VEGF-SRC SIGNALING IS ESSENTIAL FOR VASCULAR ENDO THELIAL PERMEABILITY AND OSTEOCLASTS ACTIVITY

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Objectives: To delineate the role of VEGF and c-Src signals in triggering destructive repair of osteonecrosis in vitro.

Materials and methods: The primary endothelial cells and osteoclasts were adopted in this study. Pharmacological VEGF and Src specific pp60c-srcsiRNA were used to determine the contribution of VEGF-Src signaling to vascular permeability and osteoclasts activity. Cells were treated with 50 ng/ml VEGF and/or transfected with the pp60c-srcsiRNA every other day. In parallel, equivalent PBS and non-targeting siRNA were treated in the control groups. We analyzed the endothelial permeability associated structural elements and the osteoclast formation and function.

Results: Results showed that the specific pp60c-srcsiRNA significantly reduced Src expression both in the endothelial cells and osteoclasts. For decreasing VEGF-mediating higher vascular permeability, Src blockade significantly alleviated actin stress and the formation of caveolae and vesiculo-cargo organelles (VVOs), as well as stabilized the complex beta-cat enin/VE-cadherin/Fik-1 through decreasing phosphorylation of VE-cadh erin, to keep endothelial function integrity. In addition, VEGF promoted osteoclasts formation and function, while the adhesion activity and cytoskeleton were not obviously affected by VEGF. However, Src blockade significantly destroyed the cytoskeleton resulting in a lower adhesion activity and inhibited the osteoclasts differentiation and function through decreasing the phosphorylation of Src, Pyk2 and Cbl. These findings indicated that Src blockade not only reduced the VEGF mediating vascular permeability, but also reduced osteoclasts activity.

Conclusion: VEGF-Src signaling is essential for vascular endothelial permeability and osteoclasts activity. Thus, blockade of VEGF-Src signaling may provide us a new view to develop novel strategies for preventing and treatment of destructive repair in osteonecrosis.

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Objectives: VEGF-Src signaling is essential for vascular endothelial permeability of this scaffold. The structure and mechanical properties and in vitro biocompatibility of the composite scaffold. The mechanical strength of the PLGA/TCP/Mg scaffolds was enhanced with Mg.
with increasing Mg content. The Young’s modulus of PLGA/TCP/Mg scaffolds (Mg content: 15% wt) was around 104 Mpa, statistically significantly stronger than that of Mg content: 10% wt (83Mpa) and Mg content 5% wt group (82 Mpa), as well as PLGA/Mg group, 66Mpa. All the Mg containing scaffolds were statistically significantly stronger than PLGA/TCP and PLGA group, 45 Mpa and 30 Mpa, respectively.

Conclusion: The results of CCK-8 assay demonstrated the MC3T3-E1 osteoblasts grew very well and proliferated rapidly on PLGA/TCP/Mg scaffolds compared to PLGA/TCP scaffold after 7 day culture. The in vitro study also demonstrated a good biocompatibility and bioactivity of the PLGA/TCP/Mg scaffold that was in favor of accelerating and inducing the proliferation and differentiation of osteoblasts.

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Abstracts

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SYNERGISTIC EFFECT OF DECELLULARIZED ANNULUS FIBROSUS (AF) MATRIX AND SUBSTRATE STIFFNESS ON THE GENE EXPRESSION OF RABBIT AF STEM CELLS
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Objective: Decellularized matrices (DCM) have been widely used for engineering functional tissues, mainly due to the similarity of biochemical composition and microstructure between them and native extracellular matrix (ECM) in vivo. Meanwhile, the mechanical properties of substrate play an important role in regulating cell behavior such as adhesion, proliferation, differentiation and migration. Here, we aimed to study the combined effect of DCM and substrate stiffness on the behaviors of newly identified rabbit AF-derived stem cells (AFSCs).

Methods: To this end, decellularized porcine annulus fibrosus matrix (DAFM) was covalently coupled to polyacrylamide gels (PAGs) which had elastic moduli of 2.6 KPa (Soft), 10.6 KPa (Middle), and 34.9 KPa (Rigid), respectively. As control, collagen-coated PAGs of similar stiffness were used.

