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Cell biology at interfaces

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A brief review is presented of the significant developments in the understanding of the processes involved in cell adhesion both to other cells and to substrates. The relationship between general cellular behaviour and cell adhesion is a result of the importance of the cytoplasmic cytoskeleton to most cellular processes. Interaction between a substrate and the cell is mediated through intramembranous proteins, such as the integrins. The intramembranous proteins, in turn, influence the assembly of the microfilamentous structures in the cytoplasm. Changes in the state of the microfilaments are accompanied by modifications in the behaviour of both microtubules and intermediate filaments. The expression of different types of cytoskeletal configuration result from differing types of cell–cell or cell–substratum encounters. This leads to significant changes in resultant cellular behaviour. It is argued that an understanding of changes that result from cell–biomaterial interactions, at the ultrastructural level, is necessary in order to assess the biocompatability of implant materials.

1. Introduction

Recent advances in the understanding of cell biology have led not only to a realization that cells are highly sensitive to their immediate environment but also to a greater understanding of the mechanisms involved in that sensitivity. Most normally growing cells, both in artificial culture conditions and *in vivo*, attach to surfaces of some kind. These surfaces are usually neighbouring cells, of a similar or different type, accumulations of natural extracellular materials such as collagen or non-cellular substrates, natural or artificial upon which they grow. The behaviour of a cell may well be modified according to the type of surface it encounters.

The adhesion of cells to a biomaterial surface will inevitably be a major factor in determining whether such material is accepted by the human body. The nature of the cell–implant adhesions determines whether cells remain attached to a biomaterial implant. Poor adhesion can result in detachment of the tissue cells from the implant. Such detachment can then allow infection to take place at the tissue–implant interface.

2. Cell adhesion

The phenomenon of cell adhesion has been a subject which has been of prime interest to the cell biologist for many years, as it is fundamental to the evolution of multicellular organisms. Initially, the main interest was in experiments involving the reaggregation of dissociated cells [1], but more recently much more attention has been given to the adhesion of cells to substrates. The path of development of a cell depends not only upon the chemical nature of the medium in which it is grown but also its physical reaction to any interfaces it meets. Such factors, no doubt, are of great relevance to the processes of tissue differentiation in the developing embryo [2]. Cells which are predisposed to spread upon a suitable surface will not do so on some other surfaces [3]. If a cell cannot spread then it cannot move and neither will it, in some cases, be able to divide.

In normal body cells, or cultured cells derived from non-neoplastic sources, contact with another cell can also cause an arrest of movement, by the well-established phenomenon known as contact inhibition of movement [4]. The effect that making contact with other cells, as well as with other surfaces, has upon cell growth and division is a much more complex subject. The outcome of any individual interaction will depend not only on the surface encountered but also upon the condition of the cell itself. This was the subject of intense interest in the 1960s [5, 6], when very little was known as to what mechanism might be responsible for such sensitivity to surfaces in some cell types. It became obvious, even then, that a better understanding of such mechanisms would be necessary in order to understand the phenomenon of cell adhesion.

Tissue cells are usually found adhering either directly to other tissue cells or to accumulations of extracellular proteins and carbohydrates. The nature of their immediate environment will be an important factor in determining their behaviour. Clearly, the introduction of artificial interfaces into the body present the cells of the tissue contacting such implants with messages that are likely to be radically different from those they would normally encounter. This is because the materials which have to be used are both chemically and physically alien to the tissue cells. There may be chemical removal of the implant material, to be taken up by the cells. This may or may not be toxic to the cells, as the case may be. Studying the effect of metallic implant material upon the develop-

0957–4530

ment of embryonic limbs indicates that there is at least some effect upon skeletal development. Such an effect is clearly seen when steel implant material is tested against cultured cells. What is not often realized is that the physical surface topography of the implant material, and not just its chemical properties, may be less than suitable for the formation of attachments by the cells. In order to understand this matter it is necessary to examine closer the ultrastructural anatomy of what is now understood of the cell adhesion mechanism and its links to the inside of the cell.

3. The plasma membrane

From the earliest picture we had of cells it became clear that the cell membrane formed the interface between cellular contents and its environment [7]. Much research has been done to improve our understanding of the structure and function of the plasma or cell membrane. The Singer and Nicholson fluid-mosaic model of membrane structure [8] is now well established, with the fluid biomolecular lipid leaflet in which integral membrane proteins are held by their hydrophobic regions, and the nature of these integral proteins is becoming better understood. These proteins often have attached to them carbohydrate molecules, which form an outer 'covering' to the cell membrane.

There are three main types of integral membrane proteins involved in cell adhesion: the immunoglobulin superfamily, the cadherin family (involving calcium-dependent cell-to-cell adhesion), and the integrin superfamily (involved with cell-to-substratum adhesion) [9–12]. Linking to the integrin, and other types of integral membrane proteins, both structurally and functionally, is the dynamic cytoskeletal protein system [13–16]. This gives a more precise definition of what was originally termed the cell membrane associated cortex [17], and a clear relationship between cell adhesion, the cytoskeleton and cellular morphology is well established [18].

4. The cytoskeleton

The cytoskeleton is composed of the three fundamental types of filamentous protein categories, and their associated proteins, generally referred to under the classification of microfilaments, intermediate filaments and microtubules.

