NANOPARTICLES, NANOFIBRES AND THEIR COMBINATIONS IN BONE TISSUE ENGINEERING – A REVIEW

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Nanostructured materials are considered as promising scaffolds for advanced tissue engineering. The reason is that the nanostructure of a material resembles the nanoarchitecture of the natural extracellular matrix (ECM), e.g., its organization into nanofibres, nanocrystals, nano-sized folds of ECM molecules, etc. On nanostructured surfaces, the cell adhesion-mediating ECM molecules adsorb in an appropriate geometrical orientation which gives cell adhesion receptors access to specific sites in ECM molecules, such as amino acid sequences like Arg-Gly-Asp (RGD), which serve as ligands for these receptors [1-3]. In addition, these materials enhance the adsorption of vitronectin, which is recognized preferentially by osteoblasts over other cell types [1-3]. Nanostructured materials have therefore been considered as suitable particularly for bone tissue engineering.

Our studies have focused on carbon and hydroxyapatite nanoparticles as components of substrates for colonization with human bone-derived cells in vitro. Carbon nanoparticles, namely nanocrystalline diamond (NCD) and fullerenes C60, have been used in the form of films deposited on carbon, glass, silicon and metallic substrates [3-4]. These films were of continuous (NCD) or micropatterned (C60) morphology, and have been intended for surface modifications of bone and dental implants [5], or for creating surfaces enabling regionally-selective cell adhesion and directed cell growth [6]. NCD films were also doped with boron, which resulted in improved adhesion, growth and osteogenic differentiation (measured by the production of collagen I, osteocalcin and alkaline phosphatase content) of human osteoblast-like MG 63 cells [7]. These beneficial effects can be explained by the increased electrical conductivity of boron-doped nanocrystalline diamond films, and can be further enhanced by active electric stimulation of cells.

Some nanoparticles were also incorporated into polymeric matrices, e.g. foils of a terpolymer of polytetrafluoroethylene, polyvinyldifluoride and polypropylene (carbon nanohorns, carbon nanotubes) or nanofibres prepared by an electrospinning technique from polylactide, PLA (hydroxyapatite nanoparticles) or poly(lactide-co-glycolide), PLGA (nanodiamond).

All these composite substrates promoted the adhesion, growth and osteogenic differentiation of human osteoblast-like MG 63 cells in an extent similar to or even better than standard cell cultivation substrates, such as polystyrene dishes or microscopic glass coverslips. The adhesion and growth of MG 63 cells was particularly improved on the terpolymer of polytetrafluoroethylene, polyvinyldifluoride and polypropylene enriched with 4 wt.% of single-wall carbon nanohorns or multi-wall carbon nanotubes [3, 4]. The osteogenic differentiation of MG 63 cells (measured by concentration of osteocalcin) was enhanced on nanofibrous polylactide scaffolds loaded with 15 wt% of hydroxyapatite. On PLGA nanofibrous scaffolds loaded with approx. 23 wt.% of diamond particles, the number of initially adhering MG 63 cells on day 1 after seeding and the following growth dynamics of the cells were similar to the values on pure PLGA scaffolds [8]. However, the cells on PLGA meshes reinforced with nanodiamond formed larger and more numerous talin-containing focal adhesion plaques. In addition, these plaques in cells on PLGA-nanodiamond scaffolds were localized not only at the cell periphery but also in the central part of the cells (FIG. 1).

