the scaffolds. The cells were round or near round on the scaffolds of small fiber diameter, while became spindled on the scaffolds of large fiber diameter. After 7 days, the expression of collagen-II and aggrecan genes decreased with the fiber diameter, whereas the gene expression of collagen-I increased. The related proteins level was similar to the gene expression.
Discussion and Conclusion: The scaffolds of different fiber diameters may mimic the outer, middle, and inner fibrous structures of native AF tissue. The gene expression and protein production of AFSCs on the scaffolds with different fiber diameters was similar to that of AF tissue. Specifically, the gene expression of collagen-II and aggrecan genes decreased from small diameter scaffold to large diameter scaffold, whereas the gene expression of collagen-I was the opposite case. Therefore, this study provides solid basis for the use of biomimetic scaffolds which mimic different AF zones for AF tissue engineering.
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SYNERGISTIC EFFECT OF DECELLULARIZED ANNULUS FIBROUS MATRIX AND SUBSTRATE ELASTICITY ON ANNULUS FIBROUS-DERIVED STEM CELLS

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Background: Due to the similarity of biochemical composition and microstructure between decellularized matrix (DCM) and native extracellular matrix (ECM), DCM have been widely used in tissue engineering. Meanwhile, the effects of mechanical property (e.g. elasticity) of cell culture substrate on the proliferation and differentiation of cells have also been well documented. This study aims to explore the combined effect of decellularized annulus fibrous matrix (DAFM) from porcine and substrate elasticity on the behaviors of rabbit annulus fibrous-derived stem cells (AFSCs).
Methods: DAFM was coated onto polyacrylamide gels (PAG) with elastic modulus of 2.6 kPa (soft), 10.6 kPa (middle), and 34.9 kPa (rigid), respectively. Collagen-I coated PAGs were used as control. The cell proliferation, morphology, gene expression and protein production of AFSCs were examined in both groups with different substrate elasticity.
Results: After 7 days of culture on both DAFM and collagen-I coated PAGs, AFSCs proliferated well. The cells on soft PAGs exhibited the least expression of collagen-I gene, yet the greatest expression of collagen-II and aggrecan genes. In contrast, the cells on rigid PAGs showed the greatest expression of collagen-I but the least of collagen-II and aggrecan. The gene expression of cells on middle PAGs was in between of those on soft and rigid PAGs. Expression of these genes in AFSCs cultured on DAFM-coated PAGs followed a similar substrate elasticity-dependent pattern. However, the responses of AFSCs to substrate elasticity appeared to be more prominent when they were cultured on DAFM-coated PAGs. The protein production of collagen-I, collagen-II and glycosaminoglycans (GAG) in each group was consistent with the specific gene expression of AFSCs.
Discussion and conclusion: In our pervious study, AFSCs would respond to the mechanical properties of substrate elasticity. Here, we combined the use of DAFM and scaffolds of gradient elasticity and found that the responses of AFSCs in gene expression and protein production appeared to be more prominent when they were cultured on DAFM-coated PAGs in every group with different substrate elasticity. These findings suggested that combined use of DAFM and scaffolds of gradient elasticity may represent a more efficient approach for AF tissue engineering.
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GENIPIN-CROSSLINKED DECELLULARIZED ANNULUS FIBROSUS MATRIX/CHITOSAN SCAFFOLDS FOR THE CULTURE OF ANNULUS FIBROUS-DERIVED STEM CELLS

