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Self-Assembly and Biomaterials

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An interesting field within the broad subject of biomaterials is the chemical and physical crafting of materials that can functionally substitute or help regenerate the organs and tissues of the human body. Regeneration is the new dimension of this field as opposed to the more established area of permanent implants and devices to substitute natural structures and functions. With the advent of nanoscience the field is experiencing a renaissance by embracing the vision that artificial nanostructures of the self-assembling type could be designed for highly specific functions to promote regenerative processes. Functional nanostructures could form at tissue sites through self assembly once their molecular precursors are introduced into the human body in aqueous media. The self-assembly strategy allows the introduction of these biomaterials at tissue sites in fairly non-invasive ways. Alternatively, pre-assembled nanostructures could be delivered into the bloodstream with targeting information. Potential functions include, the control of cell receptor signaling in agonist or antagonist mode, signaling through interactions with intracellular targets, delivery of small molecules or nucleic acids to cells to alter genetic programs, targeting of therapies to a subset of cells in the human body using specific molecular signals, or the performance of specific biomechanical functions. Nanostructures have been commonly investigated as drug delivery vehicles in the form of liposomes, polymersomes, and in various kinds of nanoparticles¹. In regenerative medicine targets, it has been more common to investigate bulk polymers, hydrogels, and artificial proteins². The *nanoscience school* brings an enormous opportunity to design bottom up artificial but highly biocompatible objects to guide human biology in regeneration. Regeneration is without a doubt the new medicine in this century, linked to the universal aspiration of humans for high quality of life and longevity. This brief reflection on self-assembly and biomaterials does not review the literature at the intersection of these two fields, it contemplates instead its future based on recent work.

The targets of regenerative medicine are many; the brain, the heart, joints, and the circulatory system all rank high since novel approaches at these sites would have enormous human impact. In the central nervous system it is important to cure or at least reduce the severity of Parkinson's and Alzheimer's disease, gain the ability to prevent or reverse paralysis after spinal cord injury, or regenerate structures such as the macula or the optic nerve to preserve vision. In connection with the heart and the circulatory system, the possibility to rescue or regenerate myocardium after infarct would improve the functional outcome after heart attacks, and approaches to promote arteriogenesis in peripheral vascular disease, often occurring in diabetic patients, could avoid amputation. Effectively growing cartilage, bone, tendons, ligaments, and intervertebral discs would raise human quality of life and functionality across all ages.

Nanostructures designed to have specific shapes, dimensions, internal structure, and surface chemistry could in principle emulate some of the architectural features and signaling machinery of the natural extracellular matrix in order to promote regenerative processes. Supramolecular self-assembly can combine signals and control the concentration of signals in a specific nanostructure, and even mediate interactions among nanostructures to create

more complex objects. The use of peptides to build them is logical because the known biological signals recognized by receptors and other intracellular proteins can be incorporated into the nanostructures. Also, provided an immune response is avoided, they will naturally degrade and not leave any trace after they have functioned as bioactive elements. Equally interesting is the notion of introducing saccharides and nucleic acids in the design of nanostructures for signaling or genetic manipulations. A number of peptide nanostructures have been developed and tested over the past decade including β -hairpins that upon folding create networks³, ionic self-complimentary peptides that form nanoscale fibrils⁴, and nanostructures based on molecules known as peptide amphiphiles with spherical, planar, and cylindrical shapes^{5–7}. Novel more complex forms will no doubt be invented in years to come, with the expectation that architectural complexity will be commensurate with functional sophistication.

Artificial nanoscale filaments can emulate the physical environment that cells see around them. In biology this architecture is associated with polymeric fibrils that can bundle in controlled ways to vary their diameter (e.g., collagen), by supramolecular polymers of proteins such as fibronectin, or by high molar mass polysaccharides. High aspect ratio filaments could wrap around cells covering long distances over their surfaces, act as cables that connect several cells, or mechanically support them by creating three dimensional networks (see Figure 1). If signals are to be effectively displayed in predictable fashion on well defined filament surfaces, the molecules that form the supramolecular fiber would need to have a significant internal order parameter. This is a key difference with respect to the ordinary cylindrical micelles that have been known for a long time. But if effective signaling needs to occur through interactions with cell receptors, the nanostructures should not be crystal-like but have sufficient internal dynamics to succeed at binding events. Bending ability to adjust to required distances with respect to receptors, ability to insert themselves on the complex surfaces of cells, and support various modes of motion in the signals they contain will be important to succeed at binding targets in the highly dynamic environment of cells. That is, a compromise between order parameter and dynamics should create the ideal nanoscale objects to signal cells, implying that the targeted structural space should lie somewhere between crystals and amorphous polymers. Tunable signal polyvalency in the nanostructure should be another important feature since clustering of receptors in the so called “rafts” appears to be a critical phenomenon in signaling pathways. The signals of interest in the context of nanostructures may take many forms, the simplest ones would be to mimic a natural ligand to trigger the signaling cascade, or to be an antagonist that blocks the cascade. However, the nanostructure could also signal by recruiting proteins with specific orientation in space relative to local coordinates in order to help aim their signaling domains toward receptors. Thus, one-dimensional, functionally polyvalent, and semiflexible nanostructures should be excellent artificial devices to signal cells. The schematic in Figure 2 depicts the vision of these nanostructures on cell surfaces.

