Journal of Translational Science

Mini Review

ISSN: 2059-268X

Decellularized scaffolds for neuronal regeneration

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The extracellular matrix (ECM) provides the three-dimensional structure of tissues, and is required for cell homing and cell viability, as well as for the overall homeostasis of tissues and organs [1,2]. The dynamic and complex microenvironment that the ECM generates in a specific manner for each tissue guarantees its functions [1,2]. During tissue regeneration ECM has been shown to play an essential role in controlling the tissue-stem cell compartment and to be involved in tissue regeneration outcome [3-6]. Tissue engineering combines extracellular natural and/or synthetic scaffolds (biomaterials) with stem cells and growth factors for the development of regenerative medicine strategies and the treatment of diseased tissues [7]. Despite the fact that incredible improvements have been achieved in biomaterial manufacturing, the peculiar and complex biochemistry, biomechanics and 3D organization proper of a tissue-specific ECM still cannot totally be reproduced in the lab [1,2,8]. Such complexity can however be preserved in scaffolds that take advantage of the native tissue themselves, as decellularized tissues or whole organs [9-11]. Decellularization process remove cellular and nuclear content retaining ECM mechanical integrity, biological activity and 3D architecture of the native tissue [10]. Decellularized tissues and/or organs represent alternative and promising scaffold material for the treatment of clinical cases in which extensive regeneration of an organ is required, as in cases of traumatic injuries, surgical ablation and congenital diseases [12]. Decellularized scaffolds have already been obtained from different organs and used for regenerative medicine strategies in animal models, as well as in clinical trials [12,13]. Ideally scaffold implantation should allow regeneration of the tissue of interest and guarantee the functionality of the targeted organ. In particular, support in reinnervation is a crucial aspect for the final outcome of tissue functionality in those organs in which the nervous system plays pivotal roles (i.e. muscle, heart, sensory organs). Here we will summarize the state of the art regarding the possible use of decellularized scaffolds for spinal cord and peripheral nervous system (PNS) regeneration, and tissue reinnervation.

Despite the ability of PNS to maintain a grade of regeneration after injury, central nervous system (CNS) axons do not regenerate appreciably in their native environment [14-16]. In PNS, after a nerve transection, macrophages and Schwann cells clear myelin and axonal debris, and produce cytokines that enhance axon growth [17,18]. After debris clearance, regeneration begins at the proximal end of the damage and continues toward the distal stump with a section of new tissue known as 'the bridge' and is composed of inflammatory cells, perineurial cells, fibroblasts and ECM [14,18]. For the complete functional reinnervation of the organs, axons have to extend until they reach their distal target [15]. This requires the formation of cellular cords of Schwann cells (dedifferentiated to progenitor-like cells) and fibroblasts, which transport the axons across the bridge along the surface of polarised blood vessels [14]. Differently, when CNS undergoes injury the regeneration capability is inhibited by the blood-spine barrier, which reduce the infiltration of macrophages at the site of injury, delaying the removal of inhibitory myelin and resulting in a glial scar formation [15]. However, in case of a severe injury the regeneration of spinal cord, or PNS or tissue innervation fails [15,16].

To develop new strategies for spinal cord and PNS regeneration, natural or artificial synthetic materials have been extensively used [19-24], and spinal cord biocompatible decellularized scaffolds were first obtained in 2010 [25]. Liu and colleagues provided evidence that decellularized spinal cord scaffolds seeded with human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) are able to bridge a spinal cord cavity and promote long-distance axon regeneration and functional recovery in a spinal cord injury (SCI) rat model [26]. This study supports the hypothesis that decellularized spinal cord can provide a favourable environment for host oligodendrocyte differentiation, proliferation and axonal remyelination, which promotes endogenous regeneration of neural cells [26]. However, the influence of hUCB-MSCs, which secrete a number of cytokines useful for SCI recovery [27], in the general outcome of the regeneration was not clear. More recently, the ability of decellularized spinal cord to promote regeneration *per se* (without pre-seeding cells) has been demonstrated [28,29]. Scaffolds provided the right topography and direction for nerve axon regeneration of removed T9-T10 cord segments in rats, with improvement in locomotor function compared to the untreated SCI group [28]. Xu and collaborators also showed how decellularized spinal cord can be used in combination with trophic factors to improve the regenerative properties of the implanted matrix [29]. Remarkable results in terms of functional recovery in SCI models have been obtained also using injectable decellularized extracellular matrix derived from brain [30], meninges [31] or peripheral nerve [32].

