



Recombinant spider silk matrices for neural stem cell cultures

Michalina Lewicka^a, Ola Hermanson^{a,**}, Anna U. Rising^{b,c,d,*}

^a Linnaeus Center in Developmental Biology for Regenerative Medicine (DBRM), Department of Neuroscience, Karolinska Institutet, SE-17177 Stockholm, Sweden

^b Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, The Biomedical Centre, SE-751 23, Uppsala, Sweden

^c Spiber Technologies AB, Doppingv 12, SE-756 51 Uppsala, Sweden

^d Department of Neurobiology, Care Sciences and Society (NVS), Karolinska Institutet, Novum 5th floor, SE-141 86 Stockholm, Sweden

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ABSTRACT

Neural stem cells (NSCs) have the capacity to differentiate into neurons, astrocytes, and oligodendrocytes. Accordingly, NSCs hold great promise in drug screening and treatment of several common diseases. However, a major obstacle in applied stem cell research is the limitation of synthetic matrices for culturing stem cells. The objective of this study was to evaluate the suitability of recombinant spider silk (4RepCT) matrices for growth of NSCs. NSCs isolated from the cerebral cortices of mid-gestation rat embryos were cultured on either 4RepCT matrices or conventional poly-L-ornithine and fibronectin (P + F) coated polystyrene plates. From 48 h of culture, no significant differences in cell proliferation or viability were detected in NSC cultures on 4RepCT compared to control matrices (polystyrene plates coated with P + F). The NSCs retained an undifferentiated state, displaying low or no staining for markers of differentiated cells. Upon stimulation NSCs grown on 4RepCT differentiated efficiently into neuronal and astrocytic cells to virtually the same degree as control cultures, but a slightly less efficient oligodendrocyte differentiation was noted. We suggest that recombinant spider silk matrices provide a functional microenvironment and represent a useful tool for the development of new strategies in neural stem cell research.

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1. Introduction

The properties of the extracellular microenvironment are increasingly being recognized as relevant cellular stimuli that affect proliferation, viability, and cell differentiation of immature cells [1]. However, while such properties vary significantly across tissues and developmental stages in living organisms, cell culture conditions still remain surprisingly similar using standard cell culture plates for most protocols of expanding and differentiating various types of stem cells. For example, substrate stiffness, three-dimensionality of the scaffolds, and incorporation of specific cell binding motifs influence migration as well as differentiation of

various types of stem cells in cell context-specific manner [2–5]. Thus, both the physical properties of the matrix and the stimulation by specific extracellular matrix (ECM) components are important for the cell to be able to sense the surroundings and convert the information to modulate cellular behavior such as self-renewal, migration, and differentiation.

While neural stem cells (NSCs) hold great promise in medical and therapeutic applications, e.g., for drug screening or transplantation, as well as a research tool to develop differentiation protocols to be applied to embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, virtually all published protocols of isolating, expanding, and differentiating NSCs involve animal- or human-derived ECM components [6]. This fact represents a serious obstacle in stem cell research and regenerative medicine. Further, to fully explore and utilize the potential of stem cell biology, the generation of a variety of biocompatible materials with different properties in stiffness, shapes, and other parameters is needed [7]. Accordingly, the search for alternative solutions, ideally recombinant proteins that can generate designed and defined structures, has intensified during the last years.

Spider silk has been suggested to be an ideal biomaterial [8–11], since it is both strong and extendible [12], and also seems to exhibit favorable properties when implanted in living tissues [13–15].

Abbreviations: NSCs, neural stem cell; GFAP, glial fibrillary acidic protein; SMA, α -smooth muscle cell actin; BMP4, Bone Morphogenic protein 4; TuJ1, Neuronal class III B-tubulin; BrdU, bromodeoxyuridine; P, poly-ornithine; F, fibronectin; iPS, induced pluripotent stem; ECM, extracellular matrix; 3D, three dimensional.

* Corresponding author. Department of Neurobiology, Care Sciences and Society (NVS), Karolinska Institutet, Novum 5th floor, SE-141 86 Stockholm, Sweden. Tel.: +46 709 744 888.

** Corresponding author.

E-mail addresses: Ola.Hermanson@ki.se (O. Hermanson), Anna.Rising@ki.se (A.U. Rising).