The microfilaments represent the helically polymerized form of the various forms of actin and their associated proteins, and are usually found in a state of metabolically maintained dynamically stabile equilibrium which is highly sensitive to a number of cellular control signals, such as calcium concentration [19]. Under the electron microscope the fixed and stained form of these filaments have a diameter of between 4 and 7 nm. These actin-type proteins are probably the most common type of cytoplasmic protein to be found in most living cells. As well as having many other roles, such as cell motility [20], the structural state of these proteins has a direct bearing upon cell adhesion, growth and differentiation, as they are involved with cell-surface to nuclear communication [21], and have been shown to control both cell volume [22] and DNA synthesis [23].

Intermediate filaments are formed of one of five different, but related, structural protein types, which polymerize into helical filaments. They show up in electron micrographs as filaments of about 10 to 12 nm in diameter. These are, in some instances, tissue origin type specific, for example cells of epithelial origin usually show cytokeratin while those of mesodermal contain vimentin. When mesenchymal cells are able to adhere, and spread, on a substrate then there is a pronounced increase in vimentin expression as compared with corresponding cells in a spherical non-adherent condition. Although they are the more inherently stable of the three filament types, there is some evidence that the intermediate filaments are probably controlled by the actin microfilament system [24]. Co-alignment of vimentin and microtubules has been noted [25], as well as the association of the state of cytokeratin filaments and cellular behaviour [26]. Indeed, there is also some recent evidence that the type of intermediate filament expressed may also be controlled by the physical forces acting upon cells, rather than their embryonic tissue of origin [27].

Microtubules are polymers of the protein tubulin, and can also be found in a state of dynamic equilibrium [28, 29]. The tubulin dimers are arranged into a helical tubule which has a diameter of about 25 nm. Their precise role under any particular circumstances is probably determined by the nature of associated proteins as well as the component dimers of tubulin themselves. They form the basic building blocks of structures such as cilia and flagellae. Microtubules are involved in many intracellular transport mechanisms as well as cell-shape determination, often in association with microfilaments [30]. They are known to associate directly with membrane proteins [31] and have specific motor-force generating proteins which associate with them [32, 33]. Their properties appear to be especially suited to the development of nervous tissue. They appear to be the main structural elements which are involved with major cellular events such as the formation of the mitotic apparatus.

The genetic coding for the active regions of all these cytoskeletal proteins is very tightly defined and there is little room for variation, in respect of their active domains, regardless of the source. Different types will tend to be expressed at particular times or in specific tissues, and modern electron microscopic techniques have revealed the three-dimensional arrangement of the structures they form [34, 35].

Controlling the actions of the cytoskeletal proteins are their associated proteins and agents such as nucleotide phosphates and divalent cations. Protein phosphorylation is a common feature in such systems, as is the sensitivity to calcium or magnesium ions. Almost all processes in cells are either controlled or influenced through the action or state of these ultrastructurally dynamic agents. Both wholesale cell movement and the movement of materials within cells are achieved by the functioning of these proteins. Some specialized, highly differentiated, tissues such as muscle and neural

tissue make use of the properties of these proteins on a large scale. The functional, contractile, part of the muscle is based on the interaction of actin and mysoin. Many of the key properties of nerve cells are determined by the presence of large numbers of microtubules. Processes such as cell division are not only brought about by the combined action of these proteins but also, in all probability, controlled by them as well. Cyclical variation in their configuration is likely to parallel the progression of cells through their life cycle, and any stable condition they may reach.

The cytoskeletal system is not only extremely sensitive to small changes in its controlling factors but it also appears to be the mediator of signals from the outside of the cell which will influence cell adhesion, by its association with integral membrane proteins, such as integrins [36]. Many cellular processes, and certainly those involved with and leading up to cell division and cycle control, appear to be governed by the status of the cytoskeletal system. Cell-cycle specific changes in cell surface morphology and cell shape are well documented [37, 38], and are likely to be cytoskeleton related.

The development of an organized actin-based structural network is probably a consequence of the interaction between cytoplasmic cortical flow and the cell–substratum contacts [39]. The state of organization of the actin filament network controls other facets of cellular behaviour. If inhibitors of the process which maintains microfilament integrity are administered to a culture of growing cells then individual cells are arrested at the stage when transport of thymidine is required for DNA synthesis, indicating a connection between such a transport mechanism and the state of actin microfilament network [40]. Removal of the inhibition allows the cells to resume their normal synthetic activity. There is also good evidence that even minimal changes in the nature of cellular contacts can have a dramatic effect upon the ability of cells to traverse the cell cycle in a normal fashion [41, 38].

5. Conclusions

All of the evidence now available to the cell biologist indicates that the precise chemical and physical nature of any substratum to which a cell may be required to adhere can have far-reaching effects upon the state of the complex cytoskeletal system within each individual cell. The behaviour of integral membrane proteins, probably of the integrin type, in response to the immediate external environment of the cell will have a direct bearing upon the assembly of microfilaments. The state of the microfilaments will, in turn, determine the condition of the other cytoskeletal components. The cytoskeleton itself will control the resulting shape and behaviour of the cell, including nuclear transcription and differentiation [42]. It will also control the adhesive ability of the cell [43].

Determining the precise state of the cytoskeletal proteins in a cell at any one time is not an easy task. Their inherently dynamic nature presents some difficulties for ultrastructural investigations. However, by using a combination of advanced microscopic ad immunocytochemical techniques it is possible to gather meaningful information as to the state of the cytoskeleton [44]. A real understanding of the cellular consequences of any particular type of cell–substratum interaction will only become possible when all the changes which occur to the cytoskeleton as a result of such an interaction are known.

In order to optimize cell–substrate adhesion on biomaterial implants it will probably be necessary to investigate thoroughly the cellular cytoskeletal response to any particular material to be used, using combinations of the latest ultrastructural investigation techniques.

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