IN VITRO, DONE ON STEM CELL CULTURES
BIOCOMPATIBILITY TESTING OF NERVOUS CONDUITS

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Keywords: stem cells, biocompatibility, nerve conduction

Abstract: Extensive nerve injuries, often leading to nerve defects, represent a very challenging pathology with a complex therapeutic approach: trophic molecules (1 and 2 insulin-like growing factors, NGF, BDNF, 3 and 4/5 neurotrophins), vascularized nerves, metabolic manipulation of pulsating electric fields. Nowadays, the researches are axed on peripheral nerve tissue engineering, consisting of viable Schwann or Schwann-like cells implants in nerve conduits manufactured from biocompatible natural and synthetic polymers. In our study we evaluated polymer biocompatibility, both by qualitative (Giemsa cell cytochemical staining) and quantitative methods (MTT test). According to the cytotoxicity scale from the SR EN ISO 10993-5:2003 European standard, it can be concluded that, although the synthetic polymers are slightly cytotoxic, their mixing with biopolymers led to a significat increase in viability, thus achieving a good biocompatibility, with the consequent selection of the optimal variants of polymer shuffles in order to achieve nerve conduits.

Cuvinte cheie: celule stem, biocompatibilitate, conducte nervoase

Rezumat: Leziunile nervoase extensive, soldate adesea cu defecte nervoase, reprezentă o patologie extrem de provocatoare cu o abordare terapeutică complexă: molecule trofice (factorii de creştere insulin-like 1 și 2, NGF, BDNF, neurotrofinile 3 și 4/5), nervi vascularizați, manipularea metabolică a câmpurilor electrice pulsatile. În prezent, cercetările sunt axate pe ingineria tisuară a nervilor periferici, constând în implantarea de celule Schwann sau Schwann-like viable în conducte nervoase obținute din polimeri naturali și sintetici biocompatibili. În cadrul studiului nostru am evaluat biocompatibilitatea polimerilor atât prin metode qualitative (colorarea citochimică a celulelor Giemsa) cât și prin metode quantitative (testul MTT). Conform scalei de citotoxicitate din standardul european SR EN ISO 10993-5:2003, s-a constatat că, deși polimerii sintetici sunt ușor citotoxici, amestecarea lor cu biopolimeri conduce la o creștere sensibilă a viabilității, deci o bună biocompatibilizare a acestora, cu selectia în consecinta a variantelor optim de amestecuri de polimeri în scopul obținerii de conducte nervoase.

INTRODUCTION

Composite biomaterials and particularly biodegradable/biodegradable materials present a special interest for nanotechnology, both for medicine potting, including gene therapy and for tissue engineering. Composite biomaterial biocompatibility evaluation represents a fundamental stage in the establishment of product safety and application. This fundamental process must be considered when the material has medical applications. The biological evaluation of medical devices is controlled by both European ISO 10993 and American Food and Drug Administration (FDA) standards.

Biomaterials were used in the medical domain as implantable devices, artificial organs, prostheses, stomatology, bone repair, medicine delivery systems and recently in tissue regenerative engineering (Wang et al.,2004) [1]. For medical applications, polymer biocompatibility is a key factor, referring both to minimal cytotoxicity and to biofunctionality.

For the past 15 years, the researches were concentrated on the development of an in vitro testing methodology, the currently used systems going from simple subcell fractions to primary cultures, cell lines to organotypical cultures (Bhogal et al.,2005) [2].

Cell cultures represent an important mean for the preliminary evaluation of the biomaterialul cytotoxicity. A great variety of cell types from stem cells, non-differentiated fibroblast-like cells or epithelial-like cells and also highly, tissue specific, differentiated cells can be isolated from various tissues and species. They are grown for different periods of time or/and cryopreserved for future use (Brendler-Schwaab,1994) [3]. Furthermore, mammalian cell cultures are the most indicated for cytotoxicity evaluation because the vast majority of chemical substances that can cause diseases or death in humans and animals are cell excreted (Katti et al.,2002) [4]. Cell tests can give essential information of the potential effects of a compound on a cell and represent a basis for future molecular studies.

Generally it is accepted that cytotoxicity involves the characterization of different aspects of cell functions, such as viability and proliferation, loss of plasmatic membrane integrity, decrease of cell adhesion, biosynthetic activity and altered cell morphology (Silva et al.,2005) [5]. According to the structural characteristics and biomedical applications, the tested materials can be evaluated by specific methods, either by direct contact methods, the cells being seeded directly on the material.
Morphological analysis of the cells grown for 6 days. Biocompatibility evaluation was achieved by the use of known metabolites (Coecke et al., 1999) [8]. Cell metabolic systems can be divided into three categories: (1) metabolic competent indicator cells, such as hepatocytes; (2) co-cultures, which include a metabolic non-competent indicator cell type, such as fibroblasts and a metabolic competent cell type, such as hepatocytes; (3) genetic change cell lines, that can act either as indicators of some metabolic pathways or as toxicity indicators. Co-cultures are very valuable in in vitro tests due to their capacity of in vitro cell network formation (Ward et al., 1998) [9]. Furthermore, tissue engineering recent researches were facilitated by the development of in vitro complex models (Lavik and Langer, 2004) [10].

MATERIAL AND METHODS

CN were achieved by mixing synthetic polymers [polyvinyl alcohol (PVA), polyvinyl chloride (PVC) and polyethyleneglycol (PEG)] with natural polymers (non-denatured type I collagen, hydrolyzed collagen and chitosan). It was established the optimal mix report of these polymers. The biopolymer CN achieved by us represented adequate supports for MSC, in order to test them for peripheral nerve regeneration. Nervous conduits (NC) biocompatibility screening was done on a mesenchymal stem culture. These were achieved from adipose tissue harvested by liposuction. In the first table we listed the composite materials that we had tested.