Recently, it was shown that human primary fibroblasts adhere to, proliferate, and maintain their phenotype when cultured on recombinant spider silk matrices (4RepCT) [16]. In contrast to fibroblasts however, stem cells are poised to respond strongly to the microenvironment and extracellular stimuli, and it has therefore traditionally been difficult to pinpoint the optimal conditions to maintain stem cell self-renewal, viability, and potential to differentiate into multiple adult cell types. To investigate whether recombinant spider silk matrices may represent a suitable material for stem cell culture, the performance of 4RepCT matrices for NSC culture was evaluated.

2. Methods

2.1. Production of protein matrices

The recombinant miniature spider silk protein 4RepCT was produced in *Escherichia coli* and purified as described previously [17]. A procedure for depletion of lipopolysaccharides (LPS) was also included [18]. After purification, the protein solution was sterilized by passage through 0.22 μm filter and concentrated to 3 mg/mL by ultrafiltration (Amicon Ultra, Millipore) before preparation of matrices. Two different formats were produced; film and foam according to Widhe et al. [16]. All matrices were allowed to dry over night at room temperature under sterile conditions and stored in room temperature until use. Before plating the cells, the matrices were washed twice with sterile PBS and pre-incubated with serum-free DMEM:F12 media (Sigma–Aldrich) for 1 h at 37 °C with 5% CO₂. The matrices were prepared in six-well cell culture polystyrene plates or 35 mm in diameter polystyrene plates (Sarstedt). For surface morphology, scanning electron micrographs and in depth analysis of the substrates, please see Widhe et al. [16].

2.2. Neural stem cell (NSC) culture

NSCs were isolated from the cerebral cortices of day E15.5 embryos obtained from pregnant Sprague Dawley rats largely as described previously [19,20]. Cells were mechanically dissociated followed by culture in serum-free DMEM:F12 media (Sigma–Aldrich) enriched with N2 supplements. Cells were maintained in proliferating state using 10 ng/ml fibroblast growth factor 2 (FGF2; R&D Systems) and passaged twice before usage in the experiments. NSCs were then seeded at a density of 10,000 cells/cm² and allowed to proliferate for 24–72 h prior to the experiments. The 4RepCT films and foams covered ~60% of the surface area of the cell culture dishes before NSCs were applied. NSCs used in positive control experiments were seeded on conventional polystyrene culture plates coated with poly-L-ornithine and fibronectin from bovine plasma (P + F) (Sigma–Aldrich) to which uncoated polystyrene dishes served as negative controls. In one series of control experiments, the 4RepCT films were coated with P + F (Fig. 1). In the other experiments the NSCs were seeded directly onto 4RepCT matrices, P + F coated polystyrene or uncoated polystyrene. Soluble factors were re-supplemented at 24 h and medium replaced at 48 h time intervals. To induce differentiation FGF2 was withdrawn from the cell culture media and then replaced with one or more of the following factors: ciliary neurotrophic factor (CNTF; R&D Systems), bone morphogenic protein (BMP4; R&D Systems), Wnt3a (R&D Systems), and T3 (sigma). All animal experiments were approved by the North Stockholm regional committee for animal research and ethics, Stockholm, Sweden.

2.3. Immunocytochemistry

Cell cultures were first rinsed once in Phosphate-Buffered Saline (PBS) (Sigma–Aldrich) and then fixed in 10% Formalin (Sigma–Aldrich) for 20 min at room

temperature (RT) followed by 3 \times 5 min washes with PBS/0.1% Triton-X 100 (Sigma–Aldrich). Plates were then incubated with the respective primary antibody in PBS/0.1% Triton-X 100/1% bovine serum albumin (BSA; Sigma–Aldrich) overnight at 4 °C. The primary antibodies sources and dilutions were as follows: rabbit polyclonal anti-glia fibrillary acidic protein (GFAP; 1:500; DAKO), mouse monoclonal anti-Neuronal Class III B-Tubulin (TuJ1; 1:500; Covance), mouse anti-intermediate filament protein nestin (1:500; BD Pharmingen), rat anti-MBP monoclonal (MAB386; 1:250; Chemicon). The cells were subsequently washed 6 \times 5 min each with PBS/0.1% Triton-X 100. Secondary antibodies were incubated in PBS/0.1% Triton-X 100/1% BSA at room temperature for 1 h. The secondary antibodies were species-specific labeled with Alexa-488 or Alexa 594 (1:500; Molecular Probes). Next the samples were washed 3 \times 5 min each in PBS and mounted with Vectashield including DAPI (Vector Laboratories, Inc). Fluorescent images were acquired with Axioskop software using Zeiss Axioskop2 microscope coupled to an MRm (Zeiss) camera at a magnification of 10 \times and 20 \times .