The cell signaling platform we developed for regeneration utilized molecules known as peptide amphiphiles (PAs)^{6, 8–12}. We designed specific PA structures that could form by self-assembly cylindrically shaped one-dimensional nanostructures. PA molecules contain a peptide sequence that is covalently grafted to a non-peptidic hydrophobic segment, typically alkyl segments. In aqueous environments these molecules are thermodynamically driven to self-assemble as their hydrophobic segments collapse into the core of an aggregate to minimize interfacial tension, thus exposing peptide segments on their interfaces with water. Our PA molecules combined the hydrophobic segments with peptides containing at least two domains. One domain covalently bonded to the hydrophobic segment termed the β -domain because of its high propensity to form β sheets with other molecules, and a terminal domain carrying biological information for signaling. The mechanically stiff β sheets drive the system to form a one-dimensional aggregate of molecules that disfavors crumpling into

globular objects. Once hydrophobic collapse occurs the biological signals are displayed at the aqueous interface in high density with an average orientation perpendicular to the axis of the nanoscale filament. The nanostructures are therefore cylindrical aggregates of many β sheets that display biological information around their circumference. The cylindrical supramolecular assembly requires hydration to fill volume effectively, and this contributes to internal dynamics in the nanostructure. However, there is internal order in these nanoscale filaments and this contributes to their ability to display their signals effectively. Figure 2 shows a cryo electron micrograph of the one-dimensional cylindrical nanofibers.

The future direction of the field has to consider how to organize rationally bioactive nanostructures at larger length scales. This would create systems with much more potent functionality to guide cells in regeneration. These cells could be exogenous stem cells, the resident cells of our tissues, or autologous cells that are managed through transformations *in vitro* before being transplanted. These systems could be hierarchical in structure providing the long range orientation of nanostructures that exists in the matrices of organs such as the brain and the heart, among others. These systems could also take the form of objects that emulate the structure and intelligent functions of cells. The formulation of these systems will require the bottom up strategies of nanoscience, and intense interdisciplinary activity in the scientific community. Their development will have far reaching implications in all areas of science and technology, not just the field of regenerative medicine considered in this perspective. In one recent example from our laboratory, we discovered the spontaneous rupture of a two-dimensional plaque of PA molecules into bundles of nanofibers that become unusually potent mesogens (see figure 4), forming a lyotropic liquid crystal at an extremely low concentration in water and allowing the formation of a monodomain gel of arbitrary length¹³. This hydrated array of nanostructures aligned at a specific direction over macroscopic dimensions is a good architectural model for the matrix of muscles, the spinal cord or parts of the brain. This surprising result enables the formation of a macroscopic gel made up of bundled fibers by a human hand in which the nanostructures have the same orientation over arbitrarily long distances. We proposed this process involves a two-dimensional Rayleigh instability that breaks a 2D plaque into bundles of nanofibers. The context of this discovery is the fact that polymeric mesogens have always been difficult to align relative to small molecule liquid crystals. However, in this case the assembly of small molecules into bundles of supramolecular one-dimensional nanostructures raises the relaxation times enough so that macroscopic alignment can be easily frozen without the use of large mechanical forces. The monodomain gel can not only acquire its uniaxial orientation over arbitrarily long macroscopic distances but also can entrap cells to produce a cellular wire that serves as a highway to guide the direction of cell migration or to promote high inter-cell connectivity, a feature that is of interest when dealing with electrically sensitive cells such as neurons or cardiomyocytes. These systems could deliver cell assemblies to the heart or brain that are correlated over macroscopic distances.

