Tutorial on the Biology of Nanotopography

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Tutorial Paper

***Abstract*—The aims of this short tutorial are fourfold: 1) to introduce readers unfamiliar with the field to major concepts in the field; 2) to inform the reader of major unresolved questions; 3) to inform readers of a few major sources of relevant literature; and 4) to place the subject in relation to its relevance to other areas of science and practical application.**

***Index Terms*—Adhesion, biological reactions, cell surface, nanotopography, order, physical form of nanotopography, symmetry.**

I. Nanofeatures: Their Occurrence in Biological Systems

REFERENCE TO any good textbook of cell biology [1] will show that the organelles of cells are usually on the nanoscale. Even the surfaces of cells often carry fine processes (microspikes, filopodia, fimbriae, etc.) protruding from the cell on this nanoscale. The surface of cells and the internal organelles is covered with a mosaic of molecules of many different sorts, which may be arranged into more segregated areas such as domains or rafts, also on the nanoscale. Much of the material between cells in multicellular organisms is organized into fibrils, etc., on the nanoscale. Both nanotopography and nanopatterned chemistry are encountered by cells, and they of course in turn present their own features of these two types to other cells. These interactions are mainly, and certainly initially, between the two surfaces, namely, that of the cell and that of the nanofeatured material.

If the nanofeatured material is mechanically weak or is present as nano- or microparticles, such material may be taken into the cell later by the processes of endocytosis (phagocytosis); see [1] for definitions. This initial surface–surface interaction determines the further progress of any reaction because if adhesion fails to take place, there are no subsequent events. Adhesion which is sufficient to elicit a reaction may be, however, transitory, and we do not know in most cases how short an interaction has to be to produce no effect.

The nonliving surfaces that cells encounter, such as prosthetic devices in the body or parts of process plants, usually bear nanotopographic features if only because of their methods of manufacture. Molecularly smooth surface are very difficult to make.

The prime question is whether these nanofeatures are of importance in the lives of the cells and whether they could be manipulated to yield results valuable in the treatment of disease or injury or in improving biotechnology. I am not providing answers to this because this is what current research is trying to elucidate.

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A. Are There Nanoscale Interactions in Biological Systems?

This almost seems like an unnecessary question, because in principle there must be; but the next level of question is very necessary. Just what nanoscale interactions are involved in any particular system?

One simple way to classify such interactions are those that rely on repeated structures extending over a few to many molecules where the repetition and order contribute to the interaction and those that depend upon the properties of individual molecules where the relationship of one molecule to another plays little or no role. This distinction may mark the boundary between nanoscience and molecular biology for some people. For example, I and my colleagues [2] have suggested that the adhesion of cells to nanotopography may be controlled to a considerable extent by the regularity and type of order as well as scale of nanofeatures the cells encounter. On the other hand [3], specific molecules apparently in a random or chaotic arrangement such as fibronectin may stimulate cell adhesion.

The importance of topography has also been emphasized by others working in this field [4]–[6]. One interpretation of this type of reaction to nanotopography occurs because there are small differences in chemistry between one part of the topography and another, An opposite view is that even when small local differences in chemistry are made by techniques such as nanoprinting, there is also a small difference in topography. Printing or coating a surface minimally leaves a step of the height of a monolayer of the material, and in practice there is often edge accumulation of the added material so that a step of several nanometers may develop.

B. Features of Initial Interactions of Cells With Nanotopography

The first interactions are the formation of adhesions or the failure of adhesion on contact between cell and nanofeatures in situations where both surfaces could form adhesions if they were flat or of different topography [2]. Regularly spaced pits or pillars of appropriate dimensions tend to reduce adhesion; steps and grooves of nanometric height or depth and extended length may raise adhesion [7]. The step effect often leads to cells accumulating and aligning along the steps.

C. Size and Shape Features of Nanotopography

Though much research remains to be done and many possible features have not been examined, the following appears to be true.