2.4. Proliferation studies

NSCs were incubated for 30 min in EdU (Click-iT EdU Kit, Invitrogen, Eugene, OR, USA) according to the manufacturers recommendations, followed 20 min fixation in 10% formalin. The cells were then permeabilized with 0.5% Triton-X 100 during 3 \times 5 min, and after two washes in PBS with 3% BSA, 0.5 ml of Click-iT reaction cocktail (1 \times Click-iT reaction buffer, CuSO₄, Alexa Fluor azide, reaction buffer additive) was added to each well followed by 30 min incubation at room temperature. Finally, cells were washed twice with PBS, stained 10 min with Hoechst, and one drop of mounting media was added to each well before visualizing under the fluorescent microscope.

2.5. Cell viability analysis

Live/Dead kit (Invitrogen) was used to differentiate between living and dead cells attached to the scaffolds. Plates were rinsed twice with pre-warmed PBS (37 °C) before proceeding with the assay according to the manufacturer recommendations. The assay enabled identification of live cells (green color) from dead cells (red color).

2.6. Statistics

For every experiment in all conditions studied, 5 random pictures obtained at 20 \times magnification with the Zeiss Axioskop2 microscope were picked for manual quantification. Judged by DAPI staining, each such micrograph depicted around 150 to 1000 cells in fields of a diameter of around 500 μm . GraphPad Prism 4.0 (GraphPad Software, San Diego, California) was used for statistical analysis of the data. One-way ANOVA followed by unpaired *t*-test were used for cell counts in wells with different matrix types at each culture day. *P*-values < 0.05 were considered significant.

3. Results

3.1. Neural stem cell expansion

After mechanical dispersion of NSCs derived from rat neocortex of embryonic day (E) 15, the NSCs were cultured in optimal conditions seeded on polystyrene cell culture plates precoated with poly-L-ornithine and fibronectin (P + F). This protocol is widely used by numerous laboratories since the past 15 years [3,21–24] and provides homogenous cultures of multipotent progenitors for a large number of passages [21,23,25]. As previously reported, the NSCs expanded in N2 supplemented medium in the presence of

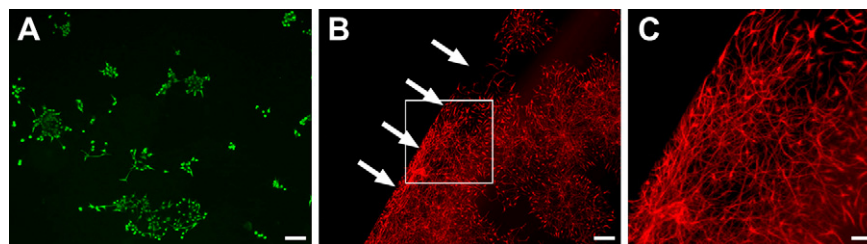


Fig. 1. NSCs maintain undifferentiated state when expanded on 4RepCT film structures and do not migrate beyond the limits of the substrate (i.e. onto the uncoated polystyrene tissue culture plate). (A) Low-power micrograph of nestin-positive NSCs expanded on 4RepCT film. (B) Low-power micrograph of GFAP-positive cells derived from NSCs expanded and differentiated on 4RepCT films. Arrows point to the border of the 4RepCT film and the polystyrene plate beneath the film. (C) Higher magnification of the marked area in (B) to visualize the morphology of the cells. Scale bars: 50 μm (A,B), 15 μm (C).

FGF2. In contrast, NSCs grew poorly or not at all on uncoated polystyrene plates or plates coated with either P or F in accordance with previous observations [3,21].

After initial expansion, we seeded NSCs after the second passage on P + F coated 4RepCT films and on uncoated 4RepCT films, respectively. Unexpectedly, we noted that NSCs seeded on uncoated 4RepCT films attached and proliferated 24–72 h after the passage (Fig. 1A). The observation that the 4RepCT matrices in themselves were sufficient to provide necessary support for cell survival and proliferation was particularly evident when analyzing the NSC cultures at the edges of the 4RepCT matrices after 72–168 h, as there was a sharp border between the 4RepCT film on which NSCs expanded and subsequently differentiated properly (see further below) and the uncoated polystyrene surface where the cells were unable to attach (Fig. 1B–C).

