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On the Social Behaviour of Cells

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Abstract

Polystyrene Petri dishes are in use in hundreds of thousands of laboratories world wide. Cell culture experiments performed in them provide fundamental information in a wide range of applications, including but not limited to testing novel biomaterials and pharmaceuticals, and stem cell research. These experiments cost billions of dollars per year. In this study we report on a potential deficiency of polystyrene Petri dishes, possibly caused by an increase in interfacial pH under relevant culture conditions and affecting cell performance. We conclude that cell performance on Petri dishes could be improved by improving the Petri dishes. As a spin-off of our study we postulate the concept that cancer cells and stem cells are social. It is impossible to validate this concept on the basis of the model established in this paper. However, the coherence of our insights may encourage further study and lead to the development of a qualitative improvement of cell culture devices, including Petri dishes and culture flasks, to the identification of potential strategies for chemotherapy and chemoprevention that could suppress progression of metastasis, and to the establishment of improved settings for tissue engineering and stem cell research. An immediate recommendation of our study is to use chemically and biologically inert substrates for important cell culture experiments, for example, nanocrystalline diamond.

Keywords: biomimetic triangle, Petri dish, stem cells, cancer, social behaviour, diamond

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1 Introduction

We wish to draw the attention to a potential deficiency in the biocompatibility of polystyrene Petri dishes: a possible increase in the interfacial pH, probably accompanied by a softening of the material surface under relevant culture conditions. This potential deficiency is of considerable biological interest. In a biomimetic approach, which attempts to mimic the native environment of a cell, the biocompatibility of a material is expressed in terms of a set of eight quantifiable determinants, including surface charge and hardness[1]. Changes in interfacial pH are manifestations of surface charge. Because surface hardness is usually a long-term determinant, its temporal and spatial constancy is normally presumed and precondition for a clear quantification of the parameter. Exceptions are biodegradable materials. Spatial gradients in surface hardness can affect the behaviour of cells, which register even minimal spatial gradients. Reportedly, 3T3-fibroblasts tended to migrate towards increasing substrate hardness[2]. Cellular locomotion induced by a spatial variation in surface hardness is called durotaxis, and reveals the existence of hardness sensing receptors. The susceptibility of cells to mechanical variations in their microenvironment (extracellular matrix – ECM) is not surprising: For instance, variations in the compliance of the ECM are interpreted by surface receptors called integrins, which connect to the cytoskeleton and translate the external mechanical information into modification of cell contractility[3]. Derailment of the mechanical properties of the ECM has consequences not only for the shape, but also for the proliferative activity of normal cells, thereby indicating that alterations of stromal compliance could prelude oncogenic conversion[4]. The ECM-cell interplay determines changes from normal to cancerous tissues. According to a recent atomic force microscopy study, cancer cells isolated from patients were about 70% less

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stiff than normal cells[5]. Mechanical microenvironment properties play further a key role in directing stem cell lineage specification[6]. Notably, 3T3-fibroblast durotaxis was observed only at very low cell densities[2]. It is assumed that durotaxis contributes to directing motile cells into stiff, fibrotic regions of tissue[7].

2 Polystyrene Petri dishes – temporal biocompatibility

Another biologically relevant gradient emerges from a temporal variation in interfacial pH, as could be caused by a swelling of the hydrophilic polystyrene surface due to uptake of water and related decrease in surface hardness. Contrary to expectation, even an increase in the surface hardness is possible, for instance, in the case of porous materials and their reinforcement by water molecules filling the pores. Since cells are sensing pH variations it is plausible to expect that they will also respond to a temporal variation in interfacial pH. Complementary, cells might register mechanical signals, possibly caused by an associated softening of the surface and/or coupled changes in surface chemistry. We believe that the dimension of the variations is at the nanoscale. Thus, experimental access to the interfacial pH, or complementary variations, is in no way trivial. Using an ultra-micro indentation system (Umis-2000, Csiro, AU), we probed the hardness of polystyrene Petri dishes (Falcon, BD-Biosciences, USA), dry and in previous contact with water. Prior to the measurement we filled dishes with ultra pure water for one and 14 days, respectively and removed the water before starting the measurements. Probably because of the extended measurement times this approach did not provide significant differences in the depth of indentation, which would indicate a softening of the material. Presumably, one would have to conduct the measurements under water, a technical condition which is hardly realizable with the standard equipment. Polystyrene tissue culture dishes are in use for almost 50 years[8]. To the best of our knowledge the constancy of their biocompatibility has never been challenged. How can we assess cell reactions that could be correlated with a temporal change in interfacial pH? The usual observation is that cells are seeded into a Petri dish sediment. In the initial phase of sedimentation gravity prevents them from establishing intimate contact with the vertical wall of the dish. However, with termination of a confluent monolayer cells located at the periphery of the dish have the choice to form a second monolayer on top of the first one, or to start climbing the vertical wall. At first sight, climbing appears less probable: adherence and migration against gravity costs the cells energy. In this situation even minimal stress – as represented by an increase in interfacial pH – should be sufficient to prevent cells from climbing. Alternatively, what could spur cells to deviate from the route of least energy and climb? According to a recent report human hematopoietic stem/progenitor cells actively migrated toward stroma cells against a gravity gradient of 5 degree inclination of the culture plate[9]. The experiment shows that suitable signals could stimulate cells to overcome spatial constraints set by gravity. On a microscopic scale cells can migrate vertically to form compact tissues[10]: layers form sequentially in organized vertical growth after the first layer becomes confluent. In this special case, vertical growth is explained on the basis of competitive bindings: the cell-cell affinity is relatively stronger than the cell-substrate affinity.

3 Nanocrystalline diamond – permanent biocompatibility

We wish to put forward a radically different cause for the growth of cells against gravity: social behaviour in which individual cells take a route that secures the survival of a community of cells. Cooperative behaviour has already been observed in the form of a coordinated beating of embryonic stem cells in aggregates of differentiating tissues known as Embryoid Bodies (EBs). It is assumed that heart muscle cells (cardiomyocytes) beat in a rhythmic pattern to pump nutrients into the increasingly larger EB[11]. In EBs formed from human Embryonic Stem Cells (hESCs) the number of beating areas significantly increased under serum-free, i.e., less favourable nutritive conditions[12]. Earlier, the phenomenon was partially explained as manifestation of the expression of cardiac genes. Analogously hESCs, when cultured in low-oxygen conditions, the resulting EBs formed a high number of hematopoietic progenitor cells, the building blocks of the vascular system[13]. The authors concluded that a lack of oxygen causes hESCs to form blood vessels in order to deliver more oxygenated blood to affected areas of the body. EB formation is encouraged by competitive binding situations in which cell-cell attraction is superior to that between cells and