Research report

An acellular cerebellar biological scaffold: Preparation, characterization, biocompatibility and effects on neural stem cells

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ABSTRACT

Biomaterial and regenerative medical research has diversified and developed rapidly. A biological scaffold consisting of an extracellular matrix (ECM) functions not only as a supportive material but also as a regulator of cellular functions. Although decellularized scaffolds have been widely applied for the repair of non-central nervous system (CNS) tissues, their efficacy in the CNS has not been extensively investigated. In this report, we describe a dynamic decellularization protocol that combined intracardial perfusion and a series of treatments to effectively remove the cellular components from the cerebellum, which is a unique and relatively simple CNS structure. The resulting cerebellar scaffold retained neurosupportive proteins and growth factors and, when tested with neural stem cells (NSCs) in vitro, was found to be cytotocompatible and to stimulate the proliferation and migration of these cells. NSCs that were cultured in vitro on the scaffold differentiated into neurons and astrocytes, as indicated by their expression of βIII-tubulin and glial fibrillary acidic protein (GFAP). Through subcutaneous and intracranial implantation experiments, this preliminary study demonstrated the in vivo biocompatibility of the cerebellar scaffold and indicated its potential for future applications. Thus, our study demonstrated that the cerebellar ECM scaffold provided tissue-specific advantages for regenerative medical applications.

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1. Introduction

Over the past decade, the field of regenerative medicine has emerged through the integration of materials science, tissue engineering, and stem-cell, molecular, and developmental biology, the goal of which is to create functional and biocompatible tissues or organs for use in repair or replacement procedures in patients based on clinically relevant approaches (Badylak et al., 2011b). One of the most important advancements in this field has been the production of biological scaffolds containing functional and structural extracellular matrix (ECM) components, which are logical and ideal scaffolds for organ and tissue regeneration (Barkan et al., 2010; Nelson and Bissell, 2006; Vorotnikova et al., 2010).

All tissues and organs consist of cells and the associated ECM, which is a product of structural and functional molecules secreted by the resident cells and constitutes a unique, tissue-specific three-dimensional environment (Calve et al., 2010; Ott et al., 2008). The native ECM dynamically interacts with the resident cells in response to variations in the microenvironment and has been shown to play an important role in the attachment, proliferation, migration, and differentiation of cells (Chastain et al., 2006; Koochekpour et al., 1995; Simon-Assmann et al., 1995; Williams et al., 2008; Yang et al., 2011). It is highly desirable to preserve the native composition and ultrastructure of the ECM during the process of tissue and organ decellularization (Ott et al., 2010; Petersen et al., 2010).

To date, acellular scaffolds have been widely applied in non-central nervous system (CNS) tissues and have been used to successfully repair or replace skin, cardiac valves, blood vessels, the bladder, the urethra, the small bowel, skeletal muscles (Badylak et al., 2011a; Bolland et al., 2007; Butler et al., 2005; Conconi et al., 2004; O’Connor et al., 2002; Palminteri et al., 2007; Parmigotto et al., 2000; Quartì et al., 2011), and even more complex organs, such as the lung and heart (Ott et al., 2008, 2010). However, due to the

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specific characteristics of the CNS and its complicated response to injury, until now, few decellularized ECM scaffolds have been tested for the repair of CNS tissue (DeQuach et al., 2011; Guo et al., 2010; Medberry et al., 2013; Ribatti et al., 2003).

The cerebellum, one of the most thoroughly studied areas of the CNS, functions in the control of smooth and skilful movements, and it is involved in higher cognitive and emotional functions (Sillitoe and Joyner, 2007; ten Donkelaar et al., 2003). The unique and relatively simple architecture of the three-layered cerebellar cortex and the well-defined afferent and efferent fiber connections have made the cerebellum a favored research area and a good candidate for studying the tissue engineering and developmental biology of the CNS (Ercog et al., 2011; Hoshino, 2012; Ito, 1999).

