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A tenascin-C mimetic peptide amphiphile nanofiber gel promotes neurite outgrowth and cell migration of neurosphere-derived cells

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ABSTRACT

Biomimetic materials that display natural bioactive signals derived from extracellular matrix molecules like laminin and fibronectin hold promise for promoting regeneration of the nervous system. In this work, we investigated a biomimetic peptide amphiphile (PA) presenting a peptide derived from the extracellular glycoprotein tenascin-C, known to promote neurite outgrowth through interaction with β1 integrin. The tenascin-C mimetic PA (TN-C PA) was found to self-assemble into supramolecular nanofibers and was incorporated through co-assembly into PA gels formed by highly aligned nanofibers. TN-C PA content in these gels increased the length and number of neurites produced from neurons differentiated from encapsulated P19 cells. Furthermore, gels containing TN-C PA were found to increase migration of cells out of neurospheres cultured on gel coatings. These bioactive gels could serve as artificial matrix therapies in regions of neuronal loss to guide neural stem cells and promote through biochemical cues neurite extension after differentiation. One example of an important target would be their use as biomaterial therapies in spinal cord injury.

Statement of Significance

Tenascin-C is an important extracellular matrix molecule in the nervous system and has been shown to play a role in regenerating the spinal cord after injury and guiding neural progenitor cells during brain development, however, minimal research has been reported exploring the use of biomimetic biomaterials of tenascin-C. In this work, we describe a selfassembling biomaterial system in which peptide amphiphiles present a peptide derived from tenascin-C that promotes neurite outgrowth. Encapsulation of neurons in hydrogels of aligned nanofibers formed by tenascin-C-mimetic peptide amphiphiles resulted in enhanced neurite outgrowth. Additionally, these peptide amphiphiles promoted migration of neural progenitor cells cultured on nanofiber coatings. Tenascin-C biomimetic biomaterials such as the one described here have significant potential in neuroregenerative medicine.

1. Introduction

Bioactive materials hold promise for promoting regeneration of the nervous system by providing both physical and biochemical cues to cells in the damaged tissue [1–4]. Biomimetic biomaterials can incorporate native cues provided by extracellular matrix (ECM) proteins [5], such as laminin and fibronectin, which can promote cellular migration and guide neurite outgrowth during neural

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development and regeneration. One successful approach to mimicking ECM protein functionality has been covalent ligation of a peptide ligand from a native protein to a material to impart biological activity, a common example being the use of the peptide RGD found in fibronectin and other proteins, to enable cell adhesion through interaction with integrins [6]. In this work, we describe the development of a biomimetic material incorporating a peptide derived from the ECM glycoprotein tenascin-C to aid in neural tissue repair.

The glycoprotein tenascin-C plays a role in regeneration of the spinal cord [7,8]. It is heavily expressed following spinal cord injury and regions of high expression show axon ingrowth [8]. Inhibition of tenascin-C expression after spinal cord injury leads to a reduction in supraspinal axon regrowth and synapse formation with spinal motor neurons and impairs locomotor recovery [7]. Tenascin-C also plays a role in guidance of neural progenitors during brain development [9]. This protein is highly expressed in the subventricular zone (SVZ), contributing to the stem cell niche by changing the response to growth factors, enhancing sensitivity to FGF2 and decreasing sensitivity to BMP4, which promotes EGF receptor acquisition [10]. Tenascin-C is also heavily expressed in the extracellular space of the rostral migratory stream [9], a pathway that migratory neuroblasts from the SVZ follow to the olfactory bulb, where they become new interneurons. Tenascin-C acts through integrin-dependent mechanisms enabling cell adhesion. Meiners and colleagues have discovered a linear peptide within tenascin-C, VFDNFVLK, that increases neurite length [11] through interactions with $\alpha7\beta1$-integrin [12]. This peptide was previously linked covalently to electrospun polyamide fibers, which were coated onto coverslips [13]. Several primary neurons (cerebellar granule neurons, spinal motor neurons, and dorsal root ganglion neurons), when cultured in vitro on the polyamide fiber coatings presenting the tenascin-C peptide, showed increased average neurite lengths [13]. Materials presenting this peptide have been shown to effect non-neuronal systems as well. In a recent report, coatings of self-assembled nanofibers presenting this peptide were shown to promote osteogenic differentiation from rat mesenchymal stem cells [14].

