higher inflammation and immune response that non-crosslinked matrices. Longer times of resorption than other non-crosslinked matrices (up to 24 wk in rats). Hernia models in rats have shown complications, seroma and inflammatory events. Similar data were obtained in a rabbit model, although with positive results in mechanical properties. A mature hernia pig model showed positive results in tissue mechanical properties after integration, although with evidence of adhesions. |
| Conexa®                 | [341]            | [183,184]      | [182,186]        | In vitro: High cytocompatibility with tenocytes, allowing the highest adhesion, proliferation and promoting the expression of tenocyte markers compared to crosslinked materials. In vivo: A subcutaneous model in vervet monkeys showed the absence of inflammatory immune reaction thanks to the cleaving of the Gal epitope (α-galactosidase processing). In a rotator cuff augmentation model in vervet monkeys, promoted remodelling in 6 mo and prevented immune reaction. Clinical data: Promoted pain relief and recovery of the motion and strength in rotator cuff massive tears in two studies of 1 and 26 patients. |
| Xenograft product | In vitro studies | In vivo studies | Clinical studies | Main findings |
|-------------------|-----------------|----------------|------------------|---------------|
| CorMatrix         | [342-344]       | [344-348]      | [206-210]        | In vitro: Suboptimal hemodynamic properties were observed in a left heart simulator. Antimicrobial activity up to 6 d was granted after antibiotic impregnation. Demonstrated good cytocompatibility with MSCs. In vivo: Positive results in terms of remodelling and functionality as cardiac patch in rat models. In larger models (pig, dog, sheep) as myocardium and vascular graft has shown good remodelling, prevention of calcification and low immunogenicity. As valve replacement in pigs showed suboptimal mechanical and functional features. Clinical data: High heterogeneity in responses has been observed and considerable complications like inflammation response, patch failures or incomplete resorption. These complications were more related to high pressure conditions. Positive results have recently been reported only in small trials. Discouraging results include: 32% recurrence in leaflet augmentation, regurgitation and inflammatory response of paediatric repaired valves after midterm follow-up. Histological data after paediatric heart surgery reported associated fibrosis, foreign-body reaction, necrosis and chronic inflammation. |
| CuffPatch         | [349,350]       | [351,352]      | –                | In vitro: Similar mechanical properties to crosslinked fresh equine pericardium. Lower mechanical properties than crosslinked dermal grafts. In vivo: Abdominal wall models in rats showed adverse immune response and M1 polarization of macrophages. |
| Endoform          | [131,134]       | [134,353]      | [154]            | In vitro: Retained soluble compounds able to modulate proteases (MMPs and elastase) action and to promote angiogenesis. In vivo: Rotator cuff augmentation model in rats showed higher histological levels of repaired tendons than sham, but not mechanical nor functional improvement. Full-thickness wound model in pigs revealed a better and faster remodelling than sham. Clinical data: Case series of 19 participants reported a 50% closure of chronic wounds after 12 wk, concluding with the potential of the material for the treatment of chronic wounds. |
| EZ Derm           | –               | –              | [135-138,354]    | Clinical data: Clinical data from the late 1980s and early 1990s yielded scattered results as full-thickness wound dressing. More recent studies of partial thickness and paediatric burns reported a firm adherence which provided beneficial conditions to the healing process, like reduced infections and evaporation, at a reduced cost. |
| Kerecis Omega 3 Wound | [355] | [356] | [156,357] | In vitro: Higher porosity, cell ingrowth and anti-bacterial properties due to the presence of omega-3 than a commercially available decellularized dermis allograft. In vivo: Shown to be safe and effective in a dura mater regeneration pilot study in sheep. Clinical data: Double-blinded and randomized trial showed equal efficacy to decellularized porcine SIS, with no adverse immune reactions to both xenografts, agreeing with recent findings in challenging ulcer wounds small studies. |
| MatriStem         | –               | –              | [146,152,153,358-362] | Clinical data: Demonstrated healing enhancement in small case series of chronic wounds and deep partial thickness burns. Healing potential and remodelling its further enhanced when employed as micronized matrix. Similar performance (<20% closure rate) to a fibroblastic cultured autograft at a considerably lower cost in the treatment of foot ulcers. Slightly better performance than allogeneic skin substitutes but a substantial lower cost revealed by a multicentre study of 13,000 cases of diabetic foot ulcers. Small series in the reconstruction of finger, vagina and urethra have shown promising results. |
