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REVIEW ARTICLE

Xenogeneic Decellularized Extracellular Matrix-based Biomaterials For Peripheral Nerve Repair and Regeneration

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Abstract: Peripheral nerve injury could lead to either impairment or a complete loss of function for affected patients, and a variety of nerve repair materials have been developed for surgical approaches to repair it. Although autologous or autologous tissue-derived biomaterials remain preferred treatment for peripheral nerve injury, the lack of donor sources has led biomedical researchers to explore more other biomaterials. As a reliable alternative, xenogeneic decellularized extracellular matrix (dECM)-based biomaterials have been widely employed for surgical nerve repair. The dECM derived from animal donors is an attractive and unlimited source for xenotransplantation. Meanwhile, as an increasingly popular technique, decellularization could retain a variety of bioactive components in native ECM, such as polysaccharides, proteins, and growth factors. The resulting dECM-based biomaterials preserve a tissue's native microenvironment, promote Schwann cells proliferation and differentiation, and provide cues for nerve regeneration. Although the potential of dECM-based biomaterials as a therapeutic agent is rising, there are many limitations of this material restricting its use. Herein, this review discusses the decellularization techniques that have been applied to create dECM-based biomaterials, the main components of nerve ECM, and the recent progress in the utilization of xenogeneic dECM-based biomaterials through applications as a hydrogel, wrap, and guidance conduit in nerve tissue engineering. In the end, the existing bottlenecks of xenogeneic dECM-based biomaterials and developing technologies that could be eliminated to be helpful for utilization in the future have been elaborated.

Keywords: Decellularization, xenogeneic extracellular matrix, peripheral nerve injury, dECM-based biomaterials, nerve repair and regeneration, tissue engineering.

1. INTRODUCTION

Peripheral nerve injury is a common clinical disease that affects 2.8% of all trauma cases globally and severely reduces patient quality of life by damaging sensory and motor functions [1, 2]. After peripheral nerve transection, surgical repair is mandatory to allow proximal axons to grow into distal degenerating nerves. At present, the preferred and most productive surgical approach for transected nerve repair is an end-to-end suturing of the injured nerve ends, providing similar stump geometry at the interface [3]. However, direct nerve anastomosis is suitable for a small gap, and surrounding tissue could invade the anastomosis site leading to adhesion and painful neuroma formation [4-8]. In cases where the gap is large, injured nerve ends must be bridged by graft to reduce tension at the site. Autografts and allografts are ideal bridging materials, while the limited donor source restricts their practical application in transected nerve repair [9, 10]. Therefore, to improve the treatment effect of transected nerves, some nerve tissue engineering materials have been developed to wrap nerve anastomosis site for anti-adhesion or to bridge nerve stumps as an alternative to autografts/allografts [11, 12]. These materials include synthetic polymer films/conduits [13-16], chitosan films [17], collagen wrappers/conduits [18, 19], and stainless steel/silicon/silk conduits [20, 21]. Although the above-mentioned materials could promote the reconstruction of nerve to some extent, these are associated with some shortcomings, such as lack of bioactivity, poor biocompatibility, or long degradation period [22]. As a consequence of this, researchers around the world are still committed to developing better peripheral nerve repair biomaterials.

Recently, animal-based studies have shown positive outcomes using xenogeneic extracellular matrix (ECM)-derived biomaterials to repair peripheral nerve injuries [23-26]. The biomaterials derived from xenogeneic tissue have abundant donor sources. Most importantly, ECM is a native material that encompasses a variety of structural and functional proteins, such as collagen, proteoglycan, and laminin fibronectin, growth factors, and so on [27]. These ECM components might provide a native-like microenvironment that would favor Schwann cell proliferation and migration as well as axon growth [28-32]. To generate xenogeneic ECM

