CARBON FIBRE RENFORCED POLYMER (CFRP) CAGE INDUCES BETTER CELLS ADHESION, SPREADING AND PROLIFERATION THAN POLI-ETERE-ETRE-KETONE (PEEK) CAGE. ANALYSIS BY A NEW CELLULAR MODEL FOR IN VITRO STUDY OF ORTHOPAEDIC BIOMATERIALS

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Introduction

In the orthopaedic perspective, tissue engineering is focused on the development of innovative materials whose action consists in recruiting bone progenitor cells and in stimulating their proliferation. In this context, it is clear that these materials should not only allow cells adhesion and proliferation, but also ensure that the attached cells will maintain the histological features of the original tissue. Employing an innovative human cellular model (patent FI2005A000038), we present the *in vitro* determination of the above parameters for different biomaterials.

Materials and Methods

Cell culture: Engineered osteoblast cells or were seeded on PEEK and carbon (CFRP) vertebral cages. Culture of the cells on glass cylinder was the negative control. Cell quantification: Samples containing an increasing number of cells, were centrifuged, washed in PBS, and resuspended in 1 ml of reading buffer (NaH_2PO_4 50 mM, Tris-HCl 10 mM, NaCl 200 mM, pH 8.0). The fluorescence of each sample was measured spectrophotometrically at λ=490 nm and used to obtain the calibration curve. The number of cells on vertebral cages and glass cylinders was determined on the calibration curve based on the fluorescence intensity. Immunofluorescence assay: Cells grown on the cages and glass cylinders were fixed with ice cold ethanol for 5'. Primary antibody diluted in PBS containing 0,2% of Triton 100 was applied on the cells for 1 hour at 37°C. After two washes at room temperature in PBS, appropriate secondary antibody was applied, and the washes repeated. Cells were then visualized by fluorescence microscopy.

Results

Engineered human osteoblast cells (patent N. FI2005A000038) which constitutively expresses high level of enhanced green fluorescent protein (EGFP), were tested by immunofluorescence analysis for expression and cellular localization of bone specific markers, such as osteopontin and osteocalcin. Cellular localization and distribution of the marker proteins were indistinguishable between parental and engineered cells. We also investigated the cytoskeletal organization, by visualizing actin fibers using TRITC-conjugated phalloydin. We observed the same architecture of cytoskeletal fibers in parental and engineerd cells.

In our essay, we considered that parental Saos-2 cells need approximately 36 hours to complete a single cell cycle, while the glass was the material used as comparative internal control (100%) of the CFRP and PEEK cages. Cultures analyzed at 36 hours indicated that the percentage of cells grown on PEEK (121%) was slightly higher than that on CFRP (116%). Cell adhesion and proliferation on the two different materials were re-evaluated at 84 h. The percentage of cells grown on PEEK increased in a fashion similar to that observed on the glass (13%), while cells on CFRP increased more than five times (53%).

The observation of the living cells directly on the cages showed a different distribution of the cells on the two different biomaterials. On the CFRP cage the cells were homogeneously distributed as a continuous monolayer, while on the PEEK cage the cells were layered in a discontinuous mode, distributed in non-homogeneous way, and associated in clusters.

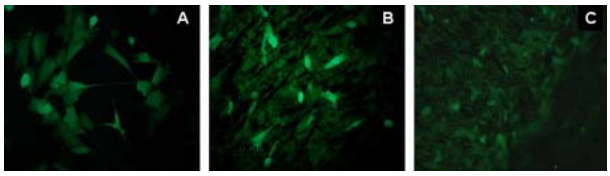

***Figure 1**. Fluorescence micrograph showing Saos-eGFP cells distribution on the CFRP panel C) and PEEK (panel B) cages, compared to the glass used as a control (panel A).*

***Table 1**. Adhesion of osteoblast cells on different artificial surfaces (number of cells/ cm^2)*

	GLASS (control)	CFRP	PEEK
36 H	65392,75 (100%)	75731,52 (116%)	79350,09 (121%)
84 H	74180,71 (13%)	115794,3 (53%)	89430,4 (13%)

Discussion

Comparative analysis carried out on parental and engineered cells demonstrated a perfect matching in expression, cellular localization and distribution of molecular markers specific for bone tissue, such as osteocalcin and osteonectin, indicating that their functional properties were not altered. Also the cytoskeletal architecture between the two cell populations was undistinguishable.

The possibility to know which cages enhance osteobalsts adhesion and proliferation could be an important step in trying to obtain a solid spine fusion. Adhesion, spreading and proliferation of osteoblast cells can dramatically differ depending on the material. Here we demonstrated that CFRP surfaces enhanced these characteristics of osteoblast cells in comparison with PEEK.

Similar assays on biomaterials are in progress using mesenchymal stem cells (MSC) to evaluate the osteoinduction, osteoconduction, and osteogenesis that are specific characteristics of MSC, when they are induced in the osteogenic lineage.

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