Decellularized nerves have also been used in animal models [33] and for clinical application [34]. In sciatic nerve replacement rat models, decellularized nerves promoted axonal regeneration and motor function recovery [35]. Interestingly, bone marrow-mesenchymal stromal cells (BM-MSCs) embedded in fibrin glue and injected around the graft helped to improve nerve regeneration and functional recovery [35]. Importantly, clinical application of a commercial decellularized nerve graft (AxoGen®, AxoGen Inc, Alachua, FL) showed promising results. Patients with digital nerve defect showed no signs of infection

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Received: January 10, 2020; **Accepted:** January 20, 2020; **Published:** January 23, 2020

or rejection after decellularized nerve implantation, and reported sensory improvement in a follow-up study [36].

The ability of decellularized scaffolds to promote *in vivo* regeneration of neuronal compartment in innervated tissues has been investigated in different organs and by using scaffolds derived from several sources. Decellularized small intestinal submucosa (SIS) scaffolds used to repair esophagus [37] and bladder [38] defects (in rats and canine models respectively) allowed *de novo* innervation of regenerated implants. Importantly, in the esophagus it was shown that decellularized SIS scaffolds promoted regeneration of synaptic active area, with functional activity of the tissue characterized by contractile responses to nerve stimulation [37]. Typical features of amyelinic fibers being surrounded in groups by single Schwann cells were identified in decellularized heart valves in pig models of valves transplantation [39]. Decellularized cornea scaffolds promoted active reinnervation of the implant by the host cells, as confirmed by the presence of multiple nerve filaments 4 months after transplantation [40].

Considering the essential role of the nervous system in skeletal muscle function, a number of studies investigated with more detail the ability of implanted decellularized scaffolds in supporting axon invasion. We recently showed that two months after implantation in mouse, a decellularized muscle xenograft promoted tissue regeneration in a volumetric muscle loss mouse model, which included neuronal compartment and active neuromuscular junction [41]. In agreement with this, other studies demonstrated that decellularized skeletal muscle scaffolds implanted *in vivo* are able to restore not only muscle mass, but also muscle innervation and functional recovery [42-44]. In particular, improved maturation of neuromuscular junctions was observed in exercised mice subject to tibialis anterior volumetric muscle loss damage [43]; in a diaphragmatic hernia mouse model, decellularized scaffold was able to guide nerve attraction and re-growth and to direct development of new neuromuscular junctions [44]. Moreover, an interesting work investigated the possibility to recreate *ad hoc* decellularized scaffold for oriented tissue regeneration [45].

In recent years, remarkable progresses have been reached in the use of biomaterials for promoting neuronal regeneration. Regardless that further studies are required to understand the role of tissue-specific decellularized matrices in neuronal regeneration, decellularized scaffolds still represent promising biomaterials for the development of alternative regenerative strategies [46]. It was recently shown that the combination of removable polymeric microfibers and decellularized matrix allowed the generation of decellularized ECM scaffolds with aligned microchannels able to guide proliferation and differentiation of nervous cells *in vitro*, and to sustain tissue repair *in vivo* [45]. The technical integration of synthetic biomaterials and decellularized scaffolds, as whole tissues, as well as injectable gel [47,48] or bio-ink suitable for bioprinting [49,50], represents a powerful instrument to finely manipulate three-dimensional scaffolds and therefore to instruct cell behaviour and improve the overall outcome of tissue regeneration.

Acknowledgments

This work was supported by STARS Starting Grant 2017 (Grant Code: LS3-19613).

Conflicts of interest

No competing interests exist.

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