In this work our goal has been to incorporate the tenascin-C derived peptide mentioned above into self-assembling nanofibers formed by peptide amphiphiles (PAs) [4,15,16] capable of forming aligned nanofiber gels that can encapsulate and support cells. PAs developed in our laboratory can be designed to self-assemble into high aspect ratio nanofibers and that gels when exposed to physiological salt solutions containing divalent cations, such as Ca^{2+} [17]. Incorporation of the peptapeptide IKVAV from the ECM molecule laminin has been used to promote differentiation of neural progenitors into neurons in vitro [18], to reduce gliosis after spinal cord injury in vivo [19], and to promote neurite growth in vitro from cell encapsulated in gels [18,20,21] and in vivo in the spinal cord [19,21]. Thermally treated nanofibers can be aligned in a single direction during gelation by application of shear stress, for example, by pipetting through a $CaCl_2$-containing gelling solution [22]. These aligned gels can encapsulate cells, promote aligned neurite growth and guide cell migration in the direction of the nanofibers [21]. In this work we have investigated PAs containing the bioactive peptide sequence derived from tenascin-C in aligned PA nanofiber gels encapsulating neurons and analyzed neurite outgrowth. We also investigated neurosphere-derived cell (NDC) migration on these aligned nanofiber gels.

2. Materials and methods

2.1. Peptide synthesis and purification

PAs were synthesized using standard solid phase peptide synthesis methods with Fmoc-protected amino acids and Rink-resin purchased from Novabiochem Corporation, as previously described [20]. Briefly, for each coupling, the Fmoc protecting group was removed by shaking the resin in 30% piperidine in *N*,*N*-dimethylformamide (DMF). Amino acids were coupled by adding the Fmoc- protected amino acids with *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and Diisopropylethylamine (DIEA) in DMF. The palmitoyl tail was added using a molar ratio of palmitic acid/HBTU/DIEA of 4:4:6. PAs were cleaved with 95% trifluoroacetic acid (TFA), 2.5% triisopropyl silane (TIS) and 2.5% H_2O. PAs were purified by preparatory scale reverse phase HPLC running a mobile phase gradient of 100% H_2O to 100% acetonitrile with 0.1% ammonium hydroxide. HPLC fractions were checked for the correct compound using electrospray ionization mass spectroscopy (ESI-MS), rotary evaporated to remove acetonitrile, and lyophilized (Labconco, FreezeZone6).

2.2. Conventional transmission electron microscopy (TEM)

Samples for conventional TEM microscopy were prepared from 0.1 wt% PA solution dissolved in milli-Q water or saline and adjusted to a pH of 7.2–7.4 by adding NaOH. The solutions were then heated to 80 °C in a water bath for 1 h, and then slowly cooled to room temperature. 2 μL of a 0.1 wt% PA solution was pipetted onto a carbon formvar grid (Electron Microscopy Sciences) and allowed to dry. The samples were then negatively stained with 2 wt% uranyl acetate solution. Each sample was imaged using a JEOL 1230 TEM operating at 100 kV.

2.3. Small-angle X-ray ccattering (SAXS) and data modeling

Samples for SAXS were prepared by dissolving 1 wt% PA solutions in a 150 mM NaCl and 3 mM KCl solution, adjusted to a pH of 7.2–7.4 by adding NaOH. SAXS measurements were obtained using beamline 5ID-D, in the DuPont-Northwestern-Dow Collaborative Access team (DND-CAT) Synchrotron Research Center at the Advanced Photon Source at Argonne National Laboratory. A double-crystal monochromator was used to select X-rays with energy of 15 keV, or a wavelength $\lambda = 0.83$ Å. The data was collected with a CCD detector positioned 245 cm behind the sample. The scattering intensity was recorded in interval $0.0005 < q < 0.23$ Å^{-1}. The wave vector defined as $q = (4\pi/\lambda)$ sin $(\theta/2)$, where θ is the scattering angle. The 2D SAXS patterns were azimuthally averaged and the background was subtracted using standard methods to produce intensity vs. q profiles using the two-dimensional data reduction program FIT2D. Data analysis was based on fitting the scattering curve to an appropriate model by a least-squares method using software provided by NIST (NIST SANS analysis version 7.0 on IGOR). The TN-C PA SAXS curve was fit to a core-shell cylinder model.

2.4. Neurite outgrowth, alignment, and cell viability assays

P19 embryonal carcinoma cells were cultured as described in MacPherson et al. [23]. Media was composed of α-MEM (Gibco), 7.5% newborn calf serum (Lonza), 2.5% fetal bovine serum (Gibco), penicillin (100 units/ml) and streptomycin (100 μg/ml) (Invitrogen). Neuronal differentiation was induced by treating P19 cells in non-tissue culture-treated Petri dishes with media containing 5 μM retinoic acid (Sigma) for four days. Neurospheres were collected from the Petri dish and allowed to settle in a centrifuge tube for 10 min. Media was removed, then trypsin/EDTA solution was added and the tube was gently agitated for five minutes. Cells were dissociated by triturating the neurospheres, and then media was added to inactivate the trypsin. Cells were centrifuged and the pellet was resuspended in media to a concentration of 25,000 cells/μl. Cells were mixed (1:4) with PA solution. 4 μl of PA/cell solution

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