Substrate topography and shape of motile cells

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A vast amount of data on cell shape and locomotion on plane glass and plastic substrates has emerged from culture studies, but the mode of control of configuration, migration and directionality of cells is not yet fully understood[1]. Nor do we know to what extent findings on cell behaviour in simplified culture conditions are relevant to living tissues, wherein cells encounter an extracellular matrix (ECM) of complex architecture and chemistry[2]. This review will consider some responses shown in culture by motile cells with fibroblastic morphology when transferred from a simple 2-dimensional substrate to 3-dimensional substrates which resemble their natural environment. We shall also present examples of the shape of migratory cells in relation to their supports in vivo.

Fibroblasts moving on a flat isotropic substrate assume a characteristic, more or less, flattened shape with a broad leading lamella at the anterior part of the cell and a narrow trailing tail. The anteriormost parts of the leading lamella show prominent protrusive activity giving rise to lamellipodia and filopodia which carry out intermittent movements[3]. Broad lamellipodia may bend upwards and move backwards creating conspicuous ruffles on the upper surface of the cell[4]. Moreover the leading lamella establishes focal contacts, or adhesions, to the culture support. As the cell moves the adhesions remain static in relation to the substrate, apparently forming a link between the substrate and the locomotor mechanism within the cell. Receding adhesions are continuously superceded by new ones at the leading edge[3,5].

However, the shape of fibroblasts may be radically altered if a flat substrate is replaced by anisotropic supports such as curved, grooved, ruled or fibrous surfaces[6-10], on which the cells usually follow the surface irregularities. Many experiments have sought to explain this substrate effect on cells - known as contact guidance[11]. For instance, it is known that fibroblasts on aligned collagen gel fibrils[9], comprising a shallow 3-dimensional substrate, assume a marked spindle shape and align themselves along the axis of fibril orientation[9,10,12]. On the other hand, when the fibrils - with maintained alignment - were flattened down on the support they evoked no orientational reponse in chick heart fibroblasts, which were well spread and exhibited the broad leading edges and ruffles seen on isotropic substrates[12,13]. Identical results were obtained in the two cases even when the surfaces of the aligned fibrils were modified by coating with a thin layer of palladium (unpublished). These experiments, which reveal a correlation between increased substrate relief and better cell alignment, would seem to support the hypothesis[7] that cells respond directly to the shape of the substrate, possibly through anisotropic distortion of cytoplasmic microfilament bundles[13], rather than to patterns of varying substrate adhesiveness.

There is evidence[14] that cells lose their migrational polarity on isotropic supports, or on narrow strips of adhesive substrates when treated with agents such as colchicine or colcemid which disrupt microtubules. Our recent results indicate that also the orientational response of cells to aligned collagen fibrils is lost when colchicine is added to the culture medium (unpublished). Despite the distinctly oriented substrate, fibroblasts spread out over the aligned fibril bundles and showed no clear polarity. Observations suggest that colchicine does not modify the adhesive property or protrusive activity of cells[14,15]. Thus, the hypothesis that contact guidance depends chiefly on properties of the cytoplasmic microfilament system[7], or on restrictions in stability of focal adhesions[8] does not seem fully to explain our results. Indeed, it would appear that the cell response to substrate shape is also subordinate to microtubules. Hence, it may well be that information on the spatial availability of adhesive substrate - possibly received by cell processes contacting their surroundings[16] - elicits an overall response involving both the microtubules and the microfilament bundles with associated focal adhesions, a response governing changes in shape of motile cells in relation to substrate topography.

The existence of such a multifactor mechanism finds some support in the literature[15,17] and may also cause the profound changes in shape shown by cells which encounter 3-dimensional culture substrates such as plasma clots[6,11] or artificial collagen gels[9]. In these fibrous matrices the cells assume markedly bipolar spindle shapes with long filopodia at each end but no apparent ruffles[9,18].

The relevance of these findings for the in vivo situation has been evaluated in a few transparent tissues which allow direct observation by light microscopy of cells in situ[1]. For studies of cell migration in opaque tissues, scanning electron microscopy (SEM) has been successfully applied to examine embryonic cells fixed in situ while undergoing directional locomotion[19-27]. The results of both approaches emphasize the importance of ECM, i.e., collagen fibrils and basal laminae, as natural substrates for embryonic cell migration; they also imply that the locomotor organelles of cells moving on ECM structures involve elongated, sometimes ramified cell processes which confer a spindle-shaped or stellate morphology.

Bard and Hay[18] succeeded in studying the migration of corneal fibroblasts both in their natural ECM - the collagen fibril network of the corneal stroma - and in an artificial collagen lattice. On both these 3-dimensional substrates the cells developed an elongate shape and extended pseudopodia and filipodia at their leading edges, creating adhesions to the ECM fibrils. No ruffling activity[4] was detected. In contrast, on a plane glass support the same type of cells were flat with broad lamellipodia which ruffled. This[18], and other studies[19-21], suggest that ruffling may be absent or play a minor role during cellular locomotion in vivo, prompting the hypothesis that this is related to the topography of the substrate used. The gently undulating under-surface of the chick embryo epiblast, which is covered by a basal lamina and sparsely distributed ECM fibrils, provides the best known example of a fairly plane substrate in vivo[21-23]. Mesoblast cells migrating in contact with this substrate extend filopodia contacting the ECM fibrils, but the cells also show occasional lamellar structures interpreted as ruffles[21-23]. As might be expected, cultured mesoblast cells showed ruffles on plane glass[23]. However, to a minor extent the cells also had ruffles on an artificial 3-dimensional Sterispon Sponge substrate, cell morphology resembling the in vivo appearance. It was suggested that morphological features in vitro, such as ruffling, might be exaggerations of the normal locomotor activity manifested in vivo because of the differences between a 2-dimensional and 3-dimensional substrate[23].