The goals of the present study were to develop a method to decellularize cerebellar tissue, to characterize the resulting scaffold with respect to its composition and its in vitro effects on neural stem cells (NSCs), and to evaluate its in vivo biocompatibility. Comparing with previously described decellularization studies (DeQuach et al., 2011; Guo et al., 2010; Medberry et al., 2013; Ribatti et al., 2003), our protocol was able to provide a cerebellar ECM scaffold that maintained the original microstructure of the cerebellum and indicated its potential for future applications.

2. Materials and methods

2.1. Preparation of the cerebellar biological scaffold

All of the surgical and experimental procedures were reviewed and approved by the Animal and Ethics Review Committee. On the basis of our repeated experimental investigation, the protocol parameters were determined, which was suitable for decellularizing mouse cerebellum. The cerebellum of 18– to 20-g C57BL/6 mice were harvested under aseptic conditions. The mice were anesthetized using chloral hydrate and were transcardially perfused with a 1.0% sodium deoxycholate solution (SDS) for 5 min (at 20 ml per min). The cerebella were removed and immediately placed in phosphate-buffered saline (PBS) at 4 °C. To maintain sterility, all of the subsequent steps were performed in a laminar flow hood. All of the non-CNS tissue was separated and removed. The following series of agitated bath treatments at 25 °C were used for decellularization: 1.0% SDS (60 min, 100 rpm), deionized water (10 min, 60 rpm), 0.02% trypsin/0.05% EDTA (30 min, 60 rpm), deionized water (10 min, 60 rpm), 1.0% Triton X-100 (60 min, 100 rpm), 1.0 M sucrose (15 min, 60 rpm), and deionized water (30 min, 60 rpm). The decellularized cerebellum were immersed in antibiotic-containing PBS (100 U/ml penicillin G, 100 U/ml streptomycin, and amphotericin B) for 72 h. The acellular cerebellar biological scaffolds were stored in PBS until used (Fig. 1).

Unless otherwise noted, all of the chemicals were purchased from Sigma, and all solutions were either filter-sterilized or autoclaved before use.

2.2. Characterization of the cerebellar ECM components

2.2.1. Characterization of the residual DNA in the cerebellar ECM

To qualitatively assess the residual DNA in the ECM scaffold, the following procedure was conducted: the scaffold was fixed using 4% neutral buffered formaldehyde and then embedded in paraffin, sectioned, and stained using 4,6-diamidino-2-phenylindole (DAPI) or hematoxylin and eosin (H&E). After digesting the comminuted ECM scaffold using 0.1 mg/ml of a proteinase K solution, the content and length of the residual DNA were quantitatively investigated. The proteins were digested until no white precipitate was found at the interface that formed following repeated phenol/chloroform extractions and centrifugations (10,000 × g), and the extract was then mixed with 100% ethanol and 3 M sodium acetate. After centrifugation, the DNA pellet was rinsed using 70% ethanol, centrifuged, and dried. The double-stranded DNA was quantified using PicoGreen (Invitrogen), according to the manufacturer’s instructions. Gel electrophoresis of the DNA extracts on a 1.0% agarose gel containing ethidium bromide (2 h at 60 V) and imaging using ultra-violet transillumination revealed the base-pair length of the DNA that remained in the cerebellar ECM scaffold. The decellularization efficacy was evaluated according to the following criteria: (1) the absence of visible nuclei in H&E- and DAPI-stained sections; (2) <50 ng dsDNA per mg of lyophilized ECM (dry weight); and (3) no DNA fragments exceeding 200 bp in length (Arenas-Herrera et al., 2011; Crapo et al., 2013).

2.2.2. Protein constituents of the cerebellar ECM

The procedure for examining the protein content was described previously (Arenas-Herrera et al., 2011; Crapo et al., 2012). Briefly, unstained sections of native cerebellum and the cerebellar scaffold were deparaffinized, rehydrated, and stained for collagens using Masson’s trichrome stain, stained for myelin using the Luxol fast blue stain, and stained for glycosaminoglycans (GAGs) using the Alcian blue/PAS stain. They were then blocked using 2% normal goat serum; incubated serially with primary antibody, H2O2, a peroxidase-conjugated secondary antibody, and diaminobenzidine; counterstained using H&E; and dehydrated using ethylene-xylene.