Results: After 7 days of culture on these PAGs, AFSCs on soft collagen-coated PAGs exhibited the least expression of collagen-I gene, while cells on rigid collagen-coated PAGs exhibited the greatest. In contrast, cells on soft collagen-coated PAGs exhibited the greatest expression of collagen-II and aggrecan genes, while cells on rigid collagen-coated PAGs had the least expression of them.

Conclusion: The gene expression of cells on middle PAGs was between those on soft and rigid PAGs. Expression of the above genes in AFSCs cultured on DAFM-coated PAGs followed similar substrate stiffness-dependent pattern. However, the responses of AFSCs to substrate stiffness appeared to be more prominent when they were cultured on DAFM-coated PAGs. Therefore, combined use of DAFM and scaffolds of gradient stiffness may provide a more efficient approach for AF tissue engineering.

Objective: Leptin has been postulated as one of the etiologic factors of AIS because of its important physiological functions in neuro-osseous development affecting skeletal growth, the onset of puberty, energy expenditure and body composition. Previous studies on the relationship between leptin and HR-pQCT derived bone quality parameters had found abnormal correlations in AIS girls, and suggested possible abnormalities in the leptin regulated bone metabolic pathways. Another study on AIS patients showed hypo-responsiveness to leptin in bone marrow derived mesenchymal stem cells. This study aimed to investigate the effect of leptin on the functional responses of osteoblasts in AIS girls, and compare with that in control subjects.

Material and Methods: In vitro assays were performed with osteoblasts isolated from 12 severe AIS girls and 6 control subjects. The osteoblasts were exposed to different concentrations of leptin (0, 10, 100, 1000 ng/ml). The effects of leptin on cell proliferation were evaluated with MTT assay after 3 days of leptin treatment; differentiation with ALP activity assay after 6 and 14 days, and with osteocalcin ELISA throughout the 35 days of culture; and mineralization with von Kossa staining after 21 and 35 days.

Results: Baseline comparison between osteoblasts from AIS and control groups showed lower differentiation and mineralization potentials in the AIS group. For functional responses to leptin, control group showed increasing proliferative response to leptin in a dose dependent manner (p=0.008), while AIS group showed no proliferative response to leptin (p=0.962). For differentiation, control group showed strong and significant trend in ALP activity to increasing leptin concentrations in both day 6 (p=0.012) and 14 (p=0.017), and secreted osteocalcin in an increasing dose dependent manner to leptin (p=0.007 in day 35), but this trend were not observed in the AIS group (p=0.05). For mineralization, the control group showed a mild rising trend to increasing leptin concentrations (p=0.002), and again no trend was observed in the AIS group (p=0.305).

Conclusion: The results in this study suggested that the osteoblasts isolated from AIS girls had low differentiation and mineralization potentials, as well as abnormally low functional responses to leptin when compared with controls. These decreases in functional responses might be due to dysfunction of leptin signaling pathway, which could include abnormalities in the leptin receptor or downstream signal molecules. This is an important finding and might serve to explain the low bone mass and deranged bone quality that is associated with AIS.

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BONY SPUR FORMATION AND DISCUSSION IN COLLAGEN-INDUCED ARTHRITIS RAT MODEL
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Introduction: A systemic chronic joint inflammation leads to profound changes in the joint architecture, which is the structural basis for progressive impairment of function. Radiographic features of RA are those of joint inflammation, periarthritis osteoporosis, uniform joint space loss, bone erosions, and soft-tissue swelling. Conversely, inflammatory joint destruction is sometimes accompanied by modeling of bony spurs, also termed osteophytes, which emerge at the joint margins in diseases, such as psoriatic arthritis and ankylosing spondylitis. The reason for the apparently divergent bone responses among various inflammatory diseases has not been fully clarified, but appears to involve differential regulation of local bone homeostasis in the course of joint inflammation. With the destruction of joint