3.2. Self-renewal and proliferation

The observation that NSCs could expand on 4RepCT films initiated a series of experiments to investigate various aspects of the biocompatibility of 4RepCT as a possible substrate for NSC cultures. We observed that NSC density 24–48 h after the seeding seemed lower on the uncoated 4RepCT films than on P + F coated structures. However, analysis of the NSC proliferation by EdU staining revealed that even if NSC proliferation showed a tendency to be lower the first 24–48 h of culturing on 4RepCT films compared to P + F coated structures, this difference was not found to be significant 72–96 h after seeding ($29\% \pm 5.5$ (P + F) vs $18\% \pm 9.5$ (4RepCT), $p > 0.05$) (Fig. 2A–C). It is thus plausible that the initial difference observed was due to differences in cell adhesion rather than proliferation. Furthermore, the NSCs retained their expression of nestin, a well-established marker of undifferentiated NSCs, throughout the culturing of the cells while displaying low or no staining for markers of differentiated cells GFAP (astrocytes), TuJ1 (neurons), MBP (oligodendrocytes) (Fig. 1A and Fig S1A–C).

3.3. Cell viability

We next investigated whether an increase in cell death could be detected in NSCs cultured on 4RepCT film structures compared to the “golden standard” coated control cultures. Staining with DAPI (binds to and stains A-T rich DNA) revealed no obvious difference in condensed or pyknotic nuclei, and the fluorescent Live/Dead assay confirmed that there were no significant differences in cell viability between NSCs seeded on 4RepCT film compared to P + F coated structures 72–96 h after seeding ($4.7\% \pm 2.8$ (P + F) vs $5.8\% \pm 2.9$ (4RepCT), $p > 0.05$) (Fig. 3A–C).

3.4. Differentiation potential and multipotency: glial differentiation

As noted above and in line with the observations of self-renewal, no signs of premature differentiation of NSCs were noted when they were growing on the 4RepCT films (Fig. 1). In order to determine if the NSCs grown on recombinant spider silk films had kept their potential to differentiate into multiple lineages, we exposed the cells to various factors for differentiation. We initially studied glial cell fates, i.e. astrocytic or oligodendrocytic cells, by immunocytochemistry of appropriate markers (GFAP for astrocytes and MBP for oligodendrocytes). NSCs expanded on 4RepCT films and in control cultures were treated with 10 ng/ml ciliary neurotrophic factor (CNTF), an interleukin-6-related cytokine that induces a rapid and efficient astrocytic differentiation of embryonic NSCs as described previously [21–23]. After 48 h of CNTF treatment, control cultures and NSCs grown on 4RepCT showed similar proportions of GFAP-positive astrocytic cells ($43\% \pm 3.9$ (P + F) vs $35\% \pm 3.5$ (4RepCT), $p > 0.05$) (Fig. 4A–C). Differentiation of embryonic NSCs into oligodendrocytic fate can be mediated by treatment of NSCs with thyroid hormone (T3) for 7 days (168 h) [21]. After 7 days of treatment, NSCs expanded on 4RepCT films showed a slightly lower proportion of MBP-positive oligodendrocytic cells compared to control cultures ($11\% \pm 2.5\%$ (P + F) vs $7.5\% \pm 2.3$ (4RepCT)), and this difference was significant ($p < 0.05$) (Fig. 5A–C). Similar observations have previously been reported by us when differentiating NSCs into oligodendrocytes on soft substrates [3]. In conclusion, the NSCs maintain the potential to differentiate into glial cell types when grown on 4RepCT films.

3.5. Differentiation potential and multipotency: neuronal differentiation

To investigate if NSCs grown on recombinant spider silk scaffold matrices can differentiate into neurons we exposed the cells to treatment with bone morphogenetic protein (BMP) 4 or co-treatment with BMP4 and the signaling factor Wnt3a for 7 days (168 h). BMP4 and BMP4+Wnt3a stimulation induces differentiation of mature, functional neuronal cells of pyramidal-like phenotype when NSCs are seeded at relatively high densities [20,26,27]. NSCs grown on 4RepCT films and exposed to 10 ng/ml BMP4 or BMP4 and Wnt3a every 24 h for 7 days differentiated into TuJ1-positive neuronal cells with characteristic morphologies to the same degree as P + F coated polystyrene cell culture plates used as controls $15\% \pm 4.1\%$ and $20\% \pm 3.6$ (P + F) vs $12\% \pm 5.6$ and $15\% \pm 6.3$ (4RepCT), $p > 0.05$ (Fig. 6A–F). A slightly less mature morphology was noted in a subset of BMP4 and Wnt3a co-treated cultures when grown on 4RepCT films (Fig. 6A–B). In summary, NSCs grown on 4RepCT maintained full potential to differentiate into neuronal cells.