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for use in therapeutic applications, the excised tissue from non-primate mammals (typically pig) must first undergo physical, chemical, or enzymatic decellularization to remove cellular components, leaving behind only the non-cellular ECM [33]. Unlike transplanted tissue, decellularized ECM (dECM) has a lower risk for immune response because almost all cellular DNA is removed [34]. The specific method of decellularization depends on tissue type; for instance, while cartilage tissue can undergo a relatively harsh treatment, lung tissue requires a more sensitive decellularization method to preserve its tissue composition [35]. With proper decellularization, the native cell microenvironment components in ECM are preserved and could support cell growth and viability [36]. However, the decellularization process does present challenges, the foremost of which is maximizing cellular material removal with minimum damage to ECM [34]. In addition, compared to synthetic materials, dECM also has some disadvantages, such as poor mechanical strength and tenability. Thus, incorporating dECM into synthetic materials presents a promising method for better mimicking a native microenvironment for the repair of a peripheral nerve injury.

Hence, this article reviews the current methods for decellularization, the biological function of the main components of ECM, and medical applications of dECM for peripheral nerve repair.

2. GENERAL METHODS OF DECELLULARIZATION

Decellularization procedure has become one popular technique for obtaining dECM-based biomaterials, and it varies according to tissue source and desired end-product morphology. The ultimate goal of decellularization is to effectively remove cellular components while maximizing the retention of ECM components. If nucleic materials in dECM are incompletely removed or degraded, it might cause host foreign body reaction after implantation, which leads to the formation of the fibrous capsule surrounding the implant site [37, 38]. It eventually limits the regenerative potential of dECM and therefore be detrimental to nerve reconstruction [39]. Moreover, the maximum retention of ECM components is conducive for biomaterial applications as it provides native microenvironment and many nutrients (collagen, glycosaminoglycan, laminin, fibronectin, TGF-β, *etc*.) for nerve regeneration [40]. The most frequently used decellularization procedures including physical, chemical, and enzymatic processes, are discussed below.

2.1. Physical Decellularization

The common methods of physical decellularization include multiple freeze-thaw cycles, osmotic lysis, sonication, and electroporation. In freeze-thaw cycles, the formation of ice crystals punctures the cell membranes to cause loss of cellular contents. This method maintains the mechanical integrity of tissue and retains components of ECM, while it alone is insufficient at removing genetic material in most cases [41]. Another method is osmotic lysis, during which tissues are placed in either a hypertonic (salt solution) or hypotonic solution (deionized water) that ruptures plasma membrane *via* osmotic pressure [42, 43]. Also, ultrasound destroys cellular structures to achieve the effect of decellularization. Electroporation is another physical method of decellularization in which weak electrical impulses mediate the formation of cell membrane micropores by disrupting transmembrane potential [44]. The formation of micropores could destabilize cells and eventually lead to cell death. Although these physical decellularization methods alone have been used successfully in a few tissues, they are often used in conjunction with chemical or enzymatic methods to effectively clear genetic material from the tissue [45, 46].

2.2. Chemical Decellularization

Chemical methods of decellularization are divided into two sub-categories based on tissue treated with either acidic/basic solvents or detergents. Tissue treated with acids/bases results in cell degradation and loss of nucleic acids. The degree of successful decellularization varies according to the type and concentration of acid/ base being used, processing time, and the type of tissue being treated. Peracetic acid and formic acid are frequently used acids for the decellularization of different tissues. However, the base is considered one harsher option than acid because it could cause a significant loss of glycosaminoglycans [47, 48]. Preservation of glycosaminoglycans after decellularization plays an important role to maintain mechanical properties of tissue (such as tensile and viscoelastic properties) [48, 49] and retain its growth factors [50, 51], the latter of which have been linked with enhanced biocompatibility *in vitro* [52].

