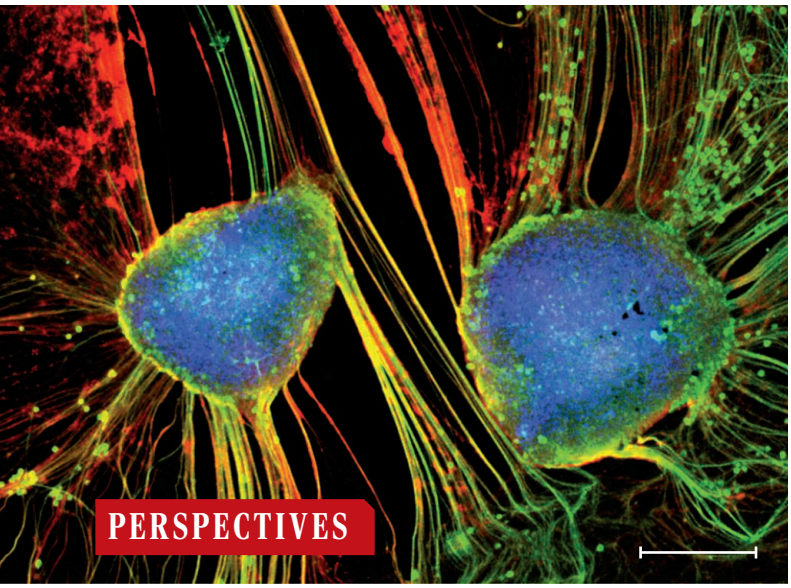

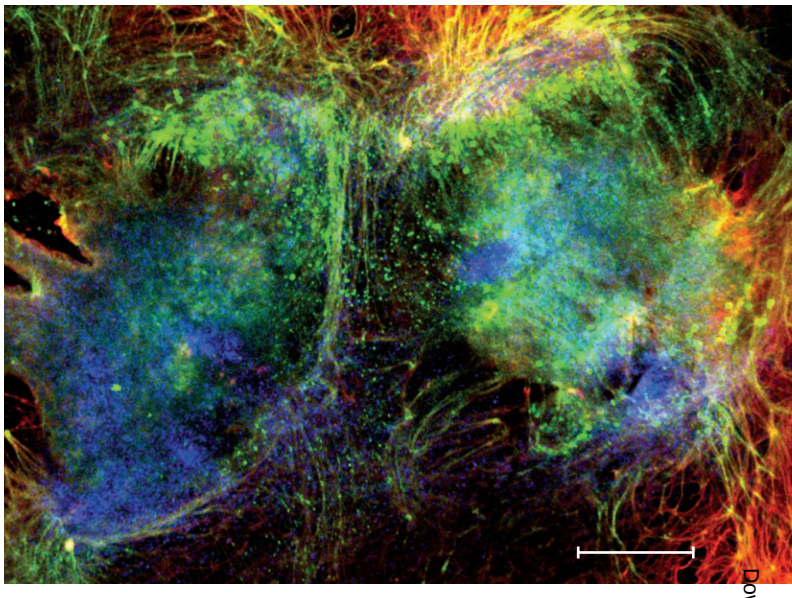

PERSPECTIVES

BIOMATERIALS

Nanomaterials for stimulating nerve growth

Flexible nanomaterials may recruit neurons or create artificial bridges to restore connectivity

By **Silvia Marchesan,[1] Laura Ballerini,[2] Maurizio Prato[1,3,4]**

Despite recent advances in supportive care for spinal cord injury (SCI), there is a great need for treatments that can improve the neurological outcome (*1*). After SCI, there is essentially no regrowth of axons beyond the point of the lesion, leaving intact, although nonfunctional, circuits below the site of injury. We discuss the potential for functional recovery from SCI by using nanomaterials to restore these dysfunctional circuits through a combination of artificial connections and devices to help stimulate motor and sensory recovery.

Because SCI interrupts axons and alters myelination, which impairs sensory and motor pathways, recovery of lesion in spinal tissue involves regeneration of the long neuronal tracts mediating such functions. To date, key neuroregenerative strategies include cell-based therapies, as well as implantable synthetic scaffolds, to promote stem cell differentiation into nerve tissue to bridge across the lesion site. Alternatively, implantable neuroprostheses can also be engineered to contain electrodes for recording and stimulation; however, the state-of-the-art is far from reaching optimal stability of the device at the synthetic-biological interface (*2*). These combined approaches represent one of the more promising avenues, although fine control over implant and long-term cell fate remain to be demonstrated. Typically, implants are composed of stiff and static materials defined at best to the micrometer scale, including silicone and stainless-steel elements, and platinum-iridium electrodes that are ultimately rejected by the human organism (*3*).

Nanomaterials have the potential to create more active scaffolds that enable intimate tissue interactions to form truly biohybridized systems. In many applications, nanostructures hold the potential to increase cell viability and adhesion to premanufactured three-dimensional (3D) scaffolds. Engineered nanomaterials coupled to microtechnology may also act as electrodes to stimulate neural tissue and record activity (*4*). Ideally, the scaffold should promote natural tissue regeneration and axon remodeling to either reconnect neurons, or at least electrically bridge between them where the contact was lost. Once functional connection is reestablished, the scaffold should be resorbed to leave only natural tissue in place. If permanent integration is needed, the electrical bridge should be free from risks of long-term side effects, something that is not currently achieved with traditional implants.