| Meso Biomatrix®   | –               | –              | [173,363]        | Clinical data: Scattered results as single-stage implant-based breast reconstruction material regarding safety for this purpose (integration, inflammation, patient comfort) |
| Medeor®           | [364]           | –              | –                | In vitro: Presence of soluble factors able to promote cell proliferation and invasion in a higher extent than other commercially available xenografts (dermis) and allografts (dermis). |
| Miromesh®         | –               | [365]          | –                | In vivo: Better integration and cell infiltration than a dermis xenograft in a hemia model in rats. |
| Xenograft product | In vitro studies | In vivo studies | Clinical studies | Main findings |
|------------------|-----------------|----------------|------------------|---------------|
| **OASIS® [132,134,143,366]** | [134] | [145,147,148,150,151,153,367] | In vitro: Retained soluble compounds and growth factors able to promote cell proliferation and angiogenesis. Higher inflammatory (M1/M2 score) response by THP-1 than bovine dermis, human dermis and a collagen scaffold. In vivo: Full-thickness wound model in pigs revealed a better and faster remodelling than sham. Clinical data: Promoted better remodelling, lower inflammation and faster healing of ulcers compared to other standards (ie wet dressings) combined with negative pressure and showed no complications. Data confirmed with histopathological studies. Similar or improved performance than hydrogel products (ie hyaluronic acid or becaplermin) in means of patient comfort and rate of healing. Slightly better performance than allogeneic skin substitutes but at a substantially lower cost revealed by a multicentre study of 13,000 cases of diabetic foot ulcers. |
| **OrthADAPT™/UNITE™ Biomatrix [341,349]** | [35,155,182,368] | In vitro: At different crosslinking treatments, no relation between crosslinking degree and mechanical properties. Supported tenocytes adhesion, but at lower extent than other non-crosslinked materials. It did not promote the expression of tendon markers in tenocytes. Clinical data: Promoted the healing of 75.7% in recurrent diabetic foot ulcers of 34 patients after 12 wk of treatment, confirming the positive results observed in a previous study in 23 patients. In tendon repair, an adverse acute reaction of one patient with an augmented Achilles tendon repair has been reported, similar to another case series of 6 patients suffering irreparable tears in rotator cuff. |
| **Osteobio®/Gen-Os [197]** | [198] | [199] | In vitro: Promoted VEGF production of periodontal ligament cells and angiogenesis in endothelial cells in a higher extent than a bovine bone xenograft. In vivo: Lower inflammatory reaction in rats muscle implantation than an allograft material. Clinical data: Small study with 15 patients showed a reduction of the hard tissue resorption improving the outcomes after tooth extraction. |
| **PeriGuard® [130,333,337,342]** | [130,333,337,342] | -- | In vitro: Suboptimal hemodynamic properties were observed in a left heart simulator. Closest mechanical properties to young aortic valves leaflets in a comparative study including other crosslinked materials. Presents higher mechanical properties and resistance to enzymatic degradation than other tissue sources (SIS) and non-crosslinked matrices. |
| **Permacol™ [120,130,337,349,350,364,369-372]** | [121,338,339,352] | [123,124,185,380-386] | In vitro: Present higher mechanical properties and resistance to enzymatic degradation than other tissue sources and non-crosslinked matrices. Supported the adhesion and invasion of MSCs, fibroblast, tenocytes, etc, but at a lower extent than other non-crosslinked matrices, cytotoxic events related to crosslinking. Elicited an inflammatory response as per cytokine production by macrophages in vitro. In vivo: Subcutaneous models showed poor cell invasion, remodelling and neovascularization, higher inflammation and immune response that non-crosslinked matrices. Longer times of resorption than other non-crosslinked matrices (up to 24 wk in rats). Hernia models in rodents and rabbits showed optimal mechanical properties performance, although poor resorption and fibrotic tissue formation. Similar data were obtained in tendon and vascular patch models. Skin regeneration models in rats revealed a very modest performance due to poor resorption and epithelization. Clinical data: Scattered results observed in pelvic wall repair (ie positive patient satisfaction and graft performance compared to synthetic substitutes after 1-year follow-up versus relation to complications and recurrence) and in hernia repair of contaminated fields (ie positive outcomes and no recurrence versus 50%-88% recurrence and 37% rate of infection). Treatment of massive tears in rotator cuff showed improvement in pain relief and shoulder functionality in small studies (5 and 10 patients). Breast reconstruction small case series showed an acceptable outcome in skin-sparing mastectomy regarding patient satisfaction scores and absence of complications. In eyelid repair, it was related to a higher rate of complications than other xenografts and materials provoked by its stiffness and low resorption. |