Chemical decellularization could also be performed through three main types of detergents, including ionic, non-ionic, and zwitterionic. All detergents have variable success in the disruption of cell-cell and cell-matrix attachments, while they could also change matrix-matrix adhesions. Sodium dodecyl sulfate (SDS) is considered to be one of the most potent ionic detergents because of its capability in the removal of cells by dissolving cell membranes. However, it also disrupts the integrity/structure of ECM that results in weak biomechanical properties of dECM [34]. Compared to SDS, Triton X-100 as non-ionic detergent has been more ECM-friendly because it can improve collagen retention of tissue while at the cost of less effective DNA removal [53]. To compensate for this, Triton X-100 has often been combined with sodium deoxycholate (SDC), which is an ionic detergent with greater biocompatibility than SDS [54]. In short, non-ionic detergents can maintain better basal lamina integrity, but ionic detergents can better remove cells [55]. Zwitterionic detergents such as 3-([3-cholamidopropyl] dimethylammonio)-1-propanesulfonate (CHAPS) have a net zero charge and possess characteristics of both ionic and non-ionic detergents [34]. Although zwitterionic detergents cause less protein denaturation than ionic detergents, they are also less capable of removing cellular material than ionic detergents [56, 57]. Some researchers have developed a novel approach for decellularizing peripheral nerve based on treatment with hypotonic and hypertonic solutions, and sub-

sequent treatment with Triton X-100 and CHAPS [58]. The resulting decellularized nerve grafts produced in this manner have favorable nerve regeneration capability used for bridging the nerve gaps [58].

In recent years, alcohol has been introduced into chemical decellularization, mainly targeting the lipids of tissues. Isopropanol-surfactant decellularization process has been proven to be effective in the decellularization of dense porcine skin while retaining higher amounts of ECM proteins than the above water/surfactant decellularization process [59].

2.3. Enzymatic Decellularization

Enzymatic decellularization agents are classified into nucleases and proteases according to the specific structures that they target. Nucleases, such as deoxyribonuclease and ribonuclease, hydrolyze phosphodiester bonds of DNA and RNA. Proteases act on proteins by hydrolyzing peptide bonds between amino acids. For example, trypsin as a serine protease can cleave the carbonyl side of lysine or arginine residues. Because of its activity on specific peptides, trypsin can severely disrupt some ECM proteins such as elastin and collagen [34]. In order to completely remove cellular components, enzymes are not used alone but as supplements to detergents to enhance the removal of antigen. For example, researchers found that Triton X-100 combined with enzyme digestion (nucleases and trypsin) was used to generate decellularized peripheral nerve showing a successful decellularization rate, fibrillar and non-fibrillar ECM molecules preservation, and adequate biomechanical properties *in vitro* [60]. Enzymatic decellularization agents are also used in conjunction with chelating agents such as ethylene diamine tetraacetic acid (EDTA), which disrupts cell-ECM adhesion by sequestering metallic ions such as calcium [34]. Among the possible combinations of chemical-nuclease-based methods, a combination of zwitterionic detergent and nuclease is currently regarded as the most suitable approach for nerve decellularization. The resulting decellularized nerve does not produce any cytotoxicity, while showing adequate removal of cells and sufficient preservation of ECM components [33].

In a word, treating tissue with the chemical and/or enzymatic agent does not result in an acceptable degree of decellularization due to limited diffusion into the tissue. Therefore, a combination of all three methods has a synergistic effect where physical agitation enhances tissue penetration depth of chemical and enzymatic agents, thereby facilitating the removal of cellular material [61, 62].

2.4. Evaluation of Decellularization Degree

Measuring the amount of double-stranded DNA (dsDNA) in dECM is the current gold standard for evaluating the degree of successful decellularization. Quantitative assay such as PicoGreen is performed to measure the amount of dsDNA in native tissue and dECM to make general conclusions on whether tissue has been sufficiently decellularized [63]. PicoGreen is a fluorochrome that selectively binds dsDNA and its fluorescence enhancement is exceptionally high when bound to dsDNA, and the unbound dye actually has no fluorescence. Crapo *et al*. have suggested three minimum criteria that tissue should satisfy to be considered successfully decellularized: (a) dECM should contain < 50 ng/mg dry tissue of dsDNA, (b) any remaining DNA fragments should be smaller than 200 base pairs, and (c) dECM stained with DAPI or hematoxylin & eosin should not have visible nuclear material [34]. Besides, it is important to evaluate the change of dECM structure and components to ensure minimal disruption of native ECM. For structure analyses, imaging techniques such as scanning/transmission electron microscopy [64] and microcomputed tomography [65, 66] could be performed to compare ECM structure before and after decellularization. For more quantitative analyses, dimethylmethylene blue DMMB assay [67], hydroxyproline assays [68], and high performance liquid chromatography [40, 69] can be performed to measure the amount of glycosaminoglycans, total collagens, and other components in dECM, respectively. These assays are well-established and help investigators to draw more concrete conclusions about the efficacy of the decellularization protocol used.