A few examples exist of motile cells which appear to change their shape in response to substrate topography in vivo. In pioneering studies reporting direct observations of living cells, Clark and Clark[28] describe how stellate fibroblasts, migrating in a fibrous network of the larval amphibian dorsal fin, flattened out and withdrew cell processes on contact with the flat substrate of blood capillary walls - a result explicable in terms of mechanics. However, the authors make the significant point that in case of occasional withdrawal of blood vessels the flattened cells did not resume their former stellate shape, and suggest that the cells differentiated into a new type on contact with the vessel. This proposal is of fundamental theoretical importance since it implies that morphological changes of motile cells in the embryo may be genetically programmed to occur at a certain stage or as a result of specific environmental influences. Apparent mechanical influences by the substrate on cell shape may thus be of secondary importance in some cases.

Recent SEM studies, nevertheless, indicate a good correlation between the regional architecture of embryonic ECM fibril networks and the shape and orientation of migrating sclerotome[24] and neural crest cells[25,26], which use the fibrils as substrates. Furthermore, neural crest cells on the neural tube of the axolotl embryo prove to have a regionally specific shape and orientation in relation to the radius of curvature of the tube[25]; it is conceivable that this is due to contact guidance by substrate curvature[7]. Moreover, a striking indication of contact guidance of cells in the embryo was described by Bard and Higginson[29], who used direct light microscopic observation to demonstrate that fibroblasts aligned themselves along orthogonally oriented collagen fibril bundles in the stroma of the embryonic chick cornea. Although these recent findings seem to imply contact steering by fibrous substrates of motile cells in vivo - which corroborates in vitro studies[9-13] - they relate to local situations and the observations hitherto do not suggest that contact guidance is the main principle for directing migrating cells in the embryo.

In summary, many cell types show a range of similar responses to substrates of different topography. Although caution must indeed be observed when extrapolating data regarding fibroblast locomotion on plane substrates in culture to the in vivo situation, few - if any - erroneous conclusions concerning the behaviour of motile cells seem to have arisen from in vitro studies. Experimental studies are now needed to modify the spatial organization of embryonic ECM fibril systems, and observations of form transitions shown by transplanted labelled cells, before we can pass judgement as to whether substrate topography or genetic programming in the specific case are determinants of cell shape in vivo. In future studies in vitro it would be useful to investigate the effects of more complex substrates, which simulate the topography and chemistry[2] of natural ones. Such experiments are encouraged by recent results indicating unexpected functional behaviour of cells cultured in artificial collagen lattices[30,31].

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Malignant and nonmalignant cells: Structural similarities and behavioural differences

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Invasiveness is the ability of cells to leave the boundaries of the tissue to which they belong, and to occupy neighbouring tissues. Invasiveness of tumours contributes largely to their malignancy both by local spread and by initiation of the formation of metastases. Because invasiveness constitutes the main difference between benign and malignant tissues, understanding of the mechanisms by which cells leave the tissue of origin and invade into the neighbouring tissues, may contribute to our knowledge of the biology of malignant cells. The role of in vitro culture in the study of some aspects of the invasiveness of malignant cells is reviewed here.

2 points are worth mentioning on beforehand. 1. There are theoretical (Nowell[1]) and experimental arguments (Liotta et al.[2], Fidler[3]) to accept that not all cells of a particular malignant tumour have or will acquire the ability to invade, so that in this respect the cell population constituting a malignant tumour is heterogeneous. This concept implies that by sampling populations of malignant cells one might overlook the activity of the invasive cells, which may represent a relatively small fraction. It may provide one possible explanation why quantitative evaluation of invasiveness is difficult (Easty and Easty[4], Hart and Fidler[5]). 2. As long as the mechanisms of invasion are unknown, invasion in vitro has to be judged on the basis of histological criteria bearing on the interaction between the tumour cells and the host tissue as it is done by clinical pathologists. Generally applicable cytological or ultrastructural criteria for distinction between invasive and non-invasive cells are not available. This implies that invasive cells have to be identified either directly whilst invading (cinemicrophotography) or indirectly through their position within the complex of tumour and neighbouring tissues. The drawback of the indirect method is that it infers kinetic features from static pictures.

Rationales for the in vitro study of invasion are isolation of the various factors that influence the invasiveness of malignant cells and direct observation of the cellular activities involved in invasion. Confrontation of biopsy specimens from animal and human tumours with a variety of embryonic and adult tissue fragments in three-dimensional culture, also called organotypical culture (Wolff et al.[6], Easty and Easty[7], Lumsden[8]), mimics the invasion of malignant cells in vivo. The argument is that the histological aspect of the frontier between tumour and host tissue is similar in three-dimensional culture and in vivo. Provided this argument is accepted, 2 conclusions can be drawn from these experiments. 1. Malignant cells conserve their invasive capacity when they are isolated from their natural host and brought into culture. 2. Invasion of malignant cells is not limited to their natural host but also occurs into tissues from different organs and from different species. The latter makes it unlikely that tumour cells become invasive because of alterations in the neighbouring tissues.

Using confrontation in three-dimensional culture, several authors (Easty and Easty[4], Schleich et al.[9], de Ridder et al.[10], Kuettner et al.[11]) have shown the invasiveness of cells from permanent lines established from animal and human tumours. These observations indicate that malignant cells do not loose their invasiveness during long-term culture. Furthermore, experiments by Barski and Wolff[12] and by Latner et al.[13] show that cultured cells become invasive when they undergo malignant alteration in vitro. Systematic analysis by independent investigators of in vitro invasiveness and ability to form invasive tumours in syngeneic animals has been done with 3 families of