Stem cell differentiation on nano-structured carbon surfaces

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ABSTRACT

Stem cells are the source of all differentiated cells in the body. However, the stimuli that control stem cell differentiation into other cell types are still not well understood. In this research, nano-structured carbon materials were prepared by using several methods: chemical vapor deposition, template mediated assembly of discotic mesophase pitch and spin coating methods. Stem cells adhesion was investigated on these nano-structured surfaces. Due to the ability of carbon nanostructures to conduct electricity, the differentiation of stem cells adherent onto the nanostructure carbon surfaces was also investigated upon application of an electrical current.

Keywords: carbon nanofiber, carbon thin film, stems cells, cell adhesion, cell differentiation

1 INTRODUCTION

Stem cells have the ability to differentiate into any cell type. This property makes them excellent candidates to restore damaged tissues and organs. Currently, the mechanisms triggering and controlling the differentiation of these cells are unknown. However, it is clear that novel biomaterials are needed to deliver stem cells to specific tissue locations and enhance their differentiation into desirable cells. It is for these reasons that advances in nanotechnology can be used in coordination with stem cell technology to heal damaged tissue (such as in the creation of scaffolds upon which stem cells differentiate *in vitro*).

There is significant evidence that properties of surfaces can affect cell functions, especially for anchorage dependent cells. The first reports showing superior cell function on nanofeatured surfaces dates back to the late 90's [1]. Nano-structured alumina and titania also enhanced osteoblast adhesion with respect to their conventional grain sized counterparts [2].

One of the important nanophase materials investigated for biomedical applications is carbon nanofibers (CNFs). Elias et al. [3] and Price et al. [4] determined significantly greater *in vitro* osteoblast functions leading to mineral deposition on carbon fibers with nanometer compared to conventional dimensions. When CNFs were incorporated into PLGA (poly-lactic-*co*-glycolic acid) at 5 wt%, it doubled osteoblast adhesion [5]. For the PCU (polycarbonate urethane) / CNF composites [6], the weight fraction of CNF in CNF/PCU composites correlated with increased *in vitro* osteoblast adhesion and decreased fibroblast adhesion. One of the main reasons for enhanced cell adhesion and activity is that nano-featured surfaces have similar properties to natural tissues (such as mimicking the nano-topography) [7].

In this research, in order to control and demonstrate stem cell differentiation *in vitro*, nano-structured carbon surfaces were chosen as a potential scaffold material due to their unique surface and electrical properties. Differences in the adhesion and differentiation behavior of these stem cells on various carbon substrates were characterized.

2 EXPERIMENTAL PROCEDURES

In this research, three different methods were used to prepare nano-structured carbon substrates for stem cell attachment and differentiation. Two of these methods, chemical vapor deposition and template mediated assembly of discotic mesophase pitch, were used to grow carbon nanofibers on anodized titania and commercial alumina templates, respectively. Bulk carbon nanofiber compacts were prepared by uniaxially pressing these individual carbon nanofibers under 6GPa of stress inside a stainless steel die into compacts. Figure 1 shows the SEM image of such CNF scaffolds used for stem cell adhesion. The third method, spin coating, was used to make semi-amorphous carbon thin film substrates. The CNFs prepared by template mediated assembly of discotic mesophase pitch were annealed at either 700^{o}C or 2500^{o}C.

Stem cells were then seeded onto these three different substrates and the differences between their adhesion and differentiation behavior was monitored. The influence of various growth factors on stem cell differentiation was also determined.

Figure 1: SEM image of CNF scaffold used for stem cell attachment and differentiation. (Scale bar: 1 micron)

3 RESULTS

The electron microscopy investigations provided evidence that the diameter of the CNF prepared by CVD process depends on the pore size of the alumina and titania template, as expected. The aspect ratios of these CNFs were controlled with the deposition time and temperature in the CVD chamber. SEM investigations showed that nanofiber compacts produced by uniaxial pressing were fully dense and non-porous. There was no difference in the roughness of the prepared scaffolds depending on the CNF diameter.

The CNFs prepared by template mediated assembly of discotic mesophase pitch showed different hydrophobicity depending on the annealing temperature. The CNFs annealed at high temperatures (such as 2500^{o}C) showed strong hydrophobic properties and the CNFs annealed at low temperatures (700^{o}C) showed hydrophilic behavior. This was explained with healing of active edge sites at higher temperatures by Hurt et al. [8]. Hydrophilicity of a surface is important in protein and cell attachment to that surface. Preferential adhesion of vitronectin on hydrophilic surfaces, which subsequently affects osteoblast adhesion, is an example to this behavior. In the given research, stem cell adhesion was also characterized depending on the hydrophilicity of the CNF surfaces.

Results of this study also demonstrated the ability to fabricate nano-structured carbon-based scaffolds that can support and direct stem cell differentiation. In this manner, the coordinated use of stem cells and carbon nano-structured surfaces is a promising technique in orthopedic and tissue engineering applications.

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