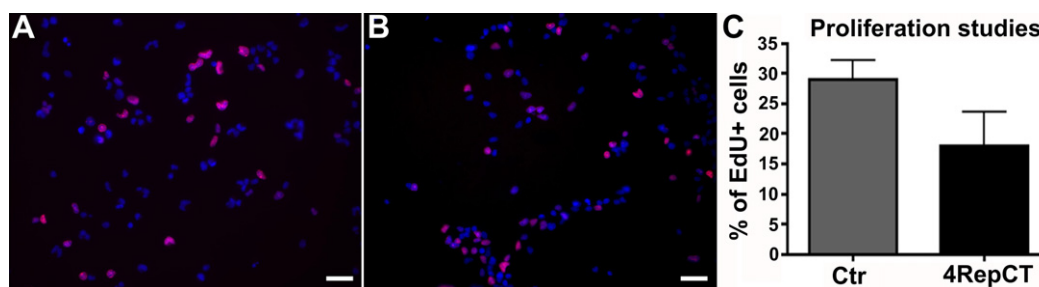


Fig. 2. After initial attachment, there was no significant difference in proliferation between NSCs growing on 4RepCT films compared to “golden standard” P+F-coated cell culture plates. Micrographs depicting EdU incorporation in NSCs expanded on (A) P+F-coated polystyrene and (B) 4RepCT films. Red = EdU-positive cells. Blue = Hoechst staining labeling nuclei. (C) Quantifications of the fraction of EdU-positive cells relative to the number of Hoechst-stained cells in the two different conditions. (1762 cells counted in control conditions and 7543 cells on 4RepCT substrates). Data represents mean \pm S.E.M. Scale bars: 30 μ m. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

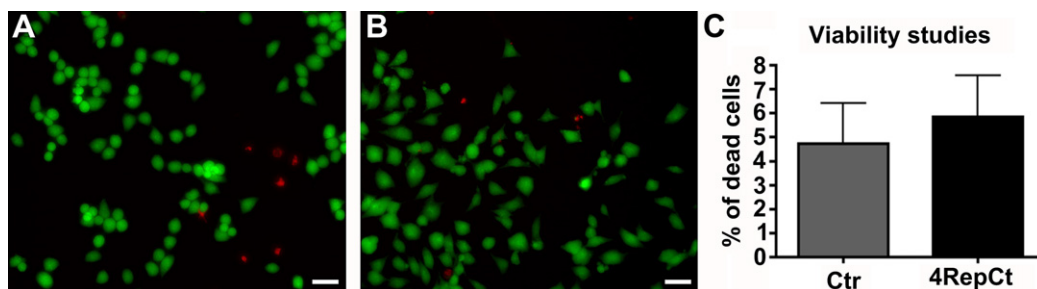


Fig. 3. There was no significant difference in cell survival or death between NSC cultures on 4RepCT films compared to P + F-coated control culture dishes. Live cell imaging demonstrating live (green) and dead (red) NSCs when expanded on (A) P + F-coatings and (B) 4RepCT film. Note that the micrographs are depicting live cells in medium and therefore are less sharp. (C) Quantifications of the fractions of red (dead) cells relative to the number of green (live) NSCs on the two different substrates. (1732 cells counted in control conditions and 3273 cells on 4RepCT substrates). Data represents mean \pm S.E.M. Scale bars: 30 μ m. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.6. 3D neural cultures by expanding NSCs on 4RepCT-based foam structures

As a proof-of-principle, we seeded the NSCs on 4RepCT foam matrices with pore sizes of around 30–200 μ m as described in detail previously [16], and differentiated these into astrocytes by treatment with CNTF to investigate if it was possible to expand and differentiate the NSC cultures on such matrices and thus create a matrix with applications for three dimensional (3D) cultures of multipotent cells. Indeed, the NSCs expanded on the foam, differentiated efficiently into astrocytes, and formed various 3D structures according to the shape of the 4RepCT foam matrices (Fig. 7A–B).

4. Discussion

The use of NSCs holds promise for improved drug screening as well as cell therapy-based treatment of many neurological disorders such as Parkinson's disease, traumatic spinal cord injuries, and peripheral nerve injuries. Many protocols for NSC culturing have been published but the common ground is that virtually all of these protocols depend on animal- or human-derived matrices [2]. Such matrices are problematic for a number of reasons; (i) they are often not mechanically robust and hence cannot be transplanted together with the cells to the location of the injured tissue, (ii) any clinical application would require the cells to be grown on defined matrices of non-animal origin due to regulatory issues, (iii) the properties of undefined matrices may vary from batch to batch making it harder to draw conclusions from experimental work, and (iv) to mimic the natural ECM the matrices need to be 3D.