Also, removal and detection of residual compounds (*e.g.* detergents, enzymes and chemicals) is an important endpoint in generating dECM, because exceeding a certain concentration of residual compounds may affect the biocompatibility of dECM [70]. Extract repeatedly with distilled water is the main method to the removal of most residual compounds in dECM. In order to assess whether the residual trace compounds in dECM will affect the biocompatibility of dECM, cytotoxicity assay as an important method should be performed to ensure the concentration of residuals in a safe range.

3. MAIN COMPONENTS OF DECM

The native ECM consists of structural and functional molecules secreted by the resident cells of each tissue and organ; therefore, the specific components and component distribution of ECM varies depending on the donated tissue source. The evidence suggests that dECM, especially those derived from site-specific tissues, is more useful for reconstructive tissues than non-specific tissue-derived materials [71-73]. The peripheral nerve-derived dECM has been proved to play an important role in regulating Schwann cell functions, including adhesion, survival, spreading, and myelination as well as in supporting neurite outgrowth [28, 74]. For instance, fibronectin promotes the greatest Schwann cell spreading area, while laminin is most effective in enhancing Schwann cell proliferation, cellular elongation, and c-Jun expression [75]. Since the composition and proportion of porcine peripheral nerve ECM are very much similar to that of humans [29], this review focuses on discussing ECM components of porcine peripheral nerves, including polysaccharides, proteins, and growth factors.

3.1. Polysaccharides

Glycosaminoglycans are a family of highly charged, unbranched heteropolysaccharides that play a key role as struc-

tural and functional components of native ECM. Due to the capacity of glycosaminoglycans to retain large amounts of water, it enables ECM hydration to endow tissues with mechanical stability [76]. Moreover, several studies have identified that glycosaminoglycans can regulate numerous physiological and biological processes by binding with other bioactive molecules, such as fibroblast growth factor (FGF), to involve downstream cellular responses [77]. Also, the ability of glycosaminoglycans to specifically interact with growth factors can protect these growth factors from proteolysis or inhibiting factors [76]. Importantly, studies have shown that glycosaminoglycan-functionalized scaffolds are effective in modulating Schwann cell behavior in terms of adhesion, proliferation and differentiation, and should be considered in strategies involved in promoting peripheral nerve regeneration [78].

3.2. Proteins

The main protein components of ECM include collagen, laminin, fibronectin, elastin, and so on. By dry weight, peripheral nerve ECM is composed of greater than 90% collagens, which not only provides structural support but also affects cell behavior by triggering intracellular signals [79, 80]. Collagens consist of three identical triple helical α chains, and are divided into different groups according to the structures they form [81]. The main sub-families are fibril-forming collagens (types I, II, III, V, and XI), collagens banded-fibrils associated (types IX, XVI, XIX, XXI, and XXII), networking collagens (types IV, VI, VIII, and X), transmembranous collagens (types XIII, XXIII and XV), endostatin precursor collagens (types XV and XVII), and other collagens. However, the large majority of collagens in peripheral nerve ECM are type I, with minor amounts of collagen types III, IV, V, and VI also present [82].

