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ITBM-RBM 26 (2005) 192–199
ITBM RBM
http://france.elsevier.com/direct/RBMRET/

Article original

In vitro methodologies to evaluate biocompatibility: status quo and perspective

Méthodes in vitro d'évaluation de la biocompatibilité : état de l'art et perspective

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Received 1 April 2005; accepted 15 April 2005

Available online 08 June 2005

Abstract

The increasing use of biomaterials in clinical medicine to augment or replace failing organ function has heightened the need to apply relevant test systems to study the safety and efficacy of new medical devices. This becomes all the more important as the field of ″tissue engineering″ develops, in which the aim is to reconstruct tissue and organ function, for example, by using the patient's own cells seeded on to a three-dimensional (3-D) scaffold structure. In the biomaterial research field, there has been a necessary expansion of the concept of biocompatibility to address not only the biosafety issue, that is, the exclusion of cytotoxic and other deleterious effects of biomaterials, but also the biofunctionality component, which concerns the fulfilment of the intended function of the applied biomaterial. Careful scrutiny of this concept leads to the conclusion that relevant test systems for biofunctionality must centre on human cells, studied under conditions relevant to the situation in the living organism for which the medical device has been constructed. Thus, progress in biocompatibility and tissue engineering would today be inconceivable without the aid of in vitro techniques. In designing such biofunctionality assays, there are certain fundamental principles which must be adhered to. A constant difficulty is the availability of sterile human tissue for such test systems. Also of paramount importance is proving the maintenance of the cell phenotype in vitro. Loss of essential characteristic functions of the cultivated cells makes extrapolatory interpretations meaningless for the clinical situation. This paper gives an overview of the basic design principles for suitable assays, and various examples covering a spectrum of applications. Relevant functional parameters will be emphasised, as well as the use of modern methods of cell and molecular biology, with measurement of these parameters at both the gene product and transcription levels. These parameters include the expression of cytokines, growth factors and cell adhesion molecules. In addition, assays can be constructed to study inflammation and the wound healing response, which includes the angiogenic reaction. Tissue remodelling around biomaterials can be studied in vitro by using cells such as fibroblasts, endothelial cells and various inflammatory cells, important parameters reflecting control of this remodelling being the matrix metalloproteinases and their inhibitors. The need for more co-culture and 3-D models is stressed and data from the authors' own laboratory are presented to illustrate these principles. Finally, the importance of signal transduction within those cells in contact with, or in the vicinity of, biomaterials is emphasised, as this knowledge offers the scientific basis for rational therapeutic intervention to suppress negative effects and enhance positive biological responses (use of drug delivery systems). In understanding these processes modern technologies using nucleic acid micro-arrays coupled with methods of bioinformatics will hopefully identify key genes which can be targeted. Well-designed in vitro assays have a central role to play in this endeavour.

Résumé

L'utilisation croissante de biomatériaux en pratique clinique pour améliorer ou remplacer la fonction d'organes défaillants a fait apparaître la nécessité d'appliquer des tests pertinents pour étudier la sûreté et l'efficacité de nouveaux dispositifs médicaux. Cela est d'autant plus

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doi:10.1016/j.rbmret.2005.04.008

