Nano to Macro: Hierarchies for Multi-Scale Biomaterial Design

Joselle McCracken Literature Seminar October 25, 2012

The significance of pursuing comprehensive characterization of hierarchical structures and exploring structure-function realities within multi-scale materials can be observed through current challenges in the field of tissue engineering. To date, multiple formulations of synthetic extracellular matrix (sECM) are in commercial use for specific research applications, and it has been demonstrated that coordination of biological systems hinges upon elaborate structural interfaces between extracellular matrix (ECM) and cellular populations.[1,2,3,4] Despite the availability of sECM products, the complexity of designing these multi-scale biomaterials that can meet the demands of diverse biological research objectives continues to drive advancements towards modular, diversifiable sECM materials.[5]

To address structural and spatial characteristics of heterogeneous ECM proteins interactions with cells, particular attention has been paid to trans-membrane integrin proteins present in cellular phospholipid bilayers. Integrin protein assemblies are critical to formation of cellular focal adhesions to ECM via the RGD peptide sequence on proteins such as collagen.[6] To study these interactions, electron beam and photolithographic techniques were used to functionalize surfaces with defined numbers of Au nanoparticles (AuNPs) in specific geometric conformations. AuNP surface area limitations prevented immobilization of more than one ECM-peptidomimetic c(-RGDfK-)-thiol functionality, resolving the number of ECM peptides that cells could 'see' over defined geometries.[7,8] Surface passivation of regions surrounding AuNPs was performed using poly(ethylene glycol) terminated siloxanes, establishing 2-D surfaces on which effects of ECM protein spatial and geometric distribution on cellular behaviors could be explicitly analyzed. Findings of this study demonstrated that there is a critical lower threshold of 6±1 ECM proteins required to trigger focal adhesions. Furthermore, it was found that classical focal adhesion geometry hinges on cells accessing an ideal number of ECM proteins for mechanical support.[9] When cells are deprived of ECM proteins, process geometries extend outward until more RGD sequences are located, and proper support is achieved.

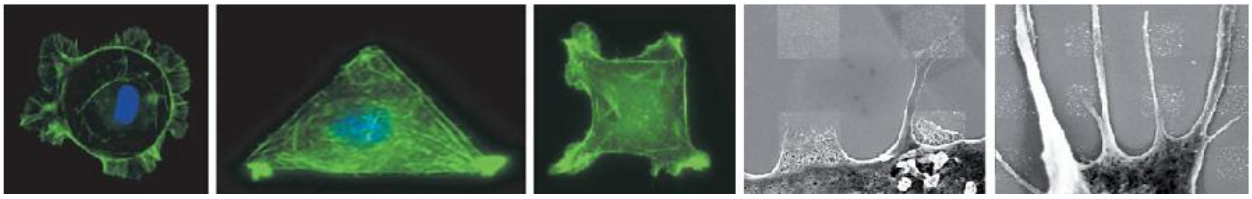

Figure 1. Cellular responses to micropatterned and AuNP-functionalized surfaces patterned with immobilized ECM proteins

Microcontact printing of ECM protein islands has also been used to study cellular responses to specific ECM geometries. Importantly, these studies showed that ECM geometries not only impact cell geometry and process formation but induce specific cellular choices.[10] For example, while stretched cells activate proliferative pathways and rounded cells activate apoptosis, a cell exhibiting intermediate characteristics will tend towards differentiation.[11] Cellular behaviors, therefore, are inextricably linked with spatial characteristics of ambient materials, and it is necessary to fully investigate the interactions underlying these innately hierarchical relationships before designing efficient 3-D tissue scaffolds.

The idea of hierarchy itself is pervasive within all facets of human society, and has enjoyed a frequent recurrence within scientific literature dating back throughout the 20th century.

Biological fields in particular focused on the aesthetic that hierarchical conceptualization of life-systems provides, when explosive progress during the second half of the 20th century in genetic understanding of cellular and organismal development required increased categorization of biomolecular architectures.[12] In those same decades, organizational benefits of hierarchical principles were readily incorporated into physical, mathematical, and computational fields,[13,14] yet the relevance of studying hierarchies for biochemical fields was not unanimously heralded, and the literature reports this dissent.[15,16] In fact, it has only been within the past decade that real efforts have been made towards rigorously defining and organizing structural hierarchies of life systems. In 2008, a notable rationale for this shortcoming was presented by Gerard A. J. M. Jagers op Akkerhuis who argued that hierarchies have been hindered by incorrect categorization of sub-ordinate levels within biological infrastructure, as well as suffering from linearization of complex ideas. In contrast to the inexact hierarchies he critiqued, Akkerhuis built upon Eigen and Schuster's 1977 Nobel-winning concept of hypercycles—or second order cycles—and published an Operator Hierarchy of living systems.[17] The Operator Hierarchy deconstructs evolution of living systems by utilizing specific, discrete closure events, such as membrane encapsulation and the enclosure of the nucleus. Within this framework, material components of living systems are considered and defined as Interactional Systems that each exhibit unique hierarchical complexity. Notably, Operator Hierarchy does not provide a concise strategy for quantitatively evaluating and predicting multi-scale structural and functional behaviors of those biomaterial systems.