A sandwich enzyme-linked immunosorbent assay was performed according to the manufacturer’s instructions (Chemicon, USA) to determine the contents of brain-derived neurotrophic factor (BDNF) and nerve-growth factor (NGF) (Kauer-Sant’Anna et al., 2007). Briefly, samples were homogenized in PBS containing 1 mM ethylene glycol bis(2-aminopropyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) and 1 mM phenylmethylsulfonyl fluoride (PMSF). The wells of 96-well flat-bottom plates were coated with samples that had been diluted 1:2 in sample diluent or, to prepare standard curves for quantification, with 7.8–500 pg of BDNF or NGF, and they were then incubated for 24 h. After coating, the sample diluent was used to wash the plates four times. After being diluted 1:1000 in the sample diluent, a monoclonal anti-BDNF rabbit antibody or a polyclonal anti-NGF rabbit antibody was applied for 3 h at room temperature. Then, the samples were incubated with 1:1000 diluted peroxidase-conjugated anti-rabbit antibody for 1 h at room temperature after washing. After the sequential addition of the streptavidin enzyme, the substrate solution, and the stop solution, the levels of BDNF or NGF were determined based on the absorbance at 450 nm. A direct relationship between the BDNF or NGF concentration and the optical density (OD) was demonstrated using the standard curves. Using bovine serum albumin as a standard, the amount of total protein present was determined using Lowry’s method.

2.3. In vitro characterization of the cerebellar ECM

Human NSC lines were used to evaluate the in vitro effect of the decellularized cerebellar ECM scaffold on their proliferation, migration, and differentiation. The derivation of the human NSC lines is described in Sun et al. (2009). Established human NSC lines are cultured on laminin (10 mg/L)-coated dishes (Iwaki) in expansion medium comprised of RHBA medium (Stem Cell Sciences Ltd., UK), recombinant mouse EGF (10 ng/ml, Peprotech), and recombinant human FGF-2 (10 ng/ml, Peprotech). The expansion medium was changed every 2 days.

We used a urinary bladder ECM scaffold as a reference material to evaluate whether the cerebellar ECM scaffold offered tissue-specific advantages in vitro. Urinary bladder matrix was prepared as previously described (Reing et al., 2009). Briefly, connective tissues were removed from the serosal surface of the bladder.
The tunica muscularis externa and tunica submucosa were then mechanically delaminated. The matrix was decellularized by treating with 0.1% peracetic acid/4% ethanol for 2 h and rinsing with PBS and deionized water. The ECM scaffolds were lyophilized, comminuted into particles (<1.0 mm) and then solubilized in 0.01 N HCl containing 1.0 mg/ml pepsin. The concentrations of protein in multiple diluents of the solubilized cerebellar ECM were assayed using the bicinchoninic acid method with a standard curve of bovine serum albumin. Then, 0.1 N NaOH was used to adjust the ECM solutions to pH 7.4; 10× PBS was used to balance the osmotic pressure; and 1× PBS was used to dilute the samples to certain concentrations. The mitogenic and chemotactic effects on NSCs were determined at concentrations of 10, 50, or 100 µg of ECM protein per ml using neutralized pepsin lacking the ECM solutions as the control. All of the in vitro assays were performed as three replicates in triplicate per condition.

A total of 1 × 10⁴ NSCs were plated per well on poly-L-lysine (PLL)-coated sterile glass coverslips to investigate the potential cytotoxic effects of the cerebellar scaffold. After 2 h, 100 µg/ml of the cerebellar ECM scaffold or PBS was added to the culture medium, and the NSCs were cultured for another 24 h. A Live/Dead Viability Kit (Invitrogen) employing 4.0 µM ethidium homodimer-1 and 4.0 µM of calcine AM was utilized to evaluate the cytocompatibility of the ECM scaffold. ImageJ (NIH) was used to quantify the viable NSCs in fluorescence images. All of the images were analyzed using the same ImageJ macro.