important que le domaine de l'ingénierie tissulaire est en pleine expansion, dans le but de reconstruire la fonction d'un organe ou d'un tissu, en utilisant par exemple, des cellules autologues ensemencées sur des structures matricielles 3-D. Dans le champ des biomatériaux, le concept de biocompatibilité recouvre non seulement l'aspect biosécurité (c'est-à-dire l'exclusion d'effets cytotoxiques ou délétères des biomatériaux) mais aussi la biofonctionnalité qui évalue la fonction attendue dudit biomatériau. Pour ce faire, les systèmes les plus pertinents sont centrés sur l'utilisation de cellules humaines, étudiées dans des conditions proches, autant que faire se peut, de celles qui prévalent in vivo et aujourd'hui les progrès réalisés sont tels qu'il serait inconcevable de s'appuyer sur les techniques in vitro. Cependant, il existe quelques principes de base à prendre en compte : disponibilité de tissu stérile humain, assurance du maintien du phénotype cellulaire in vitro sans laquelle l'interprétation extrapolée à une situation clinique n'aurait pas de sens. Cet article fournit une revue générale de ces principes et quelques exemples dans diverses applications fondés sur l'utilisation d'outils modernes de biologie cellulaire et moléculaire, incluant l'expression de cytokines, facteurs de croissance et molécules d'adhésion, ainsi que la mise au point de tests d'évaluation de l'inflammation, de la cicatrisation. Le remodelage tissulaire périimplantaire peut être étudié in vitro à l'aide de divers types cellulaires : fibroblastes, cellules endothéliales, cellules inflammatoires ; et autres paramètres reflétant le contrôle du remodelage sous l'influence des métalloprotéinases et de leurs inhibiteurs. L'importance de la signalisation dans les cellules au contact ou à proximité de biomatériaux est abordée, puisque sa modulation offre une base scientifique pour des applications thérapeutiques rationnelles afin de supprimer des effets délétères et induire des réponses biologiques positives. Pour la compréhension de ces procédés, les technologies modernes utilisant les puces à ADN couplées à la bio-informatique serviront à identifier les gènes clés.

Keywords: Cell adhesion; Cell spreading; Cell migration; Cell proliferation; Cell function

Mots clés : Adhésion cellulaire ; Étalement cellulaire ; Migration cellulaire ; Prolifération cellulaire ; Fonction cellulaire

1. The concept of biocompatibility

The biocompatibility concept has been much discussed in the past decades, but there is now general consensus that this topic has two principal components. First, there is the element of absence of a cytotoxic effect and second, there is the aspect of biofunctionality. Probably the best definition of biocompatibility is that agreed on at the ESB Consensus Conference I, namely ″the ability of a material to perform with an appropriate host response in a specific application.″ (Williams Dictionary). The corollary of this is that the type of testing method will depend on the intended function of the biomaterial being used. It then becomes obvious that the testing strategy must take into account the biological situation in which the biomaterial will find itself.

2. Established testing systems

Experimental systems for biocompatibility exist in the form of animal experimentation and in vitro techniques. The various standards organisations have established a series of guidelines, which by and large are aimed at the biosafety issues of testing. The publication ″Biological evaluation of medical devices—Parts 1–17″ from the International Organisation for Standardisation (ISO) gives a series of guidelines on the necessary testing of medical devices Thus, for example, cytotoxicity testing has been well addressed by ISO 10993-5, which presents guidelines for the choice of suitable tests and defines important principles underlying these tests. This has been detailed elsewhere [27]. In the following only in vitro methods will be discussed. The inclusion of additional animal experimental possibilities would exceed the scope of this paper.

Tissue culture offers an excellent method to screen potential biomaterials, as the methodology is generally more economical than animal experimentation. However, it must never be forgotten that tissue culture represents a reduction in the complexity of the entire organism generally to a single cell type grown as a single sheet of cells (monolayer). Thus, the buffering capacity of complex cellular and humoural systems in the intact organism are missing, so that a biomaterial may not perform well in the in vitro test, but be biocompatible in vivo. Nevertheless, this is a risk which is inherent in the system. A further problem is the fact that established (or permanent) cell lines, although the most convenient model of mammalian culture, may be less sensitive to toxic effects of biomaterials than cells which have been directly isolated from the tissues (primary culture) and further subcultivated for a few passages.

In addition to the choice between permanent cell lines or primary cells there is the choice of species. As biomaterials are intended for clinical use, the authors' laboratory uses solely cells of human origin. Various comparative studies demonstrate that human cells can react in a very different manner from cells of other mammals.

3. General principles for in vitro assays

The central pathogenetic element in biomaterial applications is that biomaterials can modulate the activation status of cells. This can occur directly, through contact between the biomaterial and cells, or indirectly, via humoural mediators induced by the biomaterial which then act on the cell. This pathobiological principle is of fundamental importance in understanding why biomaterials fail, the corollary of this being that if we possess detailed knowledge of how biomaterials interact with human fluids and cells, we should be able to tailor-make new biomaterials to carry out specific functions.