(Continues)
| Xenograft product | In vitro studies | In vivo studies | Clinical studies | Main findings |
|-------------------|------------------|----------------|------------------|---------------|
| Primatrix™        | [143]            | [144]          | [139-142]        | In vitro: Lower M1/M2 macrophage polarization score than other crosslinked and non-crosslinked xenografts. In vivo: Low immune and inflammatory response in a mice subcutaneous model. Material was remodelled by day 14. Clinical data: Several studies have shown a faster healing than standard of care and other materials such as SIS or urinary bladder in diabetic ulcers, showing no complications and a complete integration. Similar results than those offered by allogeneic skin replacements have also been reported. |
| ProCo®            | —                | —              | [214,215]        | Clinical data: Failure of the graft as vessel replacement was reported in all implantations of a small study (6 patients) due to aneurism and thrombosis, where other trial of 32 patients also showed insufficient values of primary patency in critical limb ischaemia repairs. |
| Protexa®          | —                | [387]          | [167]            | In vivo: Positive immune response and integration, although authors considered it performed at a lower level than a porcine SIS material. Clinical data: Showed good cosmetic results in a 48 patients comparative trial with the titanium mesh TiLOOP®, but with higher rate of complications. |
| Restore™          | [341,350,372]    | [180,181,351, 352,377] | [177-179,182] | In vitro: Elastic modulus comprised between human rotator cuff values, but lower strength and strain. Lower mechanical properties than dermal derived grafts in both tensile tests and suture pull-out test. Lower cytocompatibility with tenocytes than non-crosslinked dermal grafts. In vivo: Related to a M2 polarization and remodelling in an abdominal wall model in mice, although other studies in rabbits and mice also report an inflammatory reaction to the material due to ineffective decellularization, which can be improved with the use of autologous cells. In tendon repair models (eg rotator cuff in rabbits and lambs), showed an initial inflammatory response and a later complete resorption, but without recovery of mechanical properties. Clinical trials: Negative results were obtained in clinical trials; the material did not improve the healing and mechanical functionality of massive or moderate rotator cuff tears, with a high rate of complications like inflammation and immune reaction. |
| SJM Pericardial Patch | [333,342] | — | [222] | In vitro: Closest hemodynamic characteristics to normal aortic valves in a comparative study. Clinical data: Low rate of early mortality and complications and positive haemodynamic performances such as effective orifice area index in a multicentre study of 1,024 patients of 72.5 ± 9.0 y. |
| Strattice™        | [120,130,364,388,389] | [338,387,388,390-392] | [116-119, 122,161-166] | In vitro: High rate of structure preservation and similar resistance to enzymatic degradation than native tissue, although lower resistance than other dermal crosslinked xenografts. Presence of soluble factors able to promote cell proliferation in a higher extent than other commercially crosslinked xenografts (dermis) and allografts (dermis). Lower activation and production of inflammatory cytokines by mononuclear cells elicited than crosslinked dermal xenografts. Resistance to bacterial penetration due to compact structure. In vivo: Lower inflammatory response and faster degradation and remodelling than crosslinked dermal xenografts in subcutaneous models in rodents and pigs. In hernia models in rats, it has shown a positive performance regarding integration, low inflammatory response and the mechanical properties of the hernia repair, which was confirmed in an abdominal wall repair in monkeys. Clinical data: Positive outcomes for hernia repair demonstrated at a short/midterm, showing elevated (>70%) success repair in high risk population and similar rates of recovery and recurrence than those observed in synthetic partially absorbable meshes. Scattered results were observed in breast reconstruction, where several studies point its safety and efficiency with a low rate of complications, and others report a high incidence of complications (ie infection, necrosis) and unsuitability for one-stage implant-based breast reconstruction. |
| Surgimend™        | —                | —              | [168-170]        | Clinical data: Low rate of complications, high patient satisfaction and high objective satisfaction as tissue-expander breast reconstructions in studies of 28 and 65 patient, and as material for implant-based reconstruction following skin-sparing mastectomy in 118 patients. |
| Xenograft product | In vitro studies | In vivo studies | Clinical studies | Main findings |
|------------------|-----------------|----------------|-----------------|---------------|
| Surgisis®        | [120,130,370]   | [121,339,370,375] | [126-129,176,393-396] | In vitro: Lower resistance to enzymatic degradation than dermal xenografts and crosslinked grafts. Supported the invasion and growth of MSCs for stem cell delivery and elicits a lower activation and production of inflammatory cytokines by mononuclear cells than crosslinked xenografts. In vivo: Lower mechanical performance in rat and rabbit hernia models than crosslinked grafts at early stages, but a better resorption and mechanical stability of the repair at later stages motivated by the remodelling of the tissue. This remodelling was enhanced when delivering autologous MSCs. Clinical data: Suitability and safety was demonstrated for several hernia repairs (i.e. inguinal, hiatal) in several clinical trials, showing no complications nor immune rejection up to 5 y of follow-up. Contrary, its unsuitability as abdominal wall reinforcement in challenging population was reported when placed in preperitoneal sub-lay. A small trial showed positive and promising results in means of integration and aesthetics in nasal reconstruction. Few clinical trials have shown potential as nerve conduit in cubital tunnel syndrome and lingual nerve repair, where no complications and significant improvement in pain and functionality were reported, similarly to a collagen type I established product|
| SynerGraft®      | —               | —              | [216,218]       | Clinical data: High rate of complications reported as blood vessel replacement, including thrombosis and aneurism dilatation, related to immune reactions provoked by an inefficient decellularization after histopathological study |
| Tarsys®         | —               | —              | [174,175]       | Clinical data: Significant improvement of functionality in lower eyelid retraction surgery, with no recurrence surgery, low rate of complications, no infections and satisfactory cosmetic results |
| TissueMend™      | [344,350,397]   | [344,352]      | —              | In vitro: Higher mechanical properties (suture pull-out) than SIS xenografts, but lower than other crosslinked dermal grafts. No improvement in the mechanical properties observed on augmented tendons with this material in an ex vivo mechanical analysis. High cytocompatibility with MSCs for stem cell delivery purposes. In vivo: Encapsulation of the tissue was observed in an abdominal wall repair in rats, although without an inflammatory reaction elicited by other crosslinked xenografts compared. It has been effectively used as a MSCs delivery system in an infarcted heart model in mice, improving remodelling and angiogenesis |
| TutoBone®        | —               | —              | [190]           | Clinical data: A 10-year retrospective study including 556 patients reported inferior results to autografts in terms of cervical fusion, but similar to other alternative options and at a lower cost |
| Tutomesh®        | —               | [392,398]      | [172]           | In vivo: Lower collagen deposition and mechanical properties in a rabbit ventral hernia repair than a dermal xenograft. In a rat Achilles tendon repair, it showed capability to integrate and the absence of host response, but with no functional nor mechanical assessment. Clinical data: Breast reconstruction in 24 patients supported the safety of the material and technical efficiency, although post-operative seroma formation was reported as risk |
| Veritas          | [130,388]       | [388]          | [171]           | In vitro: Studies on biochemical and biophysical properties have shown low ECM structure preservation, low thermal stability and low resistance to enzymatic degradation. In vivo: Moderate inflammation in a full-thickness abdominal defect in monkeys (higher than a dermal xenograft but lower to crosslinked xenografts), but a fast resorption. Clinical data: Retrospective multicentre study on 54 patients showed low rate of complications, at a similar or lower level than those observed in allogeneic dermal matrix products |
| XCM Biologic®®   | —               | [387]          | [182]           | In vivo: In a ventral hernia rabbit model, similar results in integration when compared to other two non-crosslinked dermal xenografts, although it did not show the optimal performance regarding collagen deposition and mechanical properties. Clinical data: Higher pain relief and functionality recovery in rotator cuff massive tears than those treated with porcine SIS or equine pericardium in a study including 22 patients |
| Tissue Matrix    | —               | [388]          | —              | In vitro: Lower preservation of ECM structure and resistance to collagenase degradation than other dermal xenografts and native tissue, and higher enzymatic degradability than crosslinked xenografts. In vivo: Severe inflammatory response by the host in an abdominal wall defect in monkeys |
| XenMatrix™       | [130,388]       | [388]          | —              | In vitro: Lower preservation of ECM structure and resistance to collagenase degradation than other dermal xenografts and native tissue, and higher enzymatic degradability than crosslinked xenografts. In vivo: Severe inflammatory response by the host in an abdominal wall defect in monkeys |
also considered as a suitable and safe material for hernia repair in clinics,\textsuperscript{126,127} matching the performance of synthetic meshes.\textsuperscript{128} However, it has not shown suitability when employed as abdominal wall reinforcement in challenging scenarios, offering poor mechanical resilience ending in the discomfort of the patient and complications.\textsuperscript{129} This could be related to its lower mechanical properties and resistance to enzymatic degradation, when compared to other tissue sources like dermis or crosslinked xenografts.\textsuperscript{130}