Cell migration was evaluated using a two-chamber transwell system (8-µm pore size and 6.5-mm diameter) (Corning, NY, USA). The filters were coated for 30 min using 30 mg/ml laminin and were then allowed to dry completely. The cells were grown to 70–90% confluence, dissociated and resuspended in unsupplemented expansion medium, and re-seeded into the top chamber of a transwell device at a density of 40,000 cells per well. Expansion medium containing ECM scaffolds at various concentrations was added to the lower transwell chamber, and the transwell device was then placed in medium for 24 h in an incubator at 37°C and 5% CO₂. We scraped the cellular material from the upper (non-migratory) surface, fixed the cells on the lower (migratory) surface using methanol, and stained them using DAPI. Image J was used to determine the cell number.

The effects of the cerebellar ECM scaffold on NSC differentiation were evaluated by plating NSCs in PLL-coated plates at a density of 1 × 10⁴ cells per well. Six hours later, the wells were rinsed twice using culture medium; then, either a 50:50 solution of culture medium and the cerebellar ECM scaffold at 100 mg/ml, the urinary bladder ECM scaffold at 100 mg/ml or the neutralized ECM-free pepsin–HCl (negative control) was added, and the cells were cultured for another 48 h. The cells were fixed using 4% paraformaldehyde, washed three times using PBS, and incubated for 45 min in blocking buffer (5% goat serum, 1% BSA, and 0.2% Triton X-100 in PBS) at room temperature. After removing the blocking buffer, the cells were incubated overnight with the primary antibodies at 4°C. The following primary antibodies were used: anti-βIII-tubulin (1:200, Abcam) and anti-glial fibrillary acidic protein (GFAP) (1:250, Abcam). The next day, the cells were washed using PBS, and fluorescence-labeled secondary antibody was applied in the dark at room temperature for 1 h. The cells were then washed using PBS and stained using a DAPI solution (1 µg/ml), followed by image collection. The cell populations were quantified using Image J.

NSCs (4 × 10⁶ cells in 500 µl of medium) were injected into the cerebellar scaffold at a depth of 2.5 mm using a microsyringe over a period of 5 min. The reseeded scaffolds were cultured in expansion medium for 14 days. The blank and seeded scaffolds were all sectioned along the transverse and longitudinal planes, and the sections were examined using scanning electron microscopy (SEM). The specimens were attached to sample stubs and were sputter-coated with gold. The samples were examined and images were captured using a Hitachi-SU8000 scanning electron microscope.

2.4. In vivo investigation of biocompatibility

The acellular cerebellar scaffold was implanted subcutaneously on the dorsal side and intracranially into the frontal lobes of Sprague-Dawley (SD) rats. Twenty-four SD rats were equally divided into the subcutaneous and intracranial groups, which were each subdivided into three subgroups, namely, the sham operation, gelatin sponge, and cerebellar scaffold implantation subgroups. The rats were anesthetized using injected pentobarbital (30 mg/kg). In the subcutaneous group, we made a small skin incision (10 mm long), created a pocket through blunt dissection, subcutaneously implanted the grafts in the midportion of the back, and closed the wound. In the intracranial group, a midline incision was made on the scalp and a burr hole was drilled 5 mm lateral to the sagittal sinus at the midpoint between the bregma and lambda. Parts of the frontal lobes were resected, the grafts were implanted into the cavity, and the wound was closed. Tissue was obtained 4 weeks after the operation, and paraffin-embedded tissues were used to examine the characteristics revealed by H&E staining and the expression of CD4+ and CD8+ (Millipore, USA). The expression of CD4+ and CD8+ indicated the immunogenicity of the acellular scaffold.

2.5. Statistical analysis

The graphical representations of all data show the mean values and the standard deviation of at least three replicates conducted in triplicate. An analysis of variance (ANOVA) was used for comparisons between the experimental groups, and a two-sided t-test was used for comparisons between two groups. The differences between all the groups were compared using the log-rank test with SPSS software, version 11. A p value equal to or less than 0.05 indicated a significant difference.
3. Results

3.1. Characterization of the ECM scaffold

No residual nuclei were found in the H&E- or DAPI-stained sections of the cerebellar ECM scaffold (Fig. 2A–D). There were no residual DNA fragments exceeding 200 bp in the cerebellar ECM scaffold (Fig. 2E). The results of the dsDNA quantification showed that the cerebellar scaffold retained <50 ng dsDNA per mg of lyophilized ECM (Fig. 2F). The concentration of dsDNA in the cerebellar ECM scaffold was 34.5 ± 8.3 ng/mg.

