Negatively charged microspheres provide an additional surface for cell attachment leading to proliferation, tissue regeneration and wound healing

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Introduction

Negatively Charged Microspheres are an example of Micro technology in the field of medicine. These synthetic particles interact with cells through various mechanisms, one of them as a temporary surface for multi-point cell contact, upon which biological macromolecules and a variety of cells involved in wound healing process can attach and proliferate.

The first step after exposure of any biomaterial to a biological environment results in the rapid adsorption of proteins to its surface, particularly when the latter is covered by sulfonates. Since serum proteins like fibronectin and vitronectin play an important role in cell attachment (KhatuaD, et al. 2011; Carré A, et al. 2010), secondary phenomena such as cellular adhesion followed by cell migration, proliferation and differentiation can further take place.

The excess of protease activity can lead to a chronic non-healing situation (Matthew P. Caley et al. 2015) but when come into contact with hydrophilic polymers, Metalloproteases can be adsorbed onto their surface reestablishing the net balance by reducing the excess of extracellular matrix degradation (ECM) (Reno F, et al. 2008).

Controlling the local cytokine milieu is essential for promoting either inflammation or wound healing; therefore influencing the types and levels of cytokines produced by biomaterial adherent cells is a feasible mechanism to modulate foreign body response. In this regard, hydrophilic and anionic surfaces are particularly relevant in promoting an anti-inflammatory type of response (Brodbeck W. G., et al. 2002).

Several tests were done to demonstrate how interaction of cells involved in wound healing process with microspheres carry out critical steps that further prove to be essential for tissue regeneration and wound healing to proceed.
**Cell attachment**

Myoblasts attachment to microspheres

The formation and extension of pseudopodia is a clear evidence of changes in the cytoskeletal structure, proving that such interaction have an influence in cell shape.

Cell attachment was time and dose dependent. Total number of **cell-bound to microspheres increases** with microspheres concentration.

*Cell Attachment:* Myoblasts and beads were placed in a 35 mm Petri dish and grown. Then, cells were fixed to evaluate the interaction of beads and myoblasts using Scanning Electron Microscopy (SEM). Vascular Endothelial cells and Beads were cultured for 36 h first, and after cell culture replacement another 36 h. Then, the numbers of cells and bound beads were counted at five random fields under the microscope (x200). (A) Number of beads bound to cells (B) Percent of cells with beads.
In a healthy healing process activated and proliferating fibroblasts increase the expression of PKC-alpha (Soh JW., et al.2003) and the levels of intracellular calcium (Ko KS, et al.2001).

**Cell Activation**

Human skin fibroblasts were labelled with 2.5 mM Fluo-4 (Molecular Probe, USA) in a Ca++-containing phenol red free DMEM and grown with two different beads concentrations (0.016% and 0.0053%). Then, intracellular Ca2+ concentration were measured every 30 sec for a period of 20 min. cells were also grown with different concentration of microspheres and finally the intracellular localization of PKC alpha was explored after immunostaining of the samples by using fluorescent–labeled anti-PKC.

Resultant effect of microspheres on increasing intracellular calcium in human dermal fibroblast cells

Microspheres at concentrations of 0.016% and 0.0053% showed an increase in Ca²⁺ influx in Human dermal fibroblasts up to 15 ± 0.02% and 8 ± 0.02% (respectively) compared to the untreated control p<0.001 and p=0.035.

**Effect of microspheres on PKCa distribution in human dermal fibroblasts cells**

In control (non-stimulated) cells, PKCa activity was found mainly in nuclei and in some extend in cytoplasm (Figure A).

After co-culture of cells and beads, a decrease in PKCa activity was evident in the nuclei, and particularly in cytoplasm. While a sharp increase in the plasma membrane was observed (figure B). The PKCa activation was time and concentration dependent.
Endothelial cell proliferation

Addition of 0.5, 2 and 10 microsphere beads per cell increase significantly cell proliferation relative to the control p= 0.02, 0.033 and 0.014, respectively.

Effect on endothelial cell proliferation relative to untreated negative control (%)

Effect on fibroblast cell Thymidin incorporation

Thymidine incorporation was 27% greater in those cells cultured with beads than in control group (beads free).
Effect of microspheres on collagen synthesis in human dermal fibroblasts

co-culture of cells with Beads increases 5.3 fold the collagen synthesis to that of control (p<0.0001)

Conclusions

Negatively charged microspheres (NCM) mimic the functions of native ECM by providing a passive temporary surface for cell attachment and proliferation.

In a stagnant wound, cell interaction with NCM promotes critical components like collagen to be synthetize leading to tissue regeneration and wound healing.