CARBON NANOSCAFFOLDS FOR FIBROBLAST AND HEPATOCELLULAR CARCINOMA CELLS ADHESION, MIGRATION AND REGENERATION

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Introduction
As with many types of cancer, cell migration and adhesion is an important factor in the progression and metastasis of hepatocellular carcinoma (HCC) [1]. The carbon scaffold acts as an interim synthetic extracellular matrix (ECM) that cells interact prior to forming new tissue [2]. The scaffold should provide attachment, growth, differentiation of cell and must be porous for nutrients. The rate of degradation of the scaffold must be equal to the rate of tissue formation. The extracellular matrix and products of its degradation must be biocompatible. Strojny et al. [3] reported that diamond, graphene oxide and graphite are highly biocompatible and non-toxic for animals. The carbon nanoparticles are present for a long time after injection to tissue. This is the reason for their use as drug carriers [3]. In this study, human stromal cell line (HS-5) was used as control compared with neoplastic cells.

Material and methods
Carbon scaffolds were prepared by drops placement and desiccation of the colloids of nanoparticles of diamond (ND), fullerenes (F60), nanotubes (NT), nanotubes OH (NTOH), nanotubes COOH (NTCOOH), graphene oxide (GO), pristine graphene (GP) on the bottom of culture plates. HS-5 (ATCC, CRL-11882), HepG2 (ATCC HB-8065) and C3A (ATCC CRL-10741) were obtained from the American Type Culture Collection (ATCC). The human cell lines were maintained at 37°C under 5% CO_2 in Dulbecco's Modified Eagle Medium-Low Glucose (DMEM, Gibco, Thermo Scientific, Waltham, MA, USA) supplemented with 10% Fetal Bovine Serum (Life Technologies, Houston, TX, USA), penicillin (100 U/mL) and streptomycin (100 mg/mL) (Life Technologies). Cells were seeded on 6-well plates containing 0.1% of carbon scaffolds. The cultures with scaffolds were maintained by one day. The location, density and agglomeration of cells on the scaffolds were examined by the inverted light microscope (Leica, TL-LED, Germany) connected to a digital camera (Leica MC190 HD), using LAS V4.10 software (Leica) and compared to the control. The mean viability of HS-5, HepG2 and C3A cells on carbon scaffolds was assessed by evaluation of metabolic activity, using the PrestoBlue (Life Technologies, USA) and XTT (Roche Protocol, Germany). Cell proliferation was evaluated using a bromodeoxyuridine (BrdU) incorporation assay (BrdU colorimetric) (Roche Applied Science, Indianapolis, IN, USA).

Results and discussion
Morphology, number and differentiation of cells depend on the type of nanoscaffolds and cell lines. The interaction of carbon allotropes with cells was different. All nanoparticles were non-toxic or slightly toxic to cells used. The scaffold, prepared from ND was the most colonised by HepG2 cells, moreover, stimulated cell proliferation (large agglomerations of cells on scaffolds). HS-5 and C3A cells were single on the diamond surface. Cell lines showed a high affinity to GO scaffold. HCC cells formed smaller aggregation on the GO surface, i.e. niche provides good growth conditions. Scaffold, prepared from fullerenes, was the least colonised by HCC cells and decreased the amount of cells. However, the number of fibroblast cells was high on the fullerenes scaffold and beyond it. The most neutral for used cells was pristine graphene. Cells showed higher affinity to small aggregations than to big aggregations of GP. Scaffold constructed by carbon nanotubes, functionalised with COOH, was also well settled by cells, better than scaffold with nanotubes and nanotubes OH. Microscopic observations have been confirmed by viability and proliferation assays.

Conclusions
Nano-scaffolds, depending on the carbon allotropes, influenced behaviour, regeneration and morphology of HS-5, HepG2 and C3A cells. Furthermore, cells changed bio-function of scaffolds. Carbon nanoparticles are biocompatible and have a favourable structure to colonization by specific cells. The scaffolds-cell interaction leads to adhesion and then affects the cell division. The worst niche for the cells used were the nanotubes, which resulted from the lack of suitable functional groups. Factors such as shape, atomic hybridization and proportion of chemical bonds influence behavior of cell lines.

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