

# Screening of 3D biomaterials- A New Dimension in Biomaterials Research (or two?)

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Biomaterials play a crucial role in the healthcare industry. Applications range from medical implants, drug delivery, prosthetics and diagnostic devices, but it is in the field of tissue engineering and regenerative medicine where they have the potential to revolutionise preventative and therapeutic healthcare. The use of 3D scaffolds, produced from biomaterials, as a template for cell growth and repair forms the basis of tissue engineering. Such scaffolds have already found clinical use in preliminary proof-of-principle studies [1]; however, widespread success remains heavily dependent on understanding the basic cell-scaffold interactions. Currently, advances are limited to trial and error resulting in a lengthy and costly evaluation process which has restrictions on the number of cell types, materials and external stimuli which can be examined. Whereas cellular responses to biomaterials are known to be affected by complex, multifactorial interdependent signals, the use of combinatorial and high-throughput methodologies offer the possibility to screen the cellular response to a large number of scaffold properties in one experiment. The ability to examine the influence of multiple parameters on cell behaviour should offer a more mechanistic understanding of cell-scaffold interactions facilitating the improved design of future tissue engineered products.

## 2D screening of Biomaterials

The cell response to a biomaterial is often instigated by cell contact and adhesion to the biomaterial surface. Materials with a range of surface properties can be prepared either as a continuous gradient or as an array whereby the properties of the biomaterial are systematically varied. Gradients of surface roughness, stiffness and chemical composition have all been successfully generated using the same polymer blend system and used to study osteoblast adhesion, proliferation and differentiation; illustrating how combinatorial approaches can be used to study the complex interplay between multiple material properties [2]. Furthermore, gradients of extracellular matrix (ECM) proteins and peptides have been fabricated allowing the identification and quantification of biological activity of the protein and their effect on cell response [3].

Alternatively, polymeric microarrays have been developed whereby large libraries of materials are present on a single substrate as discrete spots each with a distinct composition. Microarrays display certain advantages over gradients namely, thousands of different molecules can be routinely examined in a fully automated fashion. An early example of a polymeric microarray was demonstrated by Mei et al and involved the use of a robotic spotter to deposit various combinations of acrylate monomers with initiator on to a cell resistant hydrogel coated microscope slide [4]. Activation of the initiator resulted in polymerisation and the formation of 576 distinct materials in triplicates. This polymeric microarray was used to study the combinatorial effect of material properties in clonal growth of human embryonic and induced pluripotent stem cells.

## Screening in 3D

Whilst admirable progress has been made in developing tools and assays for the screening of biomaterials, it should be noted that in most

tissue engineering applications, biomaterials are processed into 3D scaffolds for cell culture. It is widely recognised that cell behaviour can vary significantly on 2D surfaces and 3D scaffolds, with a number of studies indicating that cell response in 3D is more representative of *in vivo* behaviour [5]. Indeed, 3D screening is becoming an integral part in discovering new anti-cancer drugs. Tumour cells have been shown to respond in markedly different ways when cultured in 2D compared to 3D, resulting in dramatic differences in tumour cell sensitivity to chemotherapeutics when cultured in 3D [6].

With that in mind, a few recent studies have begun to develop combinatorial and high-throughput techniques to study cellular response in 3D tissue scaffolds. Chatterjee et al. fabricated macroporous 3D scaffolds with varying chemical compositions were formed via salt leaching and were used to examine the response of osteoblasts to chemical composition in 3D [7]. The same group also developed apoly (ethylene glycol) dimethacrylate hydrogel system was used to encapsulate osteoblasts and examine the effect of scaffold modulus [8]. Hydrogels were also utilised by Yang et al to study the effect of different ECM proteins on embryonic stem cell differentiation [9]. Despite some initial studies, screening of cell-scaffold interactions in 3D has not been widely adopted as automated methods for scaffold preparation and suitable biological assays do not exist yet. Most 3D culture technologies and scaffold fabrication methods are catered for research use and therefore do not scale well for screening applications that require significant 3D culture expansion and consistent cellular response.

## Functional Assays for Screening

One of the major limiting factors of screening biomaterials, both in 2D and 3D, is the lack of robust, cost-effective, biological assays. Basic information about the cell response to a biomaterial environment can be gathered from assays which measure a single end-point. For example, immunohistochemistry can be used to study differentiation or fluorescent cytotoxicity probes can be used to evaluate proliferation. However, a more thorough analysis of cellular response would require gene expression profiles via PCR methods, DNA microarrays or proteomics. In general, these methods have severe limitations to be used in a high-throughput manner, for example, the requirement for large sample size, cost and time.

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More recently, high-content imaging platforms have been developed which combine high-resolution digital imaging with powerful software algorithms thus allowing multiple and simultaneous read-outs of fluorescence or luminescence per sample in a fully automated and reproducible manner minimising user error and bias. High-content imaging opens up the possibility of real-time, direct quantitative observation of cells and can be used, not only to define cell morphology, but also as a proficient means for evaluating cell differentiation and phenotype [10]. Furthermore, cells can be manipulated with transfections to up-regulate (with DNA or RNA) or knock-down (with miRNA, siRNA or antisense) various genes allowing the systematic study of cellular responses to biomaterials.

## Conclusions

Over the last few years, a number of technologies have been developed to screen biomaterials. However, many of these screens have been limited in their scope and often only focus on the characterisation of commonly used polymer families, such as polyesters, polyacrylates and combinations thereof. With advances in materials sciences and nanofabrication techniques, novel concepts of scaffold production and characterisation should be explored in an effort to produce 3D scaffolds amenable to high-throughput analysis in an automated fashion. Advanced imaging techniques, particularly those with a high penetration depth and good spatial and temporal resolution, provide a suitable platform to study cell behaviour. Combining them with assays to study gene expression, such as high-throughput PCR, has the potential to provide more complete biomarker combinations and functional information about the cell phenotypes. Overall, the field is still in its infancy, but it has the potential to make a significant contribution to the fields of biomaterials, tissue engineering and regenerative medicine. However, for it to fulfil its potential will require the concerted efforts of the biomaterials and tissue engineering fields

to interact and work with nanotechnologists, imaging experts and computer scientists to make the necessary technological developments possible.

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