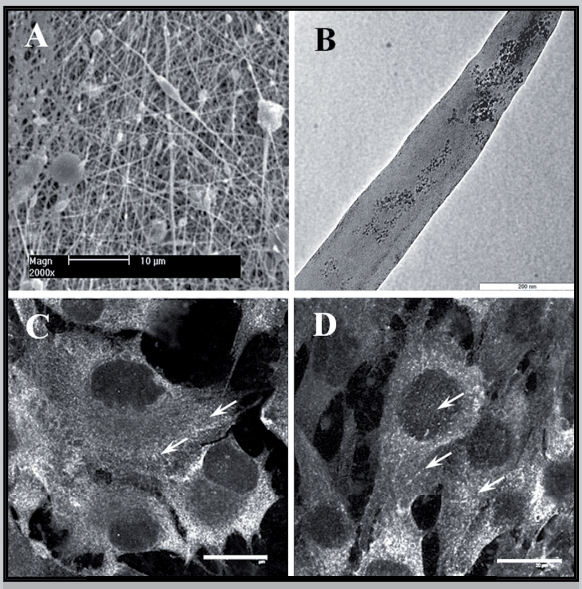

FIG. 1. Scanning electron microscope image of composite nanofibrous PLGA-nanodiamond scaffolds (A), transmission electron microscope image of a PLGA fiber loaded with nanodiamond particles (B), confocal microscope images of human osteoblast-like MG 63 cells stained by immunofluorescence against talin in 3-day-old cultures on pure PLGA scaffolds (C) and composite PLGA-nanodiamond scaffolds (D). Arrows indicate focal adhesion plaques. The bar indicates 10 µm (A), 200 µm (B) and 20 µm (C, D).

Thus, it can be concluded that nanoparticle-modified materials are more promising than their non-modified counterparts for colonization with bone cells, for construction of bone implants and for bone tissue engineering.

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POLYMER CARRIERS MODIFIED BY PLASMA AND FUNCTIONALIZED WITH Au NANOPARTICLES AS SUBSTRATES FOR MOUSE 3T3 FIBROBLASTS

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Polymers have been often applied in biology and medicine for construction of tissue replacements. However, the inert surface of the most polymers is not able to support and control cell adhesion, migration, proliferation, differentiation and other cell functions. Hence, the modification of polymer surface led to achieve appropriate properties. The polymer surface can be modified by plasma discharge by which the polymer surface chemistry and morphology is changed. Plasma treatment leads to creation of radicals, unsaturated bonds and new chemical groups, mainly oxygen containing groups. Oxidized groups increase the wettability of polymers, which supports adsorption of cell adhesion-mediating extracellular matrix (ECM) molecules in appropriate spatial conformation increasing accessibility of specific sites in these molecules by cell adhesion receptors. In addition, other surface properties of polymers are altered by plasma etching which strongly influence cell-material interaction. Radicals and unsaturated chemical bonds which are created by plasma can be utilized for grafting new chemical groups, biomolecules and nanoparticles. The biomolecules grafted on the polymer surface, such as amino acids, RGD-containing oligopeptides (i.e., ligands for integrin receptors), ECM molecules, enzymes, hormones, and also carbon and gold nanoparticles, not only have specific biological effects on cells but also change physical and chemical properties of the polymer surface, and by this way they support its bioactivity.

This study is focused on physiochemical properties and biocompatibility of modified polymers. The studied materials were poly(L-lactide) (PLLA) foils, nanofibrous PLLA meshes and polyethylene terephtalate (PTFE) foils. PLLA and PTFE foils were modified in plasma with Ar^+ ions for time intervals of 50, 100 and 300 s with power 8 W, and then grafted with Au nanoparticles.

Changes in the surface wettability were determined by reflection goniometry. The presence and concentration of Au nanoparticles were examined by X-ray Photoelectron Spectroscopy (XPS). For the biocompatibility testing, the polymers were seeded by mouse embryonic fibroblasts of the line 3T3, i.e., the cells often utilized as a feeder for keratinocytes. The cell adhesion and growth was evaluated by the number of cells, their morphology and the size of cell adhesion area in the 1st, 3rd and 6th day after seeding.

The results indicate that the water drop contact angle increases with the time of exposure to plasma, which means that the vettability decreases. However, the following exposure of plasma-irradiated polymers to a sodium citrate solution (i.e., a storage solution for Au nanoparticles) and grafting with Au nanoparticles decrease the contact angle, i.e., increase the material surface wettability. Our tests of biocompatibility indicate that the modification of the polymer surface influences positively the cell behavior. The cells adhered at higher numbers and by a larger cell adhesion area on modified polymers; it was mainly manifested on PTFE.

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