Laminin secreted by Schwann cells is mainly found in the basal lamina, and laminin-2 (a2, b1, g1) and laminin-8 (a4, b1, g1) are the major laminins of peripheral nerves [83]. The capability of the basal lamina to sustain cell differentiation, migration, adhesion activities, and axon growth after nerve injury is highly dependent on laminin [84, 85]. Fibronectin is the other major component of non-collagen proteins of peripheral nerve ECM [86]. In the nervous system, fibronectin is synthesized and secreted by Schwann cells and fibroblasts, and forms a fibrillar matrix similar to collagen and mediates cell binding [87]. Moreover, laminin and fibronectin have the characteristic of binding to other ECM components to form a firm microstructure. For instance, laminin interacts with nidogens, agrin, perlecan, fibulin-1, heparin, and sulfatides [88], whereas fibronectin binds to collagen and fibrin [89]. In addition, there are other protein molecules of ECM which are probably not very much related to axonal regeneration after nerve injury. Elastin contributes to maintaining the mechanical properties of ECM [80], and vitronectin binds to collagens and glycosaminoglycans, acting as a regulatory molecule controlling cell adhesion [90]. Nidogen-1 (also called entactin) forms non-covalent unions with laminin and collagen type IV, and might play its role as a pro-migratory factor for adult Schwann cells [91].

According to the function of these ECM proteins, they are used alone or in combination with nerve tissue engineering materials. The fibronectin mats developed as oriented substrates could improve the longitudinal migration of Schwann cells *in vitro* [92]. When fibronectin-oriented strands were introduced in nerve conduits to repair rat sciatic nerve, axonal regeneration and Schwann cell recruitment were better than when using freeze-thawed muscle grafts [93]. Collagen-laminin-based matrices have been successfully used to enhance axonal regeneration in nerve guides [94]. It has been found that dissociated sensory neurons show longer neurite extension on laminin-fibronectin-coated substrates compared to poly-L-lysine-coated surfaces [95].

3.3. Bioactive Factors

Various bioactive factors are present in peripheral nerve ECM, including nerve growth factor (NGF), FGF, platelet-derived growth factor, insulin growth factor-1 (IGF-1), heparin sulfate proteoglycans (HSPG), transforming growth factor beta (TGF-β), and so on [40, 96]. The nerve tissue-derived dECM contains these bioactive factors even after decellularization procedure, terminal sterilization, and long-term storage [69]. It is well known that NGF plays an important role in the growth, maintenance, and survival of neurons, and supports peripheral nerve regeneration in a rat model [97, 98]. Both FGF and IGF-1 encourage Schwann cells' growth and proliferation [96, 99, 100], and HSPG are known mediators of Schwann cells spreading on laminin-based surfaces [52]. However, TGF-β is secreted by macrophages and resident Schwann cells after peripheral nerve injury [101, 102]. TGF-β regulates RSC96 Schwann cells' migration and invasion to allow axonal re-growth through increasing MMP-2 and MMP-9 mRNA and protein levels and activities [103].

In conclusion, dECM is an ideal material for nerve tissue engineering because of its complex components with a variety of diverse bioactive molecules and its role in promoting nerve regeneration. Researchers have developed several types of dECM-based nerve repair materials which are discussed below.

4. APPLICATIONS OF XENOGENEIC DECM-BASED BIOMATERIALS

Porcine dECM-based biomaterials are an ideal nerve repair material as porcine tissue is very similar to human tissue in terms of its anatomical, biochemical, and cellular components [29]. For example, decellularized porcine nerves retain different kinds of functional proteins, including collagens, glycosaminoglycans, proteoglycans, and growth factors, which provide a native-like microenvironment to favor Schwann cells' proliferation and axon growth [29-32]. The commonly used xenogeneic dECM-based biomaterials are mainly classified into three categories: hydrogel, wrap, and scaffold.

4.1. Hydrogel

The method of fabricating a hydrogel using solubilized dECM is as follows: the tissue following decellularization is