Spider silk is famous for its extreme mechanical properties [12], is well tolerated when implanted in living tissue [15] and has even

been shown to enable regeneration of peripheral nerves [13,14]. Mimics of spider silk of non-animal origin can be produced in heterologous hosts (reviewed in, e.g., Rising et al. 2011 [8]). The mini-spidroin 4RepCT is produced in *E.coli* and can be processed into solid films, fibers and foams [16,28]. Previously we have shown that 4RepCT can support attachment and growth of human primary fibroblasts and that 4RepCT fibers are equally well tolerated as commercially available sutures when implanted subcutaneously in rats [16,29]. In this study, 4RepCT films and foams are employed in NSC culture in order to identify a defined culture system for NSC. The present golden standard in NSC culturing, P + F, that is of animal origin, was used as a control. It should be noted that there are animal- or human-derived components in the N2 medium used, and it is desirable in future studies to test alternative media composition.

Ligands in the ECM normally provide outside-in signals for cells mediated by an array of integrins on the cell surface. The molecular mechanisms of how the integrins transmit the signals across the cell membrane is not known in detail, but in immature neural stem cells and progenitors result in intracellular alterations of neurogenesis associated processes that influence cell migration, proliferation and survival [1,7]. Normally, binding of NSC to fibronectin and laminin is mediated, e.g., through $\alpha_5\beta_1$ and $\alpha_3\beta_1$ and $\alpha_6\beta_1$ integrin complexes respectively [1,30,31]. It is not known which integrins, if any, that are involved in the binding of NSCs to 4RepCT films and there are no apparent classical cell binding motifs in the amino acid sequence of 4RepCT. Nevertheless, primary human fibroblasts have been shown to attach, survive, proliferate and maintain their phenotype on 4RepCT films also under serum free conditions [16], indicating that the cells are able to make stable connections to the material.

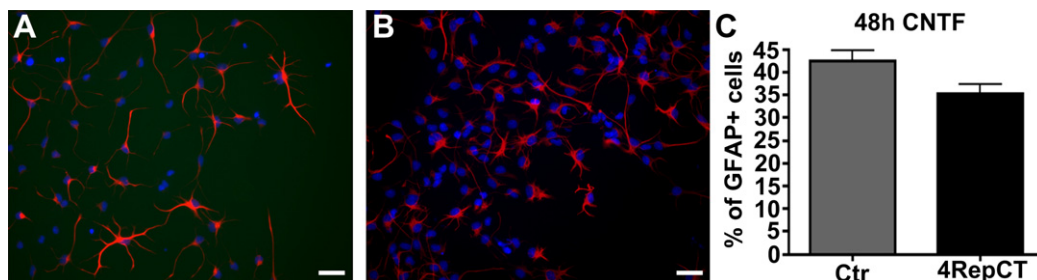


Fig. 4. NSCs retained the capacity to differentiate into astrocytes on 4RepCT films. Micrographs depicting GFAP-positive cells derived from NSCs by CNTF treatment cultured on (A) P+F-coated polystyrene dishes or (B) 4RepCT film. Red = GFAP immunoreactivity. Blue = DAPI labeling DNA/nuclei. (C) Quantifications of the fractions of GFAP-positive cells relative to the number of DAPI-stained cells on the two different substrates. (750 cells counted in control conditions and 3217 cells on 4RepCT substrates). Data represents mean \pm S.E.M. Scale bars: 30 μ m. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

