Research Highlights

Highlights from the latest articles in nanomedicine

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Dynamic cell alignment on switchable topography

Evaluation of: Lam MT, Clem CW, Takayama S: Reversible on-demand cell alignment using reconfigurable microtopography. *Biomaterials* 29, 1705–1712 (2008) – **LE McNamara, MO Riehle, R Burchmore & MJ Dalby**

Surface topography can elicit a variety of responses from cells, including alignment by certain dimensions of micro- [1] and nano-grooved [2] features, which is typically assessed by reference to cells cultured on a control planar surface. To examine the dynamic cellular response to topography, Lam *et al.* introduced a groove-like micropattern into planar poly(dimethylsiloxane) by plasma oxidation, to generate a brittle top layer, followed by unilateral compression [3]. The features could be removed by releasing the force, then reintroduced while C2C12 myoblasts remained adhered to the surface. Cells became aligned during substrate compression, redistributed to 'random' orientations after release and realigned following revival of the topography.

Myoblasts could respond reversibly to the surface changes within 24 h; however, this ability was lost on differentiation to myotubes, which retained their previous orientation. The authors proposed that a small decrease in cell redistribution during relaxation intervals, inverse to cycle number, could be owing to increased extracellular matrix production or minor impairment of substrate relaxation. Visible deformation of the substrate was encountered after approximately nine intervals, with small waves remaining following the release of compression. Repeated mechanical intervention could also promote rearrangement or aggregation of adhesive proteins along the micropattern, resulting in deposition of aligning chemical cues in the absence of topography. In addition, the cells may have

been becoming gradually desensitized to the repeated stimulation; the proportion of differentiated cells was not quantified with time but would probably also contribute to the reduction in reorientation. The decrease may have become statistically significant if examined over a longer time course with a larger sample size.

Monodisperse cell populations were examined in the study, although an interesting extension would be to investigate the effect of the switchable substrate on confluent cell layers. This would assess whether intercellular interactions could be overcome by repeated topographical stimulation, which could have more relevance to conditions *in vivo*. With refinements to improve imaging (microscopy was hindered by a thick polymer layer), substrate recovery after repeated compression and cell viability (increased cell death after approximately nine intervals was attributed to cumulative handling under sub-optimal conditions during imaging), this approach could prove fruitful for molecular and cellular studies of dynamic responses to structured surfaces. In the future, reconfigurable topographies may be useful for conditioning cells prior to implantation and as tools for mechanotransduction and biomaterials research.

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Cytoplasmic force gradient in migrating adhesive cells

Evaluation of: Iwasaki T, Wang Y-I: Cytoplasmic force gradient in migrating adhesive cells. *Biophys. J.* 94(5), L35–L37 (2008) – P Kuntanawat & M Riehle

In the report under review, a novel method to probe cytoplasmic streaming within polarized migrating adherent cells is introduced. The flow dynamics within NIH 3T3 fibroblasts was visualised *in vitro* by microinjection of a mixture of long linear polyacrylamide (PAA; molecular weight: >600 kD) as a force sensor and an optical marker, fluorescent dextran.

During polarized cell migration, the cytoplasmic PAA aggregates moved within 10 min towards the leading region of the cell and never flowed backwards. Once the cells changed direction of migration, the PAA clusters travelled to the new leading region within 30 min. Such behavior was not seen in immotile cells.

After treatment with blebbistatin, a myosin II inhibitor, the cells exhibited random migration and the aggregates of PAA did not migrate towards the leading edge. It is considered that this is due to myosin II-dependent cortical contraction,

which is hypothesized to generate the anisotropic cytoplasmic pressure. In nocodazole-treated cells (microtubule disrupting), the PAA aggregate broke into several smaller aggregates before traveling to the multiple ruffle regions of the unpolarized cells. It was postulated that the multiple-edge extension, as a result of malfunctioned microtubules, may cause changes in the force gradient pattern, thus affecting the direction of the PAA movement.

This paper presents an interesting novel method to visualize intracellular streaming but may need some experimental defining and refinement. One may raise a fundamental question about the approach: would adding foreign material to the cell affect the normal cell behavior? Although the PAA is known for its chemical passivity and stability, overly adding PAA may cause a variation in local-mechanics (i.e., viscosity) indirectly, which, in turn, could trigger biochemical alterations indirectly. Detailed biochemical and physiological observation may be required in future studies and perhaps correlated to the concentration of PAA injected.

