CARBON ALLOTROPES FOR MUSCLE REGENERATION

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Introduction

Stem cells, developing in the body, are surrounded by a specific micro-environment of the adjacent cells and components of the extracellular matrix, constructed from proteins and proteoglycans [1]. This environment of stem cells is called stem cell niche, a key factor for their further proliferation and differentiation, including *in vitro* culturing. The standard methods of cell culturing include only an effect of the two-dimensional substrate surface. The lack of the interaction between a niche and a stem cell in the 3D contact represents a significant limitation and difficulty in the *in vitro* tissue culture and especially in the *in vitro* culturing of muscles. The creation of the 3D structure that allows for culturing of muscle cells from their progenitor cells could represent a breakthrough in developing methods for *in vitro* cultivation of muscles' implants. The 3D construction composes an architectural system similar to the natural, including mechanical functions that mimic the basal lamina and extracellular tendon matrix function.
Carbon allotropes, as a 3D scaffold of the niche, can create the perfect environment for cell growth, particularly, because it is a highly biocompatible material [2]. Above all, they can be a relatively simply functionalised, hence, different organic molecules can be attached to them, in particular, proteins (amino acids), imitating natural niche cells [3].

Materials and Methods

Carbon scaffolds were prepared by layer placement and desiccation of the colloids of nanoparticles of diamond (ND), fullerenes (F60), nanotubes (NT), nanotubes OH (NTOH), nanotubes COOH (NTCOOH), graphene oxide (GO) on the bottom of a culture flask. Mesenchymal stem cells were collected from the hind limb bud of chicken embryos. On day 7 embryos were sterile removed from the eggs and a bud of the hind leg was collected, using a microscope, and placed gently in a solution of trypsin, then put aside in the refrigerator for a 24 h. In the next step, the solution was neutralized by the addition of a standard culture medium (DMEM - Dulbecco's Modified Eagle's), gently stirred and placed in flasks (BD) or dishes and culture, according to particular experiments.
Culture medium was changed every 3rd day. Cells were maintained in DMEM culture medium containing 10% foetal bovine serum (Life Technologies, Houston, TX, USA) and 1% penicillin and streptomycin (Life Technologies) at 37°C in a humidified atmosphere of 5% CO_2/95% air in a DH AutoFlow CO_2 air-jacketed incubator (NuAire, Plymouth, MN, USA). In the experiments, high or low glucose, L-glutamine, DMEM were used. At day 5 the morphology of mesenchymal and muscle cells was visualized, using SEM and light microscopes. Expression of the mRNA of chosen proteins was measured.

Results and Discussion

Morphology of mesenchymal muscle cells and their interaction with carbon scaffolds are observe using SEM and light microscopes. The interaction of nanoscaffolds with cells differed and the morphology; number and state of the development of cells were influence by carbon allotropes. The most neutral for stem cells were nano-diamond based scaffold. The scaffold, prepared from fullerenes, was the most colonised by cells, moreover, it stimulated cell proliferation. The scaffold constructed from carbon nanotubes, functionalised with COOH, was also well settled by cells, better than scaffold with nanotubes and nanotubes OH. GO scaffold stimulated differentiation of muscle stem cells and creation of the muscle tissue (FIG. 1). mRNA expression of muscle cells colonised in GO scaffold clearly showed increased expression of MyoD-marker of differentiated muscle cells.

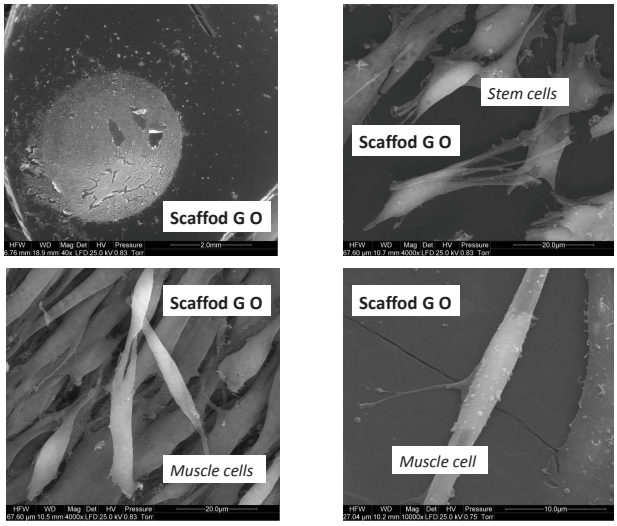

FIG. 1. Morphology of the interaction between cells and scaffolds.

Conclusions

Nano-scaffolds, depending of the carbon allotropes, influenced behaviour and morphology of muscle stem cells, moreover, their functionalization (nanotubes) changed bio-function of scaffolds and stem cells number and morphology. GO scaffold stimulated mesenchymal stem cells differentiation and beginning of the formation of muscle tissue.

Acknowledgments

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