Table no 1. Types of tested composite materials

<table>
<thead>
<tr>
<th>Crt. No.</th>
<th>NC composition</th>
<th>COMPONENT REPORT</th>
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<tbody>
<tr>
<td>1</td>
<td>Polyvinyl alcohol (PVA&lt;sub&gt;M&lt;/sub&gt;) - Collagen</td>
<td>1:1</td>
</tr>
<tr>
<td>2</td>
<td>Polyvinyl alcohol (PVA&lt;sub&gt;M&lt;/sub&gt;) - Hydrolyzed collagen (HC)</td>
<td>9:1</td>
</tr>
<tr>
<td>3</td>
<td>Polyvinyl alcohol (PVA&lt;sub&gt;M&lt;/sub&gt;) - Chitosan</td>
<td>4:1</td>
</tr>
<tr>
<td>4</td>
<td>Polyethyleneglycol (PEG) - Collagen</td>
<td>1:1</td>
</tr>
</tbody>
</table>

Biocompatibility evaluation was achieved by the morphological analysis of the cells grown for 6 days.

Morphological analysis

NC biocompatibility evaluation was achieved both in toto and on sections.

For the in toto cell morphological evaluation, the cells grown on the NC were fixed in 4% paraformaldehyde in PBS and stained with DAPI and hematoxylin eosin (H&E). The examination of the cells stained with DAPI was done at an Axiostar Plus fluorescence microscope (Zeiss).

For the morphological evaluation of the sections, the cells grown in the NC were fixed in 4% paraformaldehyde in PBS and included in paraffin. 6 µm sections were stained with hematoxylin eosin (H&E).

RESULTS

NC manufactured from 1:1 PVA<sub>M</sub> - Collagen presented a good compatibility. On their surface were identified mesenchymal stem cells (MSC) both in fluorescence microscopy by DAPI staining (Fig. 1a) and in optic microscopy by H&E staining (Fig. 1b). MSC adhered firmly on the PVA<sub>M</sub> - Collagen 1:1 nerve conduit (NC) (Fig. 1c).

Figure no. 1a-b. Human MSC on a 1:1 PVA<sub>M</sub> - Collagen nerve conduit (NC), DAPI (a) and H&E (b) in toto staining.

Figure no. 1c. Human MSC on a PVA<sub>M</sub> - Collagen 1:1 nerve conduit (NC), H&E stained section

NC manufactured from 9:1 PVA<sub>M</sub> - Hydrolyzed collagen presented also a good compatibility. On their surface were identified MSC, both in fluorescence microscopy by DAPI staining (Fig. 2a) and in optic microscopy by H&E staining (Fig. 2b). The sections showed that MSC adhered firmly to the 9:1 PVA<sub>M</sub> - Hydrolyzed collagen NC (Fig. 2c). The number of cells that adhered to the NC was bigger than in the case of 1:1 PVA<sub>M</sub> - Collagen conduit.

Figure no. 2a-b. Human MSC on a 9:1 PVA<sub>M</sub> - Hydrolyzed collagen nerve conduit (NC). In toto DAPI (a) si H&E (b) staining

Compared with the other two nerve conduits, 4:6 PVA<sub>M</sub> - Chitosan NC presented the best biocompatibility, on their surface being identified more mesenchymal stem cells both in fluorescence microscopy by DAPI staining (Fig. 3a) and in optic microscopy by H&E staining (Fig. 3b). The same aspect was observed on sections, too (Fig. 3c).

1:1 PEG – Collagen nerve conduits presented a good compatibility. On their surface were identified mesenchymal stem cells, both in fluorescence microscopy by DAPI staining (Fig. 4a) and in optic microscopy by H&E staining (Fig. 4b). The sections showed that MSC adhered firmly to the 1:1 PEG – Collagen (Fig. 4c).

In all the cases, mesenchymal stem cells that adhered to the NC had a normal morphology.

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CONCLUSIONS

- Nerve conduits (NC) achieved by the shuffle of synthetic polymers [polyvinyl alcohol (PVA), polyvinyl chloride (PVC) and polyethylene glycol (PEG)] with natural polymers (non-denatured type I collagen, hidrolyzed collagen and chitosan), having an optimal mixture report, were tested for their biocompatibility and there were selected the best biocompatible polymer shuffles in order to achieve nerve conduits, namely:
  - the PVA: hydrolyzed collagen mixture in a combination report of 9:1
  - the PVA: non-denatured collagen mixture in a combination report of 1:1
  - the PEG: non-denatured collagen mixture in a combination report of 1:1
  - the PVA: chitosan mixture in a combination report of 2:3
  - the PVC: hydrolyzed collagen mixture in a combination report of 2:3:1

- The achieved biopolymer nerve conduits represented adequate supports for MSC embedding in order to test them for peripheral nerve regeneration.
- 1:1 PVA – Collagen, 9:1 PVA – Hydrolyzed collagen, 4:6 PVA – Chitosan and 1:1 PEG – Collagen presented a good biocompatibility demonstrated by the adherence on their surface of mesenchymal stem cells that kept a normal morphology after 6 days of culture.
- From the four nerve conduits, the best biocompatibility had the 4:6 PVA – Chitosan conduit, followed by 9:1 PVA – Hydrolyzed collagen, 1:1 PVA – Collagen and 1:1 PEG – Collagen.

REFERENCES