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Background: While tissue engineering method has become an ideal approach for annulus fibrous (AF) regeneration recently, it remains challenging because of the heterogeneity of AF tissue. Decellularized extracellular matrix (ECM) has been proposed as a novel tissue-specific biomaterial for tissue engineering, yet lacks sufficient mechanical strength. Chitosan, a linear polysaccharide which possesses characteristics of chondroitin sulfate and keratin sulfate, is similar to the glycosaminoglycans (GAGs) which are rich in native AF tissue. In this study, we used Genipin to crosslink decellularized AF matrix (DAFM) and chitosan (CS) to fabricate biomimetic scaffolds for AF tissue engineering. Importantly, the DAFM/CS samples have different elasticity to mimic the outer, middle and inner zones of AF.
Methods: Tensile tests, SEM and AFM were used to study the mechanical properties and surface characteristics of DAFM samples. After being cultured on the scaffolds, the proliferation of AF-derived stem cells (AFSCs) was examined by CCK8 tests. The morphology of AFSCs was checked by SEM and cytoskeleton staining. The genes expression (Col-I, Col-II, and Aggrecan) was measured using RT-qPCR. Cell traction forces (CTFs) of AFSCs were determined using CTF microscopy (CTFM).
Results: Biomimetic DAFM-CS composites were crosslinked using Genipin to fabricate scaffolds of various elasticity (47, 75, and 120 kPa, respectively). A highly porous and interconnected network structure was observed. After AFSCs were cultured on these scaffolds for 7 days, the gene expression and protein production of collagen-II and aggrecan decreased with the elasticity of scaffold, whereas the expression of collagen-I was exactly the opposite case. Similarly, the CTFs of AFSCs gradually decreased with the elasticity of scaffold. Cytoskeleton staining and SEM imaging showed that the morphology of cells was almost round on the scaffolds of low elasticity and spindle-like on the scaffolds of high elasticity.
Discussion and conclusion: DAFM/CS composite scaffolds with different elasticity were fabricated using Genipin-crosslinking. These scaffolds mimicked the outer, middle and inner zones of native AF tissue and markedly affected the differentiation of AFSCs. Importantly, the substrate elasticity-dependent changes (cell morphology, gene expression, and CTF) of AFSCs were similar to the cellular, mechanical and biochemical characteristics of cells from outer region to inner region of native AF tissue.
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PREPARATION AND PROPERTIES OF STRONTIUM DOPED NANO HYDROXYAPATITE

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Background: Osteoporosis (OP) is defined as a skeletal disorder that is characterized by a reduction of bone mass and deterioration of bone microarchitecture, with a consequent increase of bone fragility and decrease of bone strength that could induce increased risk of fracture. Hydroxyapatite (HA) is a widely used coating material due to its satisfactory biocompatibility and osteoconductivity. Strontium (Sr), which can increase the activity of osteoblasts while decrease it of osteoclasts, has been a research focus in osteoporosis.
Subjects and Methods: Pure nano hydroxyapatite (nHA) and a series of strontium-doped nano hydroxyapatites(Sr-nHAs)were prepared by our one-step method which mainly used the principle of homogeneous phase co-precipitation. The different Sr-nHAs were designed and prepared by doping Sr ions into nHA with an atomic ratio of Sr/(Ca+Sr)=1/20 (5%), 1/10 (10%), and 1/5 (20%), respectively. The properties of prepared nHA and Sr-nHAs were characterized by using FTIR, XRD, TEM and EDS.
Results: FTIR spectra showed that absorption bands of HA characteristic vibrations were observed in the four different HA material samples. Weak bands associated with carbonate (CO_3^{2-}) were also observed. Besides, with the increase of doped Sr, the intensity of absorption peak was decreased. As compared with the standard data, typical XRD diffraction peaks of HA were clearly identified in the prepared nHA and Sr-nHAs samples. The significant broadening of diffraction peaks indicated that the powders prepared in this study were nano-sized materials. Importantly, with the increase of doped Sr, special peak position shifting was observed. TEM images showed that the synthesized nanoparticles have a tiny rod-like feature, and the size of nanoparticles increased in accordance with the Sr-doping. Finally, EDS spectra clearly showed the presence of not only Ca, O and P but also Sr in all Sr-nHA samples.
Discussion and Conclusion: HA has been widely used in bone implants including the coating of prosthesis. However, for patients with osteoporosis, pure HA has little effects on promoting bone formation and suppressing bone resorption. As a result, the loose rate of pure HA coated implants is very high. On the other hand, strontium (Sr) can simultaneously promote bone formation and inhibit bone deterioration in osteoporosis. So we hope to improve implant coating by using Sr-doped HA, a novel biomaterial. In this study, both the pure nHA and Sr-nHAs were successfully synthesized by using our method and characterized. Importantly, on basis of this work, a series of Sr-nHA coated titanium (Ti) implants were prepared. Therefore, the Sr-nHAs synthesized in this study are expected to be further explored and optimized for biomedical applications in osteoporosis.
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