The immunohistochemical analysis showed that the cerebellar ECM scaffold retained collagen and GAGs but evidently lacked obvious myelin, all of which were present in the native tissue (Fig. 3A–F). A certain amount of BDNF and NGF, which were present in the native cerebella at higher levels, was detectable in the ECM scaffold (Fig. 4A,B). The levels of the BDNF and NGF in the urinary bladder ECM scaffold were less than those in the cerebellar ECM scaffold ($p < 0.05$).
3.2. In vitro characterization of the cerebellar ECM scaffold

The cerebellar ECM scaffold was cytocompatible in vitro, as was a non-CNS ECM scaffold derived from the urinary bladder (Fig. 5A). The live/dead assay revealed that 90% of the human NSCs were viable, and their viability did not change when they were cultured with the different ECM scaffolds at 100 mg/ml (p > 0.05) (Fig. 5A). The mitogenic responses of the human NSCs to the ECM scaffolds varied with the matrix concentration and the cell-matrix combination. Both the cerebellar and urinary bladder ECM scaffolds increased the rate of NSC mitogenesis, by 48% and 20%, respectively, at the concentrations tested (p < 0.05) (Fig. 5B).

Similar to the mitogenic results, the chemotactic responses of human NSCs to the ECM scaffolds varied with the matrix concentration and the cell-matrix combination. The cerebellar ECM scaffold induced a large chemotactic response, resulting in up to an
Differentiation of human NSCs induced by the cerebellar ECM scaffold. Differentiation of the human NSCs was induced by the cerebellar ECM scaffold (B, E) and the urinary bladder scaffold (C, F) ECM, as indicated by immunofluorescence staining for βIII-tubulin (A–C) and GFAP (D–F). The negative control staining is shown (A, D). (G) Neuronal differentiation of the human NSCs was greater when the cells were exposed to the CNS-derived ECM scaffold compared with when they were exposed to the non-CNS ECM scaffold ($p < 0.05$).

The cerebellar ECM scaffold induced the neuronal differentiation of NSCs, as evident from their expression of βIII-tubulin and GFAP under the assay conditions (Fig. 6). The cerebellar ECM scaffold induced a high percentage (5.0%) of the NSCs to undergo neuronal differentiation, which was significantly higher than the negative control rate of 0.5%. The urinary bladder ECM scaffold induced neuronal differentiation at a rate significantly lower (1.5%) than that of the cerebellar ECM scaffold ($p < 0.05$) (Fig. 6G). The negative control contained cells with a typical undifferentiated morphology. Numerous cells that did not exhibit strong Tuj1 staining but clearly showed long cellular processes were observed in the cerebellar ECM group (Fig. 6B), which were likely differentiating into glial cell types, as confirmed by their GFAP staining (Fig. 6E).

Fig. 7. Scanning electron micrographs of a blank cerebellar scaffold, showing its three-dimensional network structure (A), and a cerebellar scaffold that was seeded with NSCs (B). The NSCs adhered to the decellularized scaffold, and fine, long axons grew out of the scaffold.
SEM revealed a lack of residual cells in the blank scaffold. The blank cerebellar ECM scaffold consisted of a three-dimensional network structure containing pores (Fig. 7A). When NSCs were co-cultured with this scaffold for 14 days, the NSCs attached to the scaffold and fine long axons grew out of the implanted cells and were visible at the surface of the reseeded scaffold (Fig. 7B). Some of the axons had crawled along and adhered to the surface of the scaffold, whereas other axons were suspended within the lumens and branched like vines near the walls of the scaffold. The SEM micrographs indicated that NSCs could survive in, adhere to, and extend their axons in the cerebellar scaffold.