Xenografts are also extensively used in wound treatment and skin replacement.\textsuperscript{131-134} Crosslinked porcine dermis resulted in opposing conclusions in late 1980s/early 1990s as partial thickness wound dressing.\textsuperscript{135,136} Later studies, however, have shown the beneficial effects of porcine dermis as skin substitute in the treatment of burns and surgical wounds.\textsuperscript{137,138} More recently, foetal bovine dermis has shown positive results as wound healing material in diabetic ulcers,\textsuperscript{139-141} promoting faster healing than other products such as allogeneic grafts.\textsuperscript{142} This can be attributed to the lower crosslinking content that results in faster remodelling,\textsuperscript{143} as in vitro\textsuperscript{144} and in vivo\textsuperscript{145} studies support. Porcine SIS\textsuperscript{146} and urinary bladder\textsuperscript{147} are also customarily employed in wound healing, where they have shown improved and accelerated healing of ulcers wounds\textsuperscript{148-149} and have showed similar or superior performance to biomaterials\textsuperscript{150,151} and allografts\textsuperscript{152} and at a substantial lower cost.\textsuperscript{153} Also, ovine forestomach,\textsuperscript{154} equine pericardium\textsuperscript{155} and even fish skin\textsuperscript{156} have shown promising results in small clinical trials, but further investigation is required to demonstrate safety and efficacy.

