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Review

Using extracellular matrix for regenerative medicine in the spinal cord

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ABSTRACT

Regeneration within the mammalian central nervous system (CNS) is limited, and traumatic injury often leads to permanent functional motor and sensory loss. The lack of regeneration following spinal cord injury (SCI) is mainly caused by the presence of glial scarring, cystic cavitation and a hostile environment to axonal growth at the lesion site. The more prominent experimental treatment strategies focus mainly on drug and cell therapies, however recent interest in biomaterial-based strategies are increasing in number and breadth. Outside the spinal cord, approaches that utilize the extracellular matrix (ECM) to promote tissue repair show tremendous potential for various application including vascular, skin, bone, cartilage, liver, lung, heart and peripheral nerve tissue engineering (TE). Experimentally, it is unknown if these approaches can be successfully translated to the CNS, either alone or in combination with synthetic biomaterial scaffolds. In this review we outline the first attempts to apply the potential of ECM-based biomaterials and combining cell-derived ECM with synthetic scaffolds.

1. Introduction

Unlike most other organs/tissues, the central nervous system (CNS) has a limited capacity to regenerate and traumatic injuries to the CNS are associated with a poor prognosis. Injuries to the spinal cord lead to functional motor and sensory loss by disrupting long distance projecting axons in white matter tracts, so far no widely accepted treatment strategies are available [1]. The only commonly used interventions are surgical stabilization of damaged vertebrae and intensive rehabilitation [1]. Different experimental treatment strategies are under investigation to promote recovery after spinal cord injury (SCI), with cell and/or pharmaceutical delivery as the most common approaches [2,3].

Biomedical materials are used in regenerative medicine approaches to replace or restore the anatomic structure and function of damaged or missing tissues following any injury or disease by combining the topographical cues of biomaterials with cells or bioactive molecules [1]. Tissue engineered scaffolds intended for CNS repair are often based on particular extracellular matrix (ECM) molecules (e.g. fibrin, collagen, fibronectin [4–6]), other natural polymers (alginate, agarose, chitosan [7–9]) or synthetic polymers (e.g. poly(-hydroxy acids), poly(2-hydroxyethyl methacrylate), polyethylene glycol [10–12]). While natural/synthetic polymer blends are widely accepted as potential materials [13,14] the use of ECM grafts is uncommon [15–19]. However decellularized tissue grafts used for regeneration in other systems (e.g. peripheral nerves [20,21], heart [22], and skin [23]) have shown great promise. Here we discuss the possibility of combining orientated scaffolds of synthetic biomaterials with cell-derived ECM to promote CNS repair.

2. Pathophysiology of SCI

The severity of SCI depends on the level, type and intensity of injury but includes permanent locomotor and sensory deficits, and may lead to neuropathic pain, spasticity, urinary and respiratory dysfunction, metabolic problems as well as psychological problems. The primary, mechanical injury leads to damage of numerous nerve fibre pathways in the white matter and possibly also cells in the grey matter. The mechanical disruption of the highly organized cytoarchitecture of the spinal cord has devastating effects on both the primary injury zone and the injury parenchyma. The primary injury is followed by secondary degenerative events including apoptosis, bleeding, excitotoxicity, free-radical production, inflammation, ischemia, oedema, scarring and cystic cavitation, all of which contribute to an increase in tissue loss [24,25].

Inflammatory processes are a major contributor to secondary degeneration, which take place over a time scale of hours, days, weeks and even months after the injury [26]. Microglia and also

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astrocytes represent intrinsic immunocompetent cells. If the blood-spinal cord barrier is disrupted, extrinsic inflammatory cells are recruited (neutrophils, macrophages, monocytes, lymphocytes, and natural killer cells). These inflammatory cells express proteolytic enzymes which degrade ECM proteins and contribute further to blood–brain barrier degradation and oedema. Inflammatory cells and (to a lesser degree) astrocytes express a wide range of pro-inflammatory cytokines and chemokines (e.g. interleukin (IL)-α, interferon (INF)-γ, tumour necrosis factor (TNF)-α) as well as reactive oxygen species, oxidative enzymes, and metalloproteinases, which exacerbate secondary tissue damage and the formation of an inhibitory glial scar [27,28]. However, the immune response may also contribute to tissue repair by clearance of hemorrhagic and necrotic tissue, reducing the lesion size and the release of trophic factors and cytokines which can be neuroprotective and promote axonal regeneration (e.g. interleukin (IL)-10, transforming growth factor (TGF-β)) [27,29].

The final, chronic stage of SCI is characterized by Wallerian degeneration, death of oligodendrocytes, fluid-filled cyst formation within grey and white matter and the development of the glial scar [3,25]. The glial scar represents a major mechanical and chemical barrier (e.g. chondroitin sulphate proteoglycans (CSPGs)) to axonal regeneration [30]. In lesions that spare the dura mater, the scar is composed primarily of astrocytes, but in more severe lesions (which open or rupture the meninges) invading connective tissue elements (e.g. fibroblasts, endothelial cells, ependymal cells) intermingle with the astrocytes [31,32]. Furthermore, depending on lesion type and severity, Schwann cells migrate from the adjacent nerve roots into the lesion side and come into close contact with the reactive astrocytes [31,32]. In the end, this glial scar encapsulates a cavity, which can be many times the size of the initial wound [33].