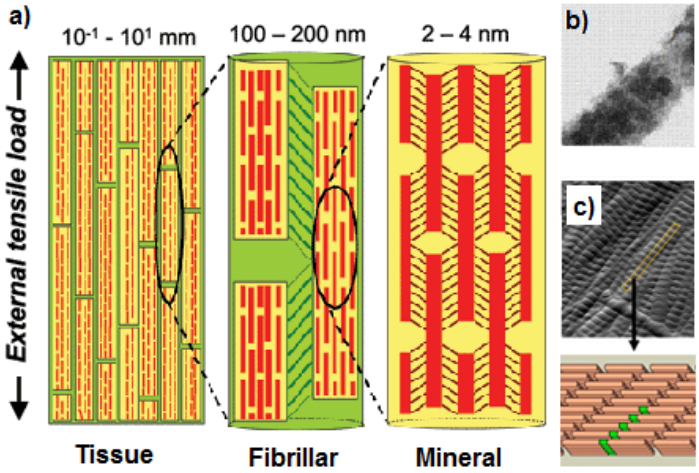

Figure 2. a) Bone structure exhibiting 3 levels of hierarchy **b)** Collagen fibril **c)** Fibril array[27]

In 2010, Cranford and Buehler authored an important step forward towards precisely this objective, by calling for a unification of biomaterial research efforts towards characterizing what they labeled the Structure-Property-Process principles of biomaterials across all length-scales, from nano to macro. Termed 'Materiomics' by the authors, such a field would utilize a multi-scale computational materials science approach to address how molecular level structural changes due to genetic defects(etc.) can contribute to changes in chemical and physical biomaterial properties and lead to mechanical failures.[18] The rationale for such a field is embedded within the mechanisms of evolution. That is, despite consisting of a comparatively limited array of simple chemical building blocks, to maximize survivability and reproductive success, it is necessary for living systems to exhibit diverse as well as robust material properties. In order to achieve these requirements, living systems engineer desirable materials not through properties of the bulk, but through properties of hierarchies.[19,20] Though still in its infancy, the application of materiomics towards disease pathology is illustrative of this principle. In the case of the genetic disease *osteogenesis imperfecta*, in which a single point mutation in ECM protein's collagen leads to systemically weak tendons, fragile bones, and deformity,[21] Buehler utilized a computational approach towards clarifying mechanisms of the genetic disease, demonstrating the importance of biomolecular pathways towards justifying how poorer collagen packing leads to lower protein interaction volumes, and lower degrees of protein cross-linking.[22]

Directly addressing the properties of biological materials that are conferred by hierarchy is another recent approach by H. Gao and others to confront challenges in tissue engineering. Bone composite consists of nano-scale crystals arranged in staggered, parallel rows separated by

soft, connective protein matrix, and composite structures of this type have been demonstrated to be convergent across multiple life systems.[23] Gao's analysis of the structure of bone and nacre attributed remarkable strength, toughness, and flaw tolerance (FT) of bone-like materials to the composite's nano-scale inorganic crystals, which fall below the critical size predicted by the Dugdale model,[24] to yield crystals insusceptible to failure via crack propagation.[25] However, some of the most interesting implications for hierarchical materials originate from Gao's work on 'fractal bone'. In this model, it is assumed that for n number of hierarchical levels, the bone composite unit cell is present at n length scales. While results of these calculations show material strength decreases by a factor of two with each level of hierarchy, FT size thresholds increase exponentially such that by 8 levels of hierarchy, theoretical FT sizes of individual mineral platelets reach up to several miles.[26] The authors concede that mechanisms of failure such as protein matrix deformation would cap these structure sizes at much lower thresholds; nonetheless, properties of hierarchy alone are shown to confer properties present on the nano-scale to macroscopic materials.

The current state of the literature in regards to the importance of hierarchy suggests that for real progress in tissue engineering applications, a greater understanding of biomaterial interactions across multiple spatial length-scales is essential, since these are precisely the terms by which biological structures evolved. Furthermore, evaluating the concept of the hierarchy as a technique for classifying and organizing material systems provides a fascinating route forward towards a more unified approach to tissue engineering and biomaterial design.

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