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Imaging and modelling tissue structure to inform the development of musculoskeletal therapies

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Abstract

Biomanufacturing is moving rapidly towards recreating the complex, hierarchical structures of native tissues. With such advances comes a need to provide a detailed characterisation of the physical interaction between synthetic structures and the biological environment, and to provide increasingly detailed and/or specific targets for synthesis/manufacturing. The musculoskeletal system is a functionally challenging environment in which to apply these synthetic constructs, reflected in the difficulties faced by current treatment approaches. Limited information on the functional role of low-level structural features provides a further challenge. Here, we discuss imaging and modelling approaches for providing this characterisation, focusing on scanning probe microscopy, nonlinear optical methods and vibrational spectroscopy for probing structure, and numerical modeling to explore the potential roles of observed structural features.

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1. Introduction

It is increasingly recognised that the extracellular matrix is a dynamic, nano- and micro-structured environment that provides mechanical, chemical and structural cues to cells [1-4]. By expanding our understanding of how cells interact with this environment, and particularly how such structures can modulate cell behaviour, it may be possible to improve current treatments or develop new ones. Attempts to recreate natural structure, or apply synthetic structures, to influence cell behaviour are widespread, and use a number of different approaches. These approaches can be broadly described as: top down – modifying the surface of a bulk material to produce a desired morphology, chemistry or surface energy (e.g. surface patterning, etching); bottom up – producing a bulk material made up of many nano/microscale structures (e.g. electrospinning, self-assembly); or a combination of the two.

As advances in biomanufacturing provide new opportunities to recreate the complex native environment of cells, or to subtly modify a material environment to elicit a certain biological response, improved characterisation techniques are required to guide and assess that development.[5] Here, we will explore some imaging and modelling approaches to this end, with a focus on sub-micron structure in the musculoskeletal system.

1.1. Surface engineering

Evidence is emerging that nanoscale surface features have interesting effects on cell behaviour [6-8], though our understanding of these cell-surface interactions is still in its infancy. Nanotechnology research has provided a means to create controlled surfaces that allow the study of these interactions, and to produce the fundamental knowledge to improve clinical outcomes [3]. In the laboratory, cell-surface interactions can be studied using lithography techniques to

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modify topology [9], combining this with surface coatings [10] or as part of a hierarchical patterning approach [11] to create highly controlled and repeatable surface features (Figure 4). A particular advantage of this approach is the ability to decouple parameters such as the size, depth, spacing, shape, and regularity of surface patterns from the complex physical and chemical environment of cell culture. A recent proof of concept study [12] has shown that patterns can be efficiently combined using new scanning probe techniques, providing libraries of up to 25 million features over the nano to microscales. This opens the possibility for large-scale screening studies that can inform the development of a new generation of biomaterials, utilising nanoscale patterns of stiffness, topology and chemistry to modulate biological response.

For *in-vivo* application such as in orthopaedic implants, large surface areas and irregular shapes make lithographic techniques impractical. Control over surface nanostructures can, however, be achieved through immersion in liquids using etching, oxidation or self-assembly to create the patterns. Oxidative nanopatterning have been shown to modify surface topology depending on the time of immersion, which can be used to encourage osteoblast and discourage fibroblast proliferation [13], aiding the integration of bone with the implant. Importantly, these techniques can be readily applied to implants and scaled for production [3]. Combining structural and biochemical stimulation, additional immersion treatments can be applied to induce collagen growth processes [14] or to add monolayers for binding bioactive compounds [15, 16] to further modulate biological response. However, such techniques are unsuitable for recreating the semi-ordered structure of the matrix, which is critical to cell response.[9]

To create semi-ordered nanostructured surfaces that can be scaled to large, clinically relevant sizes, we are using a novel method of high-energy grazing ion bombardment. [17] The extremely local (~10 nm^3) nature of the energy deposition in the high-energy regime leads to the creation of nanosized ‘hillocks’ (dots) on a surface with order determined by grazing angle (Figure 1). By varying irradiation parameters and therefore density and level of order of the hillock structures, human MSC differentiation can be directed towards bone or cartilage.

1.2. Three-dimensional structures

Driven by the biomimetic ideal of recreating the three-dimensional structure of the natural extracellular matrix [1], a range of nanostructured bulk materials have been produced for studying and applying regenerative medicine strategies. The field of nanostructured biomaterials, whether nanoscale structures (e.g. fibrils, foam struts) or nanoscale features on microscale structures, is enormous (see refs [1, 4, 18] for an in-depth description). One of the most interesting areas in this field takes a bottom-up approach to creating nanostructured biomaterials through self-assembly [19, 20], following the strategy by which all biomolecules interact and self-organise to form the structures that govern functionality. This is particularly true of the peptide-based strategies [21] that reproduce the development of the natural matrix, and therefore have potential to recreate the complex architectures and interactions in extracellular matrices. Self-assembling peptide amphiphiles have been used to form nanofibres for cartilage regeneration by displaying a high density of binding epitopes to transforming growth factor β-1 [22], and for modulating osteoblast behaviour in bone scaffolds [23]. Articular cartilage-like matrices have also been produced by combined self-assembly of collagen, hyaluronic acid and aggrecan [24].

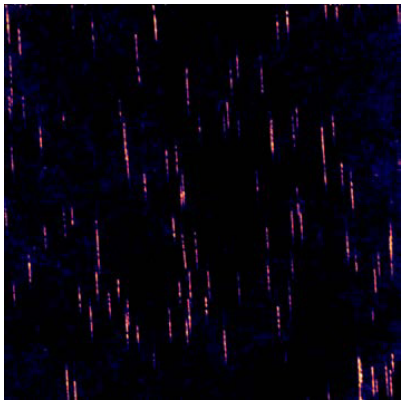

Figure 1: AFM height map of scalable nanostructured surface on TiO_2 created by glancing angle irradiation with swift, heavy ions.

Like surface modification, much of the potential for these three-dimensional nanostructured materials to advance musculoskeletal research and/or clinical treatment is in combining nanostructure with chemical and mechanical stimulation. Here, the field is evolving from an emphasis on recreating matrix porosity and mechanical stability [25-27] (that is, a passive view) towards recreating the dynamic nature of the matrix [28, 29] (an active view). Cell behaviour can be predisposed through embedding bioactive compounds within fibres [30], or hierarchically scaling the three-dimensional fibril structure [31]. Combining such approaches, particularly targeting the release of multiple therapeutic agents at optimised ratios, physiological doses, and in specific spatiotemporal patterns has considerable potential for a range of therapies [32]. Although this potential is far from being realised in the musculoskeletal environment, the large body of work on scaffold production and maturing synthesis technologies have led to consistency in structural properties. This can be used to apply the emerging knowledge of cell-surface interactions [7, 9, 12, 33] in the more complicated three-dimensional setting of the musculoskeletal system.