1) Responses both quantitatively and qualitatively vary from cell type to cell type for any one nanofeatures, Statements that some particular topography has no effect cannot be made with confidence until a very wide range of different dimensions has been examined. For example, epitenon cells do not react to 30-nm-wide pits, but blood platelets do, while epitenon cells react to 40- and 80-nm-deep pits.
2) Shape of the nanofeatures is important; see [8].
3) *Order and symmetry.* There is evidence that order and symmetry of any nanotopography can affect cell reactions [2], [7], [9].
4) *Area of features.* Little work has been done on this, but there are suggestions that nanotopographic features must be continued linearly over at least 2000 nm to have effects.
5) Further work in this area should be of value both for obtaining a better understanding of the underlying mechanisms and for discovering commercial potentials of nanotopography.

D. How Can We Decide Between Chemical Effects and Physical Effects of Topography?

Until nanoscale mapping of individual molecules becomes possible, we can only make somewhat indirect attempts at answering this question. If the same type of nanotopographic feature made in a variety of different materials has the same biological effects this makes chemical explanations unlikely, since it is improbable that surface chemistry of different materials with different surface chemistry and differing adsorption of extraneous molecules would be the same. In addition, similar results are obtained for the same type of structure in a variety of media. Nanopitted surfaces derivatized with the carbovyl-rich surface of polyacrylic acid have low adhesion for a variety of cell types of similar value to those obtained in the control polycarbonate surfaces. Derivatization with polylysine, which provides a basic amine-rich surface, does raise adhesion, but effects of nanofeatures are still detectable.

E. Cell Functions Believed to Be Affected by External Nanofeature

These include:

- adhesion [2];
- cell morphology [10];
- cytoskeletal arrangement [11];
- surface antigen display [12];
- gene display [12];
- proliferation [13].

F. Fabrication

It is very important to appreciate how any nanodevice is made and to what accuracy when biological uses and investigations are planned. The reason for this is that cells are very sensitive to nanofeatures. For example, [14] it was found that neurons would react to a 5-nm-deep groove. Randomly displayed nanotopography has little or no effect on cells [2] with results comparable with very smooth surfaces. But polished surfaces usually have linear nanotopography up to 100 nm deep, and the exact method of surface treatment may have a large effect. Bright polished metals are usually nanoscratched.

Many different methods of nanofabrication are available, but electron beam lithography followed by dry etching and replication, colloidal lithography, and nanoprinting are all appropriate methods for making materials for this area of nanotechnology (see, e.g., [15] and [16]). Polymer demixing has also been used to produce nanofeatured surfaces [7].

G. Mechanisms of Response—Speculation and Hypothesis

This subject is so young that little has been established with any certainty. However, this also means that there is much opportunity for research.

The results on shape, size, and type of packing of nanotopography on cell interactions suggest that the cells may be able to measure distances or at least relative distances on such surfaces. This would account for the different results with hexagonal and with orthogonal packing [2]. Possible mechanisms for this could be: 1) stretch receptors in the plasmalemma, in particular, the three state receptors described for chloride channels [17]) which might allow x/y axis comparisons; 2) effects on the cell nucleus and chromosome packing [18]; 3) differences and gradients in surface energy (hydrophobicity/hydrophilicity) resulting from the topography; and 4) differences in protein adsorption due to differences and gradients in surface energy.

H. Applications

The literature (see above) shows that many cell processes can be affected by the precise nanotopography of the local environment of the cell in ways specific to the order, shape, and scale of the nanofeatures. All these effects seem to result from the initial adhesion (or not) of the cells. Materials—in particular, polymers—can be used to make surfaces that can be brought into contact with cells to produce desired biological effects. For example, low-adhesion surfaces could be put on stents for vascular use or on biodegradable membranes designed to prevent unwanted tissue adhesion.

REFERENCES

[1] B. Alberts, *Molecular Biology of the Cell*. New York: Garland, 2002.
[2] A. S. G. Curtis, N. Gadegaard, M. J. Dalby, M. O. Riehle, C. D. W. Wilkinson, and G. Aitchison, "Cells react to nanoscale order and symmetry in their surroundings," *IEEE Trans. Nanobiosci.*, vol. 3, pp. 61–65, Mar. 2004.
[3] W. D. Norris, J. G. Steele, G. Johnson, and P. A. Underwood, "Serum enhancement of human endothelial cell attachment to and spreading on collagens I and IV does not require serum fibronectin or vitronectin," *J. Cell Sci.*, vol. 95, pp. 255–262, 1990.
[4] R. G. Flemming, C. J. Murphy, G. A. Abrams, S. L. Goodman, and P. F. Nealey, "Effects of synthetic micro- and nano-structured surfaces on cell behavior," *Biomaterials*, vol. 20, pp. 573–5688, 1999.
[5] A. I. Teixeira, G. A. Abrams, P. J. Bertics, C. J. Murphy, and P. F. Nealey, "Epithelial contact guidance on well-defined micro- and nanostructured substrates," *J. Cell Sci.*, vol. 116, pp. 1881–1892, 2003.
[6] S. Turner, L. Kam, M. Isaacson, H. G. Craighead, W. Shain, and J. Turner, "Cell attachment on silicon nanostructure," *J. Vac. Sci. Technol. B*, vol. 15, pp. 2848–2854, 1997.