In breast reconstruction, acellular dermal matrices are used extensively\textsuperscript{157-160} with, in general, low rates of complications.\textsuperscript{161-164} Although some adverse effects (e.g. infection, necrosis) have been reported.\textsuperscript{165-167} Similarly to skin replacement, positive outcomes have also been observed in breast reconstruction using foetal bovine dermis.\textsuperscript{168-170} Other products such as bovine pericardium,\textsuperscript{171,172} porcine peritoneum\textsuperscript{173} and porcine SIS\textsuperscript{174-176} have also been tested in small clinical trials with acceptable outcomes in breast reconstruction.

Despite all these positive patient outcomes, all studies agree that randomized clinical trials with larger number of patients are needed to further support the use of xenografts in soft tissue repair.

### 3.2 | Tendon, bone and dentistry

Commercially available xenografts in tendon regeneration are used as augmentation systems. Tendon augmentation with porcine SIS has been related to failure in clinical trials,\textsuperscript{177-179} with no improvement in healing and mechanical properties, which was associated to the inefficient decellularization and the consequent immune reaction, as reported in vivo studies.\textsuperscript{180,181} Crosslinked xenografts, such as equine pericardium, did not yield positive outcomes in clinical trials,\textsuperscript{182} which can be attributed to crosslinking-induced immune reactions. Porcine dermis xenografts (e.g Conexa\textsuperscript{®} 183,184) on the other hand have shown positive results in tendon augmentation,\textsuperscript{185-187} possibly attributed to efficient decellularization and/or crosslinking protocols and selecting a tissue with appropriate composition and mechanical resilience. In any case, overall, no definitive tendon augmentation device seems to be available. Although xenografts play a crucial role in irreparable defects augmentation, in moderate to large injuries, they have not achieved a satisfactory outcome.

Bone xenografts provide an optimal microenvironment for cell adhesion, proliferation and infiltration in vitro and de novo bone generation in vivo. This osteoinductive effect is related to the preserved micro- and macro-structure of the decellularized bone together with the partial preservation of ECM components.\textsuperscript{188} Despite their high availability, low cost and good mechanical and osteoinductive properties, only a few bone xenografts are available, which have shown limited positive clinical results,\textsuperscript{189,190} and as a result, they are rarely employed in orthopaedics.\textsuperscript{191} Nonetheless, advances in materials sciences and tissue engineering have resulted in the development of composite materials combining xenogeneic mineral matrix, synthetic and/or natural polymers, which have been tested positively in clinical trials.\textsuperscript{192-194}