digested using pepsin to create a homogeneous solubilized dECM solution that undergoes thermal gelation at physiological temperature and pH. The pepsin digestion is the most widely used method for solubilizing dECM, however, the bioactivity of pepsin-digested dECM remains controversial [104, 105]. In this regard, several studies have explored the possibility of using urea to extract soluble components of dECM [106, 107]. Urea is a chaotropic agent that disrupts hydrogen bonding resulting in the denaturation of proteins and the disruption of the interaction between lipids and proteins. Urea-extracted dECM has higher concentrations of small and moderate molecular weight proteins compared to pepsin-digested dECM, which primarily consists of collagen chains [106]. The earliest dECM-derived hydrogel was created with porcine decellularized small intestine sub-mucosa (SIS) [108]. Later, the porcine nerve-derived dECM-based hydrogel was made and has shown a good performance to promote neurite outgrowth of dorsal root ganglion (DRG) and wrapping of Schwann cells around neurites *in vitro* [69]. Due to its physical characteristics and promotion of Schwann cells' proliferation, the dECM-based hydrogel is commonly used as a lumen filler of nerve guidance conduit, or as a Schwann cells' transplantation carrier [27, 109, 110]. Recently, hydrogels as a new biodegradable and biocompatible matrix material are able to carry and deliver several factors (such as glial-derived neurotrophic factor) that are important for axonal re-growth and neuronal survival [111]. Although dECM-based hydrogel is highly malleable, it loses ECM native structure for the change of protein ultrastructure, and is also easily contaminated by bacteria because of its moisture content and the open environment in which it is used.

4.2. Wrap

For direct end-to-end suturing of the injured nerve, a nerve wrap for anastomosis site is necessary to prevent connective tissue invasion into the anastomosis site and promote nerve regeneration. After membrane tissue is decellularized, such as porcine SIS-dECM and fetal urinary bladder dECM (fUB-dECM), it is used directly to wrap the nerve anastomosis site [112]. However, dECM-based wrap derived from non-membraneous tissue, such as sciatic nerve, undergoes a series of molding process as follows: the nerve derived-dECM is transformed into a powder, the resulting powder is solubilized in 0.3% acetic acid, the digested solution is transferred to a plate, and the solution-to-membrane transition of dECM is induced by drying at room temperature [40]. At present, there are commercial dECM-based wraps used in clinical nerve repair surgery. For example, AxoGuard® Nerve Protector is a material similar to the nerve epineurium formed by incorporating the patient's own cells into dECM, which is designed to protect and isolate the nerve anastomosis during post-operative healing [113].

4.3. Scaffold

The nerve repair scaffolds are commonly used as grafts for bridging the nerve stump. The human peripheral nerve-derived scaffolds, which possess microstructure and components of a human nerve and well biocompatibility, are optimal nerve grafts for humans [114]. For example, Avance® Nerve Graft is an existing product in the market, which is manufactured using dECM obtained from the donated human peripheral nerve. Seven patients received this allograft to repair their nerve defects of 0.5-3 cm, and they recovered well without infection and rejection after surgery [115]. Except for peripheral nerves, vessels (veins and arteries) and muscles derived from humans, after decellularization, also as a conduit are widely used for connecting the proximal and distal stump of peripheral nerves in animal models and in clinical practices [115-119]. However, human tissues have limited donor sources; many researchers have found that porcine tissues might be the best alternative. As a commercial conduit, AxoGuard® Nerve Connector is derived from porcine tissues, which has been successfully used to bridge nerve stumps with less than a 5 mm gap [113]. Although these xenogeneic tissues provide a rich source of donor, the weak mechanical properties of plain dECM-based scaffolds remain a challenge to be addressed. The mechanical properties of these plain dECM-based scaffolds are affected by the manufacturing process (*e.g.*, decellularization process) and by the age and health status of the animal from which dECM is obtained. Some ways could be manipulated to tailor their mechanical properties. For instance, the mechanical properties of thin-sheet form dECM (*e.g.*, SIS-dECM and fUB-dECM) are strengthened by creating multilaminate dECM structures of a varying number of layers [120]. The mechanical properties can also be increased by cross-linking with chemicals, such as glutaraldehyde, carbodiimide, and hexamethylene-diisocyanate, or by non-chemical methods [121]. However, cross-linking reduces the degradation rate of dECM-based scaffolds *in vivo* and changes the response of host tissues from anti-inflammatory and structural remodeling response to pro-inflammatory and foreign body response [122, 123]. Moreover, combining a synthetic material with dECM may endow dECM-based scaffolds with better mechanical properties. These hybrid dECM-based scaffolds hold the advantages of both types of materials, including the mechanical and material properties of synthetic materials and bioactivities of dECM. Similarly, the scaffolds also manifest the disadvantages of both materials, for example, the host inflammatory response to many synthetic materials and the biological variability of dECM. The commonly used hybrid xenogeneic dECM-based scaffolds mainly include electrospun scaffolds and 3D-printed scaffolds.