Fig. 1 shows such a fluid-filled cavity six days after a unilateral compression injury to the rodent spinal cord. Surrounding the edge of the cavity, an intense staining of GFAP can be seen, which identifies the intermediate filaments within astrocytes. It is important to note that GFAP, a common marker for astrocytes, identifies internal filaments of this cell. The cell membrane of astrocytes, however, is markedly larger and the density of such reactive astrocytes at the edge of the cyst is particularly intense. Internally, such a cyst is almost exclusively filled with activated macrophages at this time point, emphasizing the role that inflammation plays in the cyst formation. There is also recent evidence that intermediate filaments of glial cells significantly contribute to the local stiffness of the tissue. Kas and colleagues correlated the GFAP intensity with the viscoelastic properties of glia-rich retinal slices that underwent ischemia [34]. It is an important finding since increasing the stiffness of glial scar tissue would further impede any neurite growth into this region. Conversely, the formation of a glial scar has a beneficial role during the acute phase (1–2 weeks) after SCI. When reactive astrocytes were eliminated or prevented from contributing to scar formation after CNS injury, the result was a failure in blood–brain barrier repair, accompanied by massive inflammatory cell infiltration, increased loss of neurons and oligodendrocytes, and the eventual worsening of the functional outcome [35,36].

The lack of any functionally significant axon regeneration is mainly due to an imbalance of local axon growth-promoting and growth-inhibitory mechanisms. Neurotrophic factors and guidance

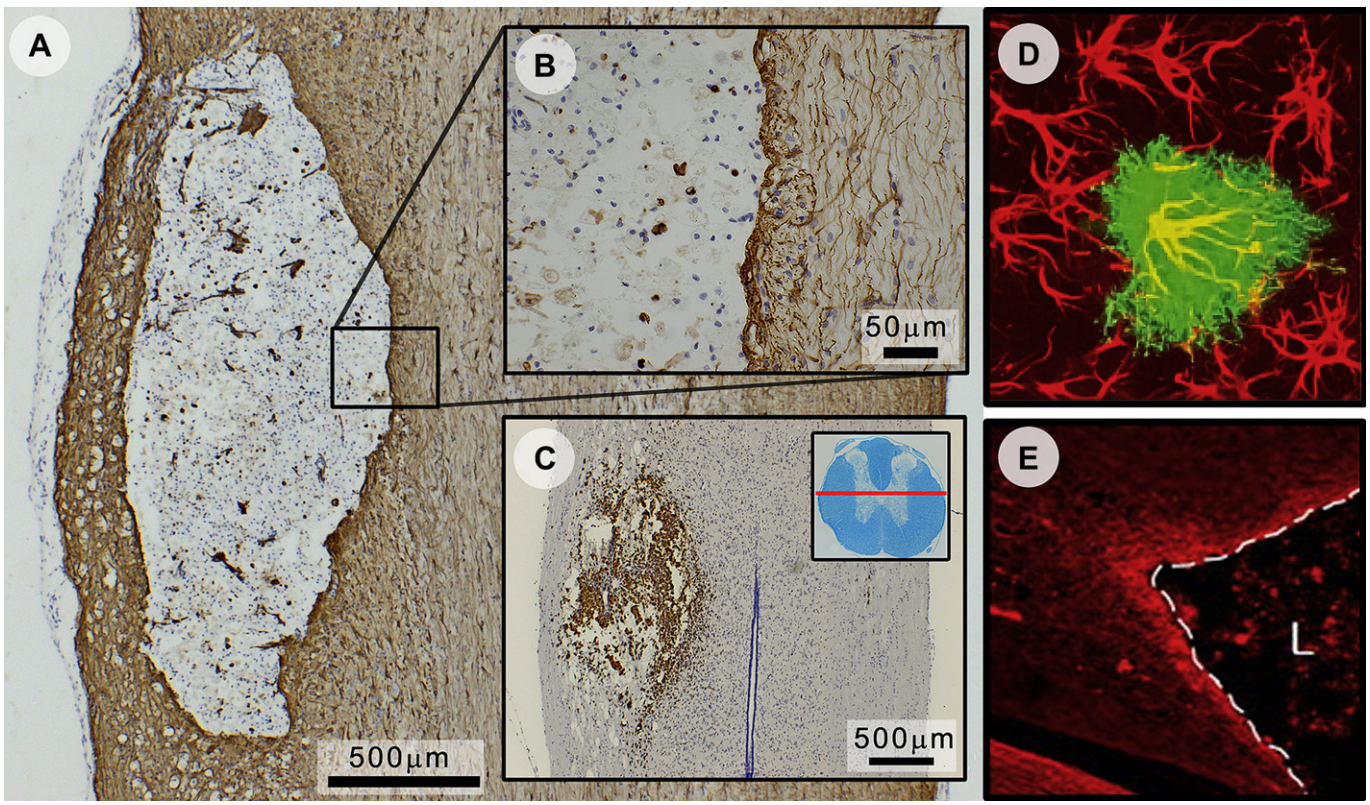

Fig. 1. A–C: Immunohistological sections of the rodent spinal cord six days after injury showing a debris-filled cyst, surrounded by a dense glial scar, which is impermeable to regenerating axons. B: Higher magnification of the glial scar with GFAP staining. C: Glial scar stained for CD68+ cells, which are activated macrophages/microglia. The insetin C is luxol fast blue that stained myelin and visualizes the white and grey matter, with the red line showing the plane of sectioning for A–C. D: injection of a single astrocyte with a florescent dye demonstrates the volume of an astrocyte. E: Glial scar at 4 weeks stained for neurocan, an ECM known for inhibiting neurite outgrowth. A–C: Unpublished Images. D: Republished with permission of the Society for Neuroscience, from Wilhelmsson et al. [210]; permission conveyed through Copyright Clearance Center, Inc. and E: Reprinted from Huang et al. [211] with permission from Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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