In another example a hierarchically structured membrane of ordered nanostructures was formed by contact between two aqueous solutions, one containing small molecules and the other biopolymers¹⁴. In this example a hierarchical structure is formed as a result of the combination of specific molecular interactions and ionic osmotic pressure differences at the liquid-liquid interface. We believe these membranes in microcapsule architecture are good candidates to build cell-like objects for biomedical applications and other technologies. There is also great need to develop nanostructures that will take targeted drug delivery into the next level. In spite of a significant amount of work on the use of nanostructures to target and deliver drugs, the advances have not produced groundbreaking results that demonstrate strong efficacy in *in vivo* models. Future systems with nanoscale design could also include complex nanostructures that can enter cells and “transform” or “repair” cells through their ability to interact specifically with intracellular sites in organelles or the nucleus. An

example could be the exciting possibility of nanostructures that carry both proteins and genes to genetically re-program cells into pluripotency with high accuracy and reproducibility. So far it has been a surprise to see what bottom up design of nanostructures can actually accomplish for regeneration, and a lot more innovation will still be possible at the intersection of self-assembly and biomaterials. As a deliverable from nanotechnology, it would be difficult to imagine something better than offering humans longevity accompanied by high quality of life.

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References

1. Geng Y, Dalhaimer P, Cai S, Tsai R, Tewari M, Minko T, Discher DE. *Nature Nanotechnology*. 2007; 2:249–255.
2. Langer R, Tirrell DA. *Nature*. 2004; 428:487–492. [PubMed: 15057821]
3. Schneider JP, Pochan DJ, Ozbas B, Rajagopal K, Pakstis L, Kretsinger J. *J Am Chem Soc*. 2002; 124:15030–15037. [PubMed: 12475347]
4. Zhang S. *Biotechnology Advances*. 2002; 20:321–339. [PubMed: 14550019]
5. Yu Y-C, Berndt P, Tirrell M, Fields GB. *J Am Chem Soc*. 1996; 118:12515–12520.
6. Hartgerink JD, Beniash E, Stupp SI. Self-Assembly and Mineralization of Peptide-Amphiphile Nanofibers. *Science*. 2001; 294(5547):1684–1688. [PubMed: 11721046]
7. Bitton R, Schmidt J, Biesalski M, Tu R, Tirrell M, Bianco-Peled H. *Langmuir*. 2005; 21:11888–11895. [PubMed: 16316129]
8. Rajangam K, Behanna HA, Hui MJ, Han X, Hulvat JF, Lomasney JW, Stupp SI. Heparin Binding Nanostructures to Promote Growth of Blood Vessels. *Nano Letters*. 2006; 6(9):2086–2090. [PubMed: 16968030]
9. Tysseling-Mattiace VM, Sahni V, Niece KL, Birch D, Czeisler C, Fehlings MG, Stupp SI, Kessler JA. Self-Assembling Nanofibers Inhibit Glial Scar Formation and Promote Axon Elongation after Spinal Cord Injury. *J Neurosci*. 2008; 28(14):3814–3823. [PubMed: 18385339]
10. Huang Z, Sargeant TD, Hulvat JF, Mata A, Bringas P Jr, Koh C-Y, Stupp SI, Snead ML. Bioactive Nanofibers Instruct Cells to Proliferate and Differentiate During Enamel Regeneration. *Journal of Bone and Mineral Research*. 2008; 23(12):1995–2006. [PubMed: 18665793]
11. Shah RM, Shah NA, Del Rosario Lim MM, Hsieh C, Nuber G, Stupp SI. Supramolecular Design of Self-Assembling Nanofibers for Cartilage Regeneration. *Proc Natl Acad Sci*. 2010; 107(8):3293–3298. [PubMed: 20133666]
12. Mata A, Geng Y, Henrikson K, Aparicio C, Stock S, Satcher RL, Stupp SI. Bone Regeneration Mediated by Biomimetic Mineralization of a Nanofiber Matrix. *Biomaterials*. 2010; 31(23):6004–6012. [PubMed: 20472286]
13. Zhang S, Greenfield MA, Mata A, Palmer LC, Bitton R, Mantei JR, Aparicio C, de la Cruz MO, Stupp SI. A Self-Assembly Pathway to Aligned Monodomain Gels. *Nature Materials*. 2010; 9(7):594–601.
14. Capito R, Azevedo H, Velichko Y, Mata A, Stupp SI. Self-Assembly of Large and Small Molecules Into Hierarchically Ordered Sacs and Membranes. *Science*. 2008; 319(5871):1812–1816. [PubMed: 18369143]

Figure 1.
Schematic representation of filamentous nanostructures surrounding cells for signaling events. The filaments could mechanically support and couple groups of cells.

Figure 2. Schematic representation of a supramolecular one-dimensional nanostructure displaying biological signals perpendicular to its long axis that bind to receptors on the cell membrane.

Figure 3.
Cryo transmission electron micrograph of peptide amphiphile nanofibers (micrograph obtained by Honggang Cui).

Figure 4. Schematic representation of bundled nanofibers with long range alignment derived from the spontaneous rupture of a two-dimensional aggregate of molecules.