4.3.1. Electrospun Scaffolds

To improve the property of electrospun nerve scaffolds, many attempts have been made to combine synthetic polymer and dECM. dECM breaks up into particles and dissolves in isopropanol following addition of polycaprolactone (PCL) to develop hybrid dECM-containing electrospun scaffolds through the blending of electrospinning [124]. The resulting dECM-containing electrospun scaffolds show higher glycosaminoglycans and collagen content compared to PCL scaffolds. Similarly, Schwann cells are seeded on D,L-

polylactic-polyglycolic acid-silk fibroin-collagen (PLGA-SF-COL) scaffolds fabricated through the blending of electrospinning, and the results confirm that PLGA-SF-COL scaffolds are more suitable for nerve tissue engineering in comparison with PLGA scaffolds [125]. Besides the blending of electrospinning, surface conjugation has been used to modify the surface of electrospun polyhydroxyalkanoate with dECM particles [126]. These electrospun scaffolds have been proved to promote peripheral nerve regeneration *in vivo* and *in vitro* due to their common characteristics, including good mechanical properties, biomimetic microstructure, and abundant bioactive molecules.

4.3.2. 3D-printed Scaffolds

3D printing of PCL fibers is a relatively well-characterized method to create porous scaffolds for tissue engineering applications [127, 128]. Such 3D printing technology is rapidly emerging as a key scaffold fabrication strategy for mimicking native tissue complexity. Meanwhile, dECM bio-ink derived from animal organs has received great attention because of its excellent biocompatibility. Many researchers have found that the versatility and flexibility of the bio-printing process have been developed with tissue-specific dECM bio-inks, including adipose, cartilage, liver, and heart tissues [84, 85, 129]. In recent years, 3D printing technology has been used in peripheral nerve repair application [130]. However, the addition of dECM alone without any modification would reduce the printability and mechanical properties of dECM-based biomaterials, thereby limiting the chance of producing an ideal nerve conduit. Recently, polydopamine (PDA) has been introduced for 3D-printed dECM-based biomaterials' modification due to its optimal cell adhesion behaviors [131-133]. A novel dECM and polydopamine (PDA)-coated 3D-printed PCL-based conduits have been fabricated, whereby the PDA surface modification acts as an attachment platform for further dECM attachment [134]. The resulting conduits possess strong mechanical properties as compared to the nerves of humans or animals. This property makes conduits more tear-resistant for surgical procedures and handling. Moreover, PDA improves the hydrophobicity of conduits and increases dECM coating amount on them. The PDA-modified dECM-based biomaterials have been proved to possess a higher level of laminin and collagen, which further enhances Schwann cells' adhesion, proliferation and differentiation, and increases expression of cell-specific neuronal-markers; Nestin, TUJ-1, and MAP2 for promoting nerve regeneration *in vivo* [134].

CONCLUSION AND FUTURE PERSPECTIVES

Xenogeneic tissues have a wider range of donated sources than autologous/allologous tissues for humans, so more and more researchers use dECM derived from xenogeneic tissues or organs to develop various peripheral nerve repair biomaterials according to different clinical requirements. Many studies have shown that xenogeneic dECM-based biomaterials have numerous superiorities and potentials, both in pre-clinical and clinical applications, due to biological advantages. However, the existent challenges are still hindering the more profound applications of xenogeneic dECM-based biomaterials, such as how to retain the maximized active components in initial preparation, sterilization, preservation, and other processes. The tissue sources and storage conditions, and variability in tissue processing (from isolating tissue to decellularizing) could influence the quality of dECM, resulting in low reproducibility even within the same tissue type. Therefore, with further research, many operational processes have been optimized. For instance, different decellularization methods following flexible guidelines have been devised to remove cellular components of native tissue while preserving higher amounts of ECM components [39]; however, it is difficult to draw conclusions on which method is best for a specific application. Hence, balancing the constraints between removing enough cellular components and retaining ECM components is a challenge that requires further investigation.