3.3. In vivo biocompatibility

Within the subcutaneously implanted group, neutrophilic granulocytes, lymphocytes, and fibroblasts were found in the control subgroup and the experimental subgroup animals (Fig. 8A–C). The degree of cellular infiltration in the experimental and control subgroups was not significantly different 4 weeks after implantation. CD4+ and CD8+ monocyte infiltration was observed 4 weeks after implantation; however, no obvious increase was found in the experimental subgroup (Fig. 8D–I).

Similar to the results obtained in the subcutaneously implanted group, the immune response of the host brain to the cerebellar scaffold in the intracranially implanted group was not significantly different between the subgroups 4 weeks after implantation (Fig. 9A–C). In contrast to the control material, a fraction of the cerebellar bioscaffold remained 4 weeks after implantation and had partially integrated into the host brain. However, there was no obvious increase in the number of host cells that had infiltrated the implanted bioscaffolds compared with that of the control. The gelatin sponges were observed in the cerebral cavity 4 weeks after their implantation. None of the subgroups exhibited large numbers of CD4+ or CD8+ monocytes after implantation. Compared with that of the control subgroup, the staining intensity of the positive cells in the experimental subgroups was not stronger (Fig. 9D–I).

4. Discussion

The adult human CNS has a limited regenerative capacity despite the existence of NSCs in both the brain and spinal cord (Alvarez-Buylla and Garcia-Verdugo, 2002; Alvarez-Buylla et al., 2002; Gage, 2000). The in vivo maintenance and regulation of stem cell behavior in the CNS is partially attributable to the characteristics of the NSC niche, which is a complex microenvironment responsive to extracellular cues (Crapo et al., 2014; Kazanis and ffrench-Constant, 2011; Massirer et al., 2011). Therefore, prospective NSC therapeutic materials often include a biomimetic component that is intended to support the post-transplantation survival and integration of the exogenously delivered NSCs (Discher et al., 2009; Potter et al., 2008).

With the exception of cases of repairing the dura mater, the development and application of tissue-derived ECM scaffolds for CNS reconstruction have rarely been reported (Bejjani and Zabramski, 2007; Volpato et al., 2013). ECM bioscaffolds produced by the decellularization and antigen depletion of mammalian tissues largely retain the structural and functional complexities of their tissues of origin, which play an important role in many aspects...
of neural development, both in vitro and in vivo (Prestwich and Healy, 2015).

We found that the characteristics of a brain-derived ECM scaffold, which is very weak and readily disintegrates, led to difficulties in rinsing and recovering the ECM matrix (Crapo et al., 2012; DeQuach et al., 2011; Volpato et al., 2013). Residual cellular material within the ECM scaffold, particularly DNA, may cause cytotoxic incompatibility in vitro and adverse host responses in vivo upon the reintroduction of cells (Arenas-Herrera et al., 2013; Badylak et al., 2011b; Ott et al., 2008). In our initial trials to decellularize brain material, we used only a series of agitated bath treatments (using detergents such as SDS and Triton X-100), which did not eliminate as much cellular content as expected. Using our intracardial perfusion-serial agitated bath-treated method, we were able to isolate a cerebellar ECM scaffold that maintained the original microstructure of the cerebellum. Although it was not possible to completely remove all of the cellular materials, regardless of the technique used, our combination of qualitative and quantitative strategies avoided adverse responses to the scaffold we produced.

There are relatively small amounts of fibrous proteins, such as fibronectin and collage, in the brain ECM, but there are relatively larger amounts of proteoglycans and GAGs (e.g., hyaluronic acid) (Crapo et al., 2012, 2014; Medberry et al., 2013; Volpato et al., 2013), which have been shown to affect NSC proliferation, to regulate the formation of synapses, and to stabilize the connections made. GAGs have been shown to play an important role in cellular behavior, either alone or through cooperation with other molecules, including proteins. Using our decellularization protocol, more than 95% of the DNA was removed, and many of the important ECM components, such as GAGs and proteins, were retained to a certain extent relative to their levels in native tissue. The GAGs and proteoglycans in the cerebellar scaffold we produced may be important mediators of cellular behavior.