In dentistry, xenografts are used as bone-filling materials, with data to-date showing superiority in clinical outcomes even over autologous treatments.\textsuperscript{195} These materials are normally deproteinized with high temperature processing, maintaining the mineral component and microstructure of the bone.\textsuperscript{196} Bovine, porcine and equine origin grafts have shown positive results in vitro,\textsuperscript{197} in pre-clinical models,\textsuperscript{198} and in clinical setting.\textsuperscript{199-201} Porcine non-crosslinked dermis has also been used successfully as augmentation/contention system in clinical trials,\textsuperscript{202,203} largely attributed to its integration with the surrounding soft tissue, which prevents the second operation that synthetic materials require for removal.\textsuperscript{204}

### 3.3 | Cardiovascular

In cardiac graft replacement or patching, decellularized porcine SIS is one of the most employed material\textsuperscript{205} but only few clinical studies can be considered in a class III relevance, and the reported class IV correspond to case series or small trials.\textsuperscript{206,207} In addition, high heterogeneity in responses has been observed, together with considerable complications, such as inflammatory response\textsuperscript{208} and/or graft failures.\textsuperscript{209,210} These complications were often related to high pressure conditions, which could be indicative of the low performance due to insufficient mechanical properties of the source of tissue, as observed in vitro.\textsuperscript{205} Therefore, crosslinked materials with enhanced mechanical properties, like bovine pericardium, that include processing steps preventing crosslinker-related complications (e.g calcification\textsuperscript{211}) have been developed, which have shown positive short-term results in paediatric cardiovascular applications,\textsuperscript{211-213} but long-term assessment is required.

Vascular replacement xenografts (e.g crosslinked bovine vein, bovine ureter) have mainly resulted in failure.\textsuperscript{214-216} These poor outcomes were related to inappropriate processing, which resulted in insufficient mechanical properties to support the pressure of the circulatory system,\textsuperscript{217} and low antigen removal.\textsuperscript{218} Having said that, recent reports on bovine artery crosslinked with starch dialdehyde...
(as opposed to GTA) have shown positive results as haemodialysis access\textsuperscript{219} or as lower extremity bypass.\textsuperscript{220} However, their implantation is not a generalized procedure, as their performance has not improved synthetic grafting. Further efforts in the processing technology and recellularization of the grafts are needed to improve the current outcomes.\textsuperscript{221}

In the field of valve replacement, the use of the stented valves is a common procedure, which utilizes metal/polymeric stents and processed animal tissue (eg bovine or porcine cardiac tissue processed with glutaraldehyde and anti-calcification solvent). Stented valves were established in clinical practice due to their effective performance in adult patients and low rate of complications\textsuperscript{222,223} and reduced long-term calcification.\textsuperscript{224} Stentless analogue valves have been also designed to obtain hemodynamic patterns closer to physiological levels and are clinically available,\textsuperscript{225} demonstrating similar clinical success to stented ones in a long-term basis and less complications in the early stages after implantation.\textsuperscript{226,227} However, no reliable valve replacement graft is available for paediatric patients with congenital heart diseases,\textsuperscript{228} where decellularization and recellularization is the main option to overcome the limitations that tissue grafts present in valve repair.\textsuperscript{229,230}

4 CONCLUSIONS AND FUTURE PERSPECTIVES

The low availability of autologous and allogeneic tissues, coupled with advances in decellularization, crosslinking, sterilization and preservation, have made numerous, primarily, porcine and bovine xenografts commercially and clinically available for a diverse range of tissue engineering and regenerative medicine applications. In general, non-crosslinked grafts (in particular, grafts from young animals) demonstrate better resorption for tissues that do not require mechanical resilience. Crosslinking, although significantly improves mechanical properties, is often associated with immune response and calcification, imposing the need for development and assessment of new methods. As immune rejection remains the major concern of xenografts in clinical practice, genetically engineered pigs with reduced immunogenicity could become the ideal source for xenografts in the years to come. Overall, although pre-clinical and clinical studies have demonstrated, in most cases, safety and efficacy, the field urgently requires randomized double-blinded clinical trials to safely conclude on the potential of a specific xenograft for a specific clinical indication. Considering the, unmatched by human-made devices, physicochemical and biological similarity of xenografts to human tissues, we believe that xenografts will continue gaining pace in modern regenerative medicine.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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