Another problem hindering the application of xenogeneic dECM-based biomaterials is the absence of Schwann cells and their ability to continuously produce neurotrophic factors, thus dECM-based biomaterials lack consistent biological microenvironment provided by an autologous nerve [135, 136]. Xenogeneic dECM-based biomaterials combined with bioactive molecule sustained release system and/or cells (*e.g.*, Schwann cells) as innovative nerve repair materials might resolve the above-mentioned issue and prove beneficial for peripheral nerve regeneration. In addition, the most significant deficiency of dECM-based biomaterials is the potential immune response which might negatively affect their repairability [137]. For example, many ECM glycoproteins and proteoglycans of porcine are rich in the expression of gal epitopes which will not be completely eliminated during the preparation of dECM-based biomaterials, so the risk of host immune rejection and homologous incompatibility still exists [138, 139]. Recent studies have found that cell-derived dECM, such as Schwann cell- and bone marrow stromal cell-derived dECM (BMSC-derived dECM), can overcome the drawback of this xenogeneic tissue-derived dECM [140, 141]. Purified BMSCs co-cultured with a chitosan nerve guidance conduit (NGC) and silk fibroin filamentous fillers, and intracellular components are removed to obtain a BMSC-derived, dECM-modified neural scaffold, which is implanted into rats to bridge a 10 mm-long sciatic nerve gap and has a good performance [140]. In future research, cell genetic modification technology might also be introduced to develop more and more novel cell-derived dECM-based biomaterials that could meet various peripheral nerve repair needs.

The manufacturing processes deprive xenogeneic dECM-based biomaterials of natural ECM microstructure, limiting our ability to engineer dECM-based biomaterials to adequately mimic tissue-specific ECM architecture biochemistry, also limiting their more advanced applications in the clinic. Therefore, many researchers are constantly improving the technology for developing xenogeneic dECM-based biomaterials, adopting more methods combined with a broader knowledge background to integrate it into the history of biomedicine, such as 3D printing technology. Although

dECM is an ideal 3D printing bio-ink, its low viscosity and slow cross-linking speed make it inappropriate for the layer-by-layer process for the construction of 3D structures *via* bio-printing. The main component of dECM bio-ink is thermoresponsive collagen; thus, its rheological property continuously varies during the printing process, which makes it difficult to maintain consistent printability over long processing times [142]. Also, it is difficult to obtain repeatable physical properties because of the heterogeneity of the biochemical composition of tissue and the process conditions of preparing dECM bio-ink. For example, pepsin digestion for solubilizing decellularized tissue, which is a time-consuming process of preparing conventional dECM bio-ink, could result in the denaturation of some biochemical components of dECM, including protein and growth factors, and loss of mechanical strength of dECM [129, 143-146]. Thus, weak mechanical properties, low repeatability of physical properties, reduction of biochemical components, and slow cross-linking speed of the conventional dECM bio-ink are the major obstacles to its application to 3D bio-printing technology. In this regard, several researchers introduced new technologies to improve the mechanical properties and 3D printability of the conventional dECM bio-ink [147]. These resulting dECM-based bio-inks prepared by loading the dECM microparticles into a gelatin mixture could provide an optimized physically and biologically suitable microenvironment for cell growth. Thus, the future of dECM-based biomaterials in applications of peripheral nerve repair is promising.

To summarize, a large portion of the current work still focuses on the development of decellularization methods, while utilization of dECM native biological characteristics will be the key to providing viable therapeutic applications for nerve regeneration. The specific component of dECM-based biomaterials that promotes the cell behaviors, tissue regeneration, and angiogenesis is currently unclear, and related cellular and molecular mechanisms are also worth studying in the future.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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