Stiffness, mechanical compliance with the neural tissue, and size are the guiding rules to developing 3D implants able to reach unprecedented biological integration. An implantable bridge must match the shape and elasticity of human tissue for long-term, high-quality performance. Neural tissue is soft; its shear modulus is between 0.1 and 10 kPa, depending on age and anatomic region, similar to the softest gelatins. Recently, neural implants were prepared with elastic materials designed to meet the mechanical properties of spinal and brain tissues. Subdural implantation of an electronic device into the dura mater—that is, the outermost of the three membranes that envelop the brain and spinal cord and is located immediately under the bone—provides an intimate interface for the integration of electronics and microfluidics to stimulate neurons (*3*). For additional technological advancements to reach patients, optimal electrode densities and embedded electronics for real-time reading and writing into the nervous system will be needed.

Supramolecular hydrogels have the viscoelastic and dynamic character of neural tissue but typically lack the high conductivity required for interfaces with electrically active tissues, as well as the high resilience to sustain the mechanical stress that the spinal cord faces. Hydrogel integration with nanomaterials such as carbon nanotubes (CNTs)

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PHOTOS: S. USMANI *ET AL.* (6)

Flexible CNT networks can provide a scaffold that promotes neuronal growth (right; compare with left image without scaffold). Their electrical conductivity can be used to help stimulate neurons. Scale bars, 500 μm.

could impart the needed properties, and CNT-based scaffolds revealed a remarkable ability to guide the connectivity of neural networks. However, the chemical functionalization needed to lessen the aggregation-induced cytotoxicity of CNTs can reduce their electrical conductivity, and thus must be designed ad hoc (*5*).

Chemical vapor deposition (CVD) can create a nanoporous network of unfunctionalized CNTs that is well organized through space in a scaffold and avoids the issue of CNT aggregation when used in solution. The successful integration between networks of neurons and CNTs can be maximized in three dimensions, as shown by the growth of a neural web on a CVD-derived mesh of pure CNTs that supports the reconnection of segregated spinal cord segments. Its conductivity increases linearly from 3.5 to 25 S m^{-1} when compressed and more contacts are made. Such CNT scaffolds, besides inducing a low inflammatory response when implanted, possess an ideal morphology for the biointegration with regrowing neuronal fiber (see the photos) (*6*). An elongated morphology indeed appears to be key for efficient communication with neurons toward their integration into hybrid systems.

Graphene has been introduced in flexible and stretchable electronics for wearable devices and implants in both ex vivo and in vivo contexts, proving superior performance, in terms of sensitivity and resolution, over conventional methods (*7*). Properties similar to those of the CNT networks can be extended to graphene that is electrospun into nanofibers, which were implanted to provide axonal guidance through their elongated morphology, and even attract migrating neuroblasts from their brain niche (*8*). This recruitment provides a needed capability; the spinal cord is a source of stem cells, but at present there is no way to direct them to the lesion site.

Injectable nanostructured bioelectronics are needed to develop high-density neural-electronic interfaces, with semiconductor nanomaterials such as silicon nanowires, CNTs, and graphene showing great promise (*4*), provided that they are outer-functionalized with softer components to match neural tissue elasticity. For instance, addition of a biomimetic peptide allows biointegration without compromising cell activity (*9*). Self-assembling peptides and peptide amphiphiles can present minimalist bioactive motifs with high density to promote neural stem cell differentiation for SCI (*10*). They self-organize into supramolecular hydrogels capable of dynamic behavior (e.g., self-healing) with the additional benefit of high degrees of internal order, which are ideal for the biomimicry of hierarchical architectures that occur in tissues (*11*).

Combined approaches are an effective way to maximize recovery. Experiments in small mammals showed that pharmacological intervention synergizes with physical training to induce treadmill locomotion, given that the body weight is supported. In addition, the residual regenerative capacity of intraspinal circuits to bypass lesions can be experimentally improved via activity-based plasticity processes, motivating active participation of the animal—for instance, by encouraging movement to reach a food reward, versus mindless treadmill locomotion (*12*). A study on paralyzed primates established a brain-spine interface that restored weight-bearing locomotion by means of a wireless system consisting of microelectrode arrays implanted in the brain cortex and a stimulating implant in the spinal cord (*13*). Clinical studies on paraplegic patients, where long-term training by brain-machine interfaces was combined with virtual reality and robotic actuators, reported the occurrence of partial neurological recovery. This recovery was associated to the resurgence of lost motor imagery in the cortex (*14*).

Implantable structures have the potential to artificially bridge neurons or recruit neurons to restore connectivity. Injectable nanoelectronics that cooperate with the patient's brain to restore spinal function may generate hybrid structures able to cross the barriers between artificial and natural systems. Many challenges remain in learning how to restore neuronal systems through designed nanostructures, but the very open design space of these approaches will help enable successful outcomes. ■

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CHEMISTRY

From sequence to color

Tyrosine-containing tripeptides form eumelanin-like pigments with tunable properties

By **Marco d'Ischia**[1] *and* **Phillip B. Messersmith**[2]

Black eumelanin pigments play a key role in determining skin and hair color in humans and other animals. In the body, they are produced by enzymatic conversion of tyrosine to dopaquinone, followed by oxidative polymerization (*1*). Synthetic eumelanin-type biopolymers—such as mussel-inspired polydopamine (*2*)—have optical, electronic, and free-radical properties that make them attractive for materials and biomedicine applications. However, it remains difficult to control and tune the properties of eumelanin-type biopolymers. On page 1064 of this issue, Lampel *et al.* (*3*) show that tyrosine-containing tripeptides can serve as tunable precursors for polymeric eumelanin-like pigments, with properties that depend on the peptide sequence and the degree of supramolecular order that it imparts.

"...understanding how peptide/protein components control the self-assembly and solubility of eumelanin-type pigments may lead to important scientific and technological advances..."

Development of eumelanin-based technologies requires a deep understanding of the mechanisms that govern the formation of the disordered oligomer assemblies that make up the supramolecular architecture of eumelanins. Such understanding may lead to a rational strategy for controlling and tuning

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Science

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