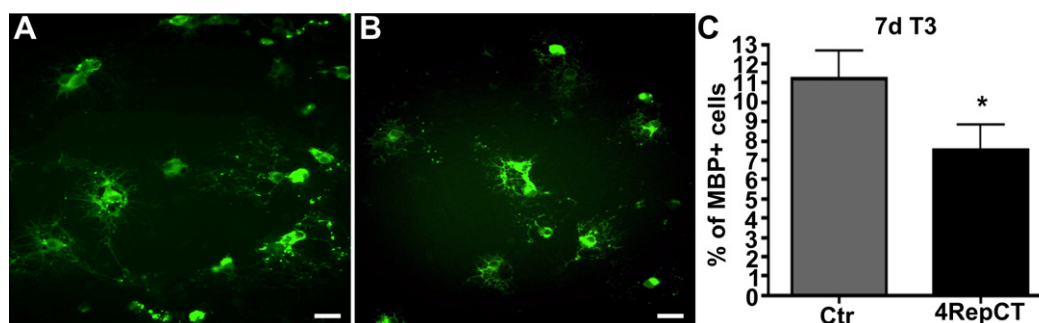


Fig. 5. NSCs retained their capacity to differentiate into oligodendrocytes on 4RepCT films albeit with a slightly lower efficiency. Micrographs depicting MBP-positive cells derived from NSCs by T3 treatment cultured on (A) P+F-coated control culture plates or (B) 4RepCT film. (C) Quantifications of the fractions of MBP-positive cells relative to the number of DAPI-stained cells in the two different conditions. (1586 cells counted in control conditions and 2676 cells on 4RepCT substrates). Data represents mean \pm S.E.M., * $p < 0.05$. Scale bars: 20 μ m.

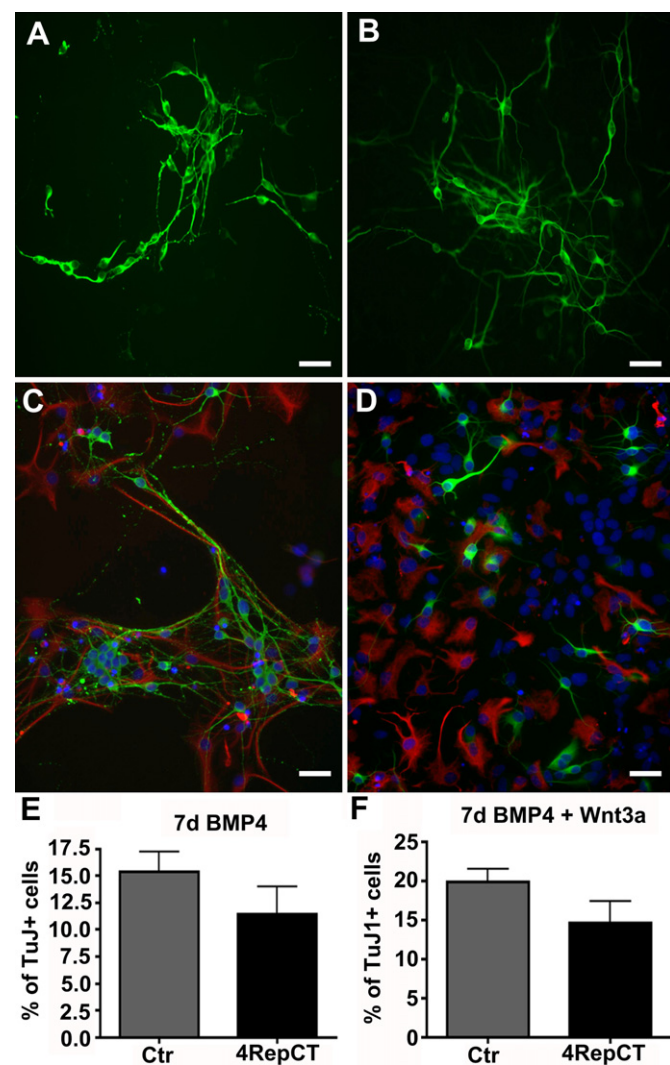


Fig. 6. NSCs retained their potential to differentiate into mature post-mitotic neuronal cells on 4RepCT films in response to BMP4 and co-treatment of BMP4 and Wnt3a respectively. Micrographs demonstrating neuronal cells derived from NSCs after treatment with BMP4 only (A,B) or co-treatment with BMP4 and Wnt3a (C,D) when grown on control culture plates (A,C) or 4RepCT (B,D). Note the slightly more immature morphology of BMP4/Wnt3a co-treated cells on 4RepCT compared to control. Green = TuJ1-staining. Red = GFAP immunoreactivity. Blue = DAPI. (E,F) Quantifications of the fractions of TuJ1-stained cells relative to the number of DAPI-stained cells after treatment with either BMP4 only (E) or co-treatment with BMP4 and Wnt3a (F) on the two different substrates. (1235 (E) and 1581 (F) cells counted in control conditions and 2072 (E) and 2441 (F) cells on 4RepCT substrates). Data represents mean \pm S.E.M. Scale bars: 20 μ m. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In the present study, we show that FGF2-expanded embryonic NSCs remain undifferentiated and maintain cell viability on 4RepCT films. However, a slightly prolonged time required for attachment was observed compared to when grown on P + F coatings, which could be a result of extracellular cues lacking in the 4RepCT matrices or that the cells start to produce their own extracellular matrix under the process of attaching to the 4RepCT films. The latter implies that the cells would attach via newly synthesized ECM proteins to the 4RepCT films and would explain why the cells

