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A dynamic duo

Self-assembly of nanofibers facilitates repair of spinal cord injury in mice

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The extracellular matrix (ECM) is a vital component of all tissues which contributes to the physical and chemical cues that can affect cell fate. The design of materials to encourage repair of tissue following injury is a long-standing goal of regenerative medicine. Supramolecular polymers based on reversible non-covalent interactions form fibrous materials that can act as simple, but tailored ECM mimics. On page XXX of this issue, Álvarez *et al.* (*1*) show that tuning the dynamics of bioactive supramolecular polymers correlates with the degree of regeneration and functional outcome following acute spinal cord injury (SCI) in mice.

Structurally, the ECM comprises a nanoscale fibrous network composed of a complex and dynamic collection of structural proteins (*e.g.,* collagen, fibronectin, laminin) along with glycosaminoglycans (*e.g.,* heparin, hyaluronic acid) and signaling proteins (*e.g.,* growth factors, enzymes), which are constantly being remodeled based on interactions with the resident cells. The signals provided by the ECM can have multiple, sometimes opposing, roles on the local tissue environment, beneficially aiding regeneration of damaged tissue or conversely, negatively amplifying a diseased tissue environment. This is especially true following SCI where a complex ECM microenvironment of proteins and proteoglycans can inhibit axon regrowth, but also act to isolate the injury site, preventing further damage (*2*, *3*). This response contributes to the difficulties in treating SCI, emphasizing the importance of understanding the role of the ECM in tissue environments and designing synthetic mimics of the ECM to promote regenerative cell behavior(*4*).

Supramolecular polymers are a promising class of materials that are composed of well-defined monomeric subunits, which self-assemble into nanofibers through reversible non-covalent interactions to form a mesh-like network that structurally mimics the ECM. Peptide amphiphiles (PAs) are designer molecules that form supramolecular polymers that contain a hydrophobic segment at the N-terminus, a β-sheet like peptide sequence, and a charged solubilizing peptide sequence at the C-terminus (see the figure). The design of the PA allows for incorporation of a bioactive peptide sequence (epitope) at the C-terminus, which is displayed on the outside of the nanofiber (*5*). Compared to covalent polymers, PAs are injectable, degradable, and display high epitope density (*6*).

PAs functionalized with a laminin derived peptide (IKVAV) have previously been shown to promote differentiation of neural progenitor cells *in vitro* and suppress astroglial differentiation (*6*). This was attributed to the availability of the IKVAV epitope, owing to its density on the PA nanofibers. Later work demonstrated restoration of partial function in a mouse model of SCI (*7*). Álvarez *et al.* add to our understanding of how supramolecular polymers efficiently interact with neural cells and promote regeneration, highlighting the importance of supramolecular assembly dynamics.

Due to their reversible non-covalent bonds, supramolecular polymers exist in an equilibrium between monomeric and aggregated states. Disruption of the β-sheet region increases the dynamics of the PAs within the supramolecular structure (*8*). Álvarez *et al.* characterize a library of PAs with a mutated β-sheet tetrapeptide sequence by changing the glycine, alanine, and valine content. These mutations affect the organization of the PA within the supramolecular polymer and the equilibrium between the monomer and the supramolecular polymer. They show that IKVAV-presenting PAs with a high degree of dynamics were more effective at promoting neuronal differentiation of neural progenitor cells *in vitro*. Extending their studies to a severe contusion mouse model of SCI, the authors include a PA displaying a fibroblast growth factor-2 (FGF2) mimetic peptide (YRSRKYSSWYVALKR), a multi-functional growth factor with important roles in adult neurogenesis. The FGF2-presenting PAs are mixed with IKVAV presenting PAs to make co-assembled nanofibers that display both epitopes. They find that the co-assemblies of FGF2 PAs with a mismatched β-sheet sequence (*i.e.*, VVAA mixed with AAGG) showed higher total axon regrowth compared with controls, reduced glial scarring around the lesion, improved angiogenesis, improved neuronal cell survival and higher locomotor recovery. Further characterization showed that using an FGF2 PA derivative with a mismatched β-sheet forming peptide sequence showed higher degrees of dynamics within the supramolecular polymer.

The increased dynamics of the supramolecular polymer may be more effective owing to its interactions with cells, such as through effective integrin clustering or possibly differences in the mechanics perceived by the cells. It is however, currently not possible to attribute the dynamics of the co-assemblies to SCI recovery in the mouse models via direct observation. Developing methods to study how bioactive materials interact with biological systems is important to improve understanding of the cell-material interface. Previous work on related supramolecular polymers have shown the importance

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of characterizing the exchange mechanisms directly using super-resolution microscopy, which revealed monomer exchange within disordered domains (*9*, *10*). Site-directed spin labeling electron paramagnetic resonance spectroscopy (SDSL-EPR) was used on related derivatives to characterize the supramolecular dynamics *in vitro* (*8*). Advances in SDSL-EPR *in vivo* could possibly be used to characterize the dynamics of these materials in biological environments. Perhaps a supramolecular systems chemistry approach might be utilized for kinetic control of the supramolecular polymer (*11*). Additionally, advances in protein structure prediction and understanding protein structure dynamics may provide opportunities to engineer the dynamics of mimetic materials (*12*). The study of Álvarez *et al.* offers exciting opportunities for kinetically controlled supramolecular polymers, and the pathway complexity of these self-assembled structures needs to be considered and characterized (*13*).

Ultimately, effectively promoting SCI regeneration will likely require a material with multiple bioactive sequences that mimic other pro-regenerative circuits in biology. Understanding these systems requires methods to kinetically control organization of multicomponent supramolecular networks and characterize their composition and behavior (*14*, *15*). Moving forwards, these intriguing materials have potential for translation, because they originate from well-defined chemical molecules whose degradation products can be identified, aiding in the characterization of the safety profile of the materials when continuing in chronic SCI models of other animals and potentially humans.

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