Neuronal differentiation of hMSCs in response to nanotopography

Evaluation of: Yim EKF, Pang SW, Leong KW: Synthetic nanostructures inducing differentiation of human mesenchymal stem cells into neuronal lineage. *Exp. Cell Res.* 313, 1820–1829 (2007) – RJ McMurray, N Gadegaard & MJ Dalby

Nanopatterned synthetic biomaterials have a key role in tissue engineering because they provide the necessary platform for cell growth. *In vivo*, such nano-scale features are presented to cells in their

native environment. It is thought that these features may change cell–extracellular matrix contact and hence impact the behavior of the cell. Key cellular processes, for example, adhesion, proliferation and, more recently, the differentiation of mesenchymal stem cells (MSCs), can be affected by both micro- and nano-scale features [1]. It is the availability of adult stem cells, as well as their being less controversial than embryonic stem cells, which makes them a desirable target for use in regenerative medicine. Mesenchymal stem cells have the

ability to form adipocytes, osteoblasts and chondrocytes; however, they are now thought to possess increased pluripotency than when first studied, with the ability to also generate neurons [2]. In a study by Yim *et al.*, hMSCs were shown to differentiate down a neurogenic lineage when cultured on polydimethylsiloxane patterned with 350 nm-wide gratings. Flat polydimethylsiloxane and glass coverslips were used as controls. MSCs were also grown on both flat and patterned surfaces in the presence and absence of retinoic

acid (RA) to induce neuronal cell development. In comparison with MSCs grown on the control surfaces, the MSCs grown on the gratings appeared aligned along the gratings and were significantly more elongated.

Fluorescence microscopy also identified the mature neuronal markers, microtubule-associated protein (MAP)2 and β -tubulin III, which were only identified in cells grown on the flat controls when cultured with RA. To further validate the results, gene-expression analyses were carried out. RT-PCR identified upregulation of the neuronal markers, SOX2, MAP2, neurofilament and tyrosine hydroxylase. However, myosin light chain and myf5, both muscle markers, and CD34, a vascular marker, were also identified. These results were then compared with those of the MSCs cultured with RA. These

showed further upregulation of SOX1 and SOX2 but MAP2, neurofilament and tyrosine hydroxylase were not affected. Analysis of MAP2 expression by qRT-PCR showed MSCs grown on the nanogratings had significantly increased levels both in the presence and absence of RA when compared with the day 7 and 14 flat controls. Microarray analysis of 474 genes was also carried out, which identified the nanotopographical regulation of genes related to neuronal differentiation and function.

This study also determined that the 350 nm gratings produced the most significant effect on stem cell differentiation compared with those in the microscale.

One drawback of the study is that the MSCs used were at passage 6–9. It is thought that MSCs may begin to undergo spontaneous differentiation in culture after passage 3; therefore, it may

be beneficial to study the MSCs at this stage to accommodate for this. One key take home point of the study, however, is that the MSCs grown on nanogratings without RA produced the same, if not a more significant, cell response as that of those grown on flat controls with RA.

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Nanoparticulate toxicology: cellular responses

Evaluation of: Stone V, Johnston H, Clift MJ: Air pollution, ultrafine and nanoparticle toxicology: cellular and molecular interactions. *IEEE Trans Nanobioscience* 6(4), 331–340 (2007) – **A Andar & M Riehle**

The article described gives an overview of toxicological effects that can be caused by exposure to nanoparticles and ultrafine particles. It outlines particulate air pollution and ultrafine particle research, which provides the base evidence for information that can be used for nanotoxicology research.

The production and use of nanoparticles in industrial and consumer products has increased in recent years [1]. An example of this is the Mitsubishi Corporation, which opened a plant in Japan (Frontier Carbon Corporation) to generate carbon fullerenes on a large scale to produce scratch-proof bowling balls, with carbon fullerene production quantities of 4/40 tons/year. This paper suggests that the assessment of nanoparticle risk

needs to be taken into consideration if nanoparticle production and exploitation occurs at such an exponential rate.

Adverse effects on health owing to air pollution have also been highlighted in this article and it describes PM₁₀ particles (particles measuring 10 μ m or less). The PM₁₀ standard was designed to identify those particles likely to be inhaled by humans and PM₁₀ has become the generally accepted measure of particulate material in the atmosphere in the UK and in Europe [101]. It is claimed that PM₁₀ particles exert toxicity through a proinflammatory mechanism, meaning that, once they enter our lungs, they would be capable of promoting inflammation in the lung epithelial cells and macrophages. PM₁₀ exposed to lung epithelial cells and macrophages would generate proinflammatory cytokines, such as IL-8 and tumor necrosis factor- α . The authors also point out that lung epithelial cells show increased transcription of proinflammatory mediators, which are mediated by

increases in intercellular calcium and oxidative stress. Intercellular oxidative stress can activate inflammation through various intercellular signaling pathways that control the expression of the genes involved in inflammation. The genes that regulate the inflammation are under the influence of the transcription factor NF- κ B and activator protein (AP)1, which are highly reactive to nanoparticles.

The authors have done well to stress the need for a toxicological approach towards nanomaterial production. Exposure to nanoparticles can occur as a consequence of their generation from a number of different production sources. It can occur in occupational, consumer and environmental or even accidental and intentional situations. They are also being used in nanomedicine (e.g., cadmium sulphide quantum dots and cadmium selenide quantum dots) and studies have shown that they are taken up by cells by endocytosis and have been observed within cell vesicles [2].

Nanomaterials have many novel properties and can vary not only in size but also in shape, composition, structure surface area and surface coating. Therefore, there is a necessity to confer their mechanisms of cellular and molecular toxicity. A database of nanomaterials in relation to their

dermal exposure, inhalation, ingestion and distribution of particles through the bloodstream to different organs can be related to the amount of particle exposure and dosage rate (similar to radiation protection) and would ensure the safe use of nanomaterials for future applications.

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