However, current decellularization protocols lack the specificity to remove growth-inhibitory molecules while retaining growth-promoting molecules (DeQuach et al., 2011; Volpato et al., 2013). Similar to the case with acellular peripheral-nerve grafting materials, myelin was not completely removed from the CNS-derived ECM scaffold, which would hamper its benefits after transplantation into the CNS.

The effects of CNS-derived ECM scaffolds on human NSCs may be critical for constructive CNS remodeling. An earlier study showed that post-stroke implantation of an ECM scaffold into a lesion not only supported the transplantation of exogenous NSCs but also acted as a lesion filler and recruited endogenous cell populations into the gel-filled lesion (Medberry et al., 2013). While the development of exogenous cell-based therapeutics for the treatment of CNS pathologies continues to appear promising, clinical treatments using tissue-derived ECM scaffolds alone may be more acceptable due to the potential tumorigenicity of exogenous NSCs (Ben-David et al., 2011). Safety concerns aside, the efficacy of NSCs in CNS therapies may be enhanced by or even require the co-delivery of cell-supportive materials such as the ECM (Bible et al., 2012). Mitogenic and chemotactic responses of NSCs to various ECM scaffolds may differ due to their unique protein profiles and biomolecular constituents, including GAGs, myelin, and collagen, all of which
vary according to the source of the ECM (Crapo et al., 2014; Volpato et al., 2013). The evidence for the neuronal differentiation and neurite extension of NSCs seeded in the cerebellum-derived ECM scaffold suggested that this scaffold may evoke a tissue-specific response in human NSCs. Although the mechanisms by which ECM scaffolds promote a constructive and functional remodeling response are only partially understood, an ECM scaffold implanted in a minimally invasive manner may provide a stem-cell-friendly microenvironment at a CNS injury site that would induce the endogenous NSCs to migrate to the site, proliferate, differentiate to replace the lost neural cells, produce functional tissue, and ultimately, improve the functional outcome.

Biocompatibility is one of the general issues that must be considered when choosing biomaterials and designing scaffolds ( Arenas-Herrera et al., 2013; DeQuach et al., 2011; Guo et al., 2010). Ideally, the scaffolds used in regenerative medicine would minimize adverse tissue reactions in vivo and promote optimal cell adhesion, migration, and axonal outgrowth. The results of this study showed that the microstructure of the cerebellum-derived ECM scaffold was similar to that of native cerebellar tissue and that NSCs could adhere, proliferate, extend axons, and maintain a high level of viability in the cerebellar scaffold. The data indicated that the porous and fibrous cerebellar scaffold favored the infiltration of endogenous cells. The subcutaneous and intracranial implantation experiments demonstrated the safety and biocompatibility of the cerebellar scaffold. Therefore, our preliminary study supports the potential use of this scaffold for tissue-specific regenerative repair and minimally invasive therapy. However, it is critical to investigate the in vivo biocompatibility, degradation time, and cellular infiltration of this scaffold in long-term studies.

However, because our approach did not yield an optimal neural-regeneration scaffold, improvements are required to make the scaffold more similar to the native ECM, such as optimizing its internal architecture and providing more stimulatory factors.

5. Conclusion

In this study, we demonstrated that our dynamic decellularization protocol, which combined intracardial perfusion and a series of agitated bath treatments, effectively decellularized the cerebellum. The resulting acellular ECM scaffold retained neurosupportive proteins, growth factors, and its native structure. This multi-functional scaffold was cytocompatible and appeared to interact with NSCs in an appropriately biomimetic manner. The scaffold also promoted the differentiation of NSCs into neurons and astrocytes, as indicated by their bIII-tubulin and GFAP expression in vitro. The subcutaneous and intracranial implantation experiments demonstrated that the cerebellar scaffold was biocompatible in vivo. Thus, the results of our study demonstrated that the cerebellar scaffold provided an excellent mimic of the native cerebellar ECM and that it may facilitate the development of regenerative medical approaches to CNS reconstruction.

Disclosure

The authors declare no conflict of interest.

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