[7] A. S. G. Curtis, B. Casey, J. D. Gallagher, D. Pasqui, M. A. W. Wood, and C. D. W. Wilkinson, "Substratum nanotopography and the adhesion of biological cells. Are symmetry or regularity of nanotopography important?," *Biophys. Chem.*, vol. 94, pp. 275–283, 2001.

[8] A.-S. Andersson, F. Blasckhed, A. von Euler, A. Richter-Dahlfors, D. Sutherland, and B. Kasemo, "Nanoscale features influence epithelial cell morphology and cytokine production," *Biomaterials*, vol. 24, pp. 3427–3436, 2000.

[9] J. O. Gallagher, K. F. McGhee, C. D. W. Wilkinson, and M. O. Riehle, "Interaction of animal cells with ordered nanotopography," *IEEE Trans. Nanobiosci.*, vol. 1, pp. 24–28, Mar. 2003.

[10] M. J. Dalby, M. O. Riehle, H. Johnstone, S. Affrossman, and A. S. G. Curtis, "*In vitro* reaction of endothelial cells to polymer demixed nanotopography," *Biomaterials*, vol. 23, pp. 2945–2954, 2002.

[11] M. J. Dalby, S. Childs, M. O. Riehle, and H. J. H. Johnstone, "Fibroblast reaction to island topography: Changes in cytoskeleton and morphology with time," *Biomaterials*, vol. 24, pp. 927–935, 2002.

[12] M. J. Dalby, S. J. Yarwood, H. H. Johnstone, S. Affrossman, and M. Riehle, "Fibroblast signaling events in response to nanotopography: A gene array study," *IEEE Trans Nanobiosci.*, vol. 1, pp. 12–17, Mar. 2002.

[13] M. J. Dalby, M. O. Riehle, H. J. H. Johnstone, S. Affrossman, and A. S. G. Curtis, "Polymer-demixed nanotopography: Control of fibroblast spreading and proliferation," *Tissue Eng.*, vol. 8, pp. 1099–1107, 2002.

[14] A. M. Rajnicek, S. Britland, and C. D. McCaig, "Contact guidance of CNS neurites on grooved quartz: Influence of groove dimensions, neuronal age and cell type," *J. Cell Sci.*, vol. 110, pp. 2905–2913, 1997.

[15] C. D. W. Wilkinson and A. S. G. Curtis, "Nanofabrication and its applications in medicine and biology," *Dev. Nanotechnol.*, vol. 3, pp. 19–31, 1996.

[16] P. Hanarp, D. Sutherland, J. Gold, and B. Kasemo, "Nanostructured model biomaterial surfaces prepared by colloidal lithography," *Nanostruct. Mater.*, vol. 12, pp. 429–432, 1999.

[17] U. Ludewig, M. Pusch, and T. J. Jentsch, "Two physically distinct pores in the dimeric CIC-O chloride channel," *Nature*, vol. 383, pp. 340–343, 1996.

[18] A. R. Leitch, T. Schwarzacher, W. Mosgoller, M. D. Bennett, and J. S. Heslop-Harrison, "Parental genomes are separated throughout the cell-cycle in a plant hybrid," *Chromosoma*, vol. 101, pp. 206–213, 1991.

[19] M. J. Dalby, M. O. Riehle, D. S. Sutherland, H. Agheli, and A. S. G. Curtis, "Use of nanotopography to study mechanotransduction in fibroblasts—Methods and perspectives," *Eur. J. Cell Biol.*, vol. 83, pp. 159–169, 2004.

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