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Hybrid electrospun scaffolds for enhanced collagen formation in regeneration processes: electron and confocal microscopy analysis

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Background

Extracellular matrix (ECM) of bone tissue is a natural composite containing organic and inorganic components, that can be biomimicked using electrospun fibers. Addition of ceramic filler into the polymer fibers can enhance the mechanical properties as well as improve bioactivity. However, to obtain the best hybrid scaffold the uniform distribution of particles and reduction of aggregations are necessary [1]. Here, the careful design of composite fibers can trigger cellular adhesion, proliferation and tissue regeneration via enhance collagen formation in the in vitro cell culture [2]. Therefore, the goal of this work is to optimize and verified production of hybrid electrospun fibers for effective bone tissue scaffolds including visualization using advance electron and confocal microscopy analysis.

Methods

Blend and co-axial electrospinning were employed to manufacture bone scaffolds of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) with incorporated titanium dioxide nanoparticles (TiO_2) using TechNOVA electrospinning device [3]. Morphology of obtained fibers was evaluated by scanning electron microscopy (SEM) with secondary electron detector (Merlin, Zeiss). Distribution and aggregation of TiO_2 within polymer fibers were studied by SEM imaging with back scattered detector (BSE), energy dispersive X-Ray spectroscopy (EDS, detector Brucker). Additionally similar analysis was also performed at the cross-sections prepared by focused ion beam (FIB, Crossbeam 350, Zeiss). In vitro cell culture study was performed with osteoblast cells up to 14 days. Cell proliferation and morphology in response to various scaffolds was verified after 1, 3 and 7 days of incubation. The collagen formation was confirmed by immunofluorescence staining and visualized by confocal laser scanning microscopy (CLSM, LSM 900, Zeiss).

Results

Over 20% increase in fiber diameter was observed when PHBV was enriched with TiO_2 nanoparticles, reaching 3.84 µm for sample prepared by co-axial process. Blend fibers had smooth surface with visible large particles aggregations, locally exceeding 5 μm^2, in majority covered by polymer. On the other hand, the fibers prepared using co-axial nozzle had core-shell morphology, with polymer fibers homogenously decorated with ceramic particles with significantly smaller aggregations up to 0.3 μm^2. In response to core-shell fibers cells exhibited significantly higher proliferation after 7 days with excellent spreading within the scaffold structure. Additionally, they produced vast network of collagen fibers as the important stage of ECM formation, which was not observed in such extent for any other tested scaffold.

Conclusion

Current study shows the importance of electrospinning strategy for single-step production of bioactive scaffolds supporting bone regeneration. For cellular guidance it is essential not only to provide composite morphology but also to ensure proper particles distribution and availability, which was achieved by co-axial electrospinning. The presence of ceramic nanoparticles, effectively integrated onto the surface of polymer fibers, enhances cells adhesion, ingrowth and promotes the formation of ECM, which are necessary in tissue regeneration processes.

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Keywords:

Organic-inorganic composites, bone, collagen staining

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