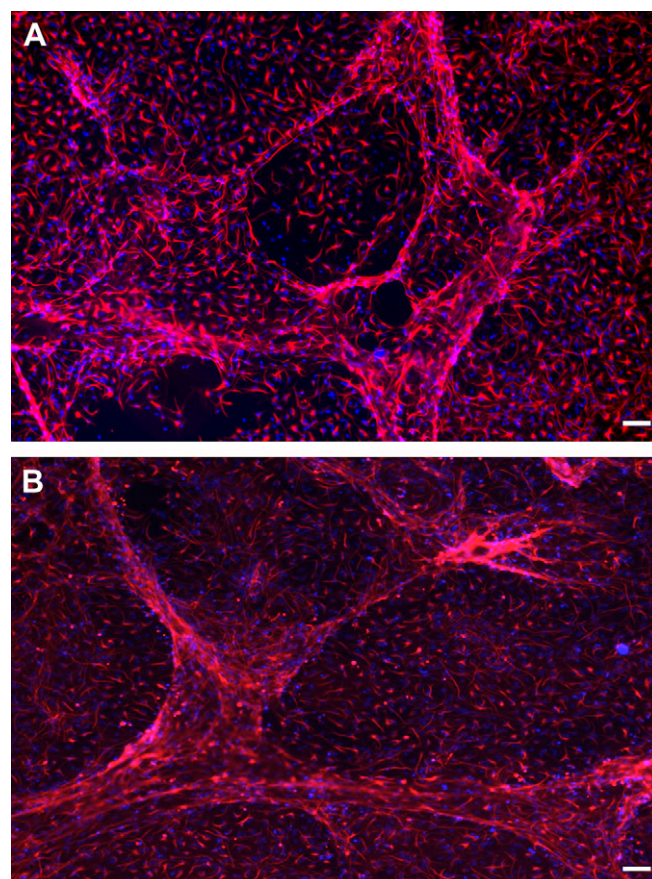


Fig. 7. NSC cultures aligned with 3D structures of 4RepCT (foam) and retained differentiation potential into astrocytes. (A,B) Low-power micrographs displaying GFAP-positive cells derived from NSCs when expanded on 4RepCT foam structure. Red = GFAP immunoreactivity. Blue = DAPI. Scale bars: 50 μ m. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

behave normally in all other aspects we have monitored. It is well known that proteins rapidly coat a biomaterial surface [32], but it cannot be the sole mechanism of attachment since the majority of biomaterials fail to support NSC attachment and growth. Clearly, more extensive studies are needed to determine the detailed mechanisms of NSC attachment to 4RepCT films. Yet, the NSCs retained self-renewal and their capacity to differentiate into multiple cell fates when expanded on 4RepCT films, showing that the multipotency of the NSC remained intact during the culture period observed.

There are a few reports on defined 3D materials for NSC culture. Silva et al. demonstrated the formation of hydrogels consisting of a network of nanofibers by self-assembly of amphiphilic peptides that support growth of embryonic NSC from mice [33] and similarly, Cunha et al. showed that adult mouse NSC could be cultured in nanofiber networks of self-assembled peptides [34]. In contrast to the mechanical robustness and versatility (e.g., films, foams and meter-long fibers) of 4RepCT structures [16,28], self-assembling peptides are predestined to form hydrogels of entangled nanofibers of about a few micrometers in length [33]. For certain applications alternative and solid 3D structures are needed, like, e.g., long and organized fibers for directed axonal outgrowth. In this study we show that the 4RepCT foam structure supports NSC attachment, survival and differentiation into astrocytes and may be a good candidate to use in future studies in of NSC in 3D.

5. Conclusions

The performance of the 4RepCT matrices in this study largely equals the golden standard coating of embryonic NSCs (P + F), but compared to these, 4RepCT matrices have additional qualities in terms of mechanical properties, biosafety and three-dimensionality. In summary, our experiments demonstrate that 4RepCT provides a novel substrate for efficient culturing of NSCs with no negative effects on cell viability, self-renewal, or differentiation potential, except for a slightly less efficient oligodendrocyte differentiation. The results constitute proofs-of-principle in stem cell culturing and provide the basis for further deepened investigations of alternative tailor-designed structures to optimize stem cell culturing.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biomaterials.2012.07.021>.

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