for a surface delivery of biomolecules stimulating a cell behavior to accelerate an osteosynthesis on a metal surface [4, 5]. The surface nanotopography and the surface biomolecules encapsulation are inspired to create the lab-on-a-chip system and the bioconstuction on the basis of bone implant with a nanostructured TiO_2 coating and human stem cells and to culture tissues and organs in vitro [6]. Here, the aim of research is to investigate the surface nanotopography influence of the mesoporous coatings on the basis of TiO_2 on the viability and proliferation of human bone marrow-derived mesenchymal stem cells (MSCs). We focus on the mesoporous nanostructures, a pore diameter 40-70 nm. The order TiO_2 nanotubes films (TNT) and the disorder mesoporous TiO_2 films (TMS) were obtained by the methods of electrochemical anodization and sonochemical treatment, respectively. The chemical deposition of nanoscale hydroxyapatite (HA) was applied to modify the surface of nanostructured TiO_2 films with remaining their nanotopography (HA-TNT, HA-TMS). The viability and proliferation of MSCs were evaluated via a MTT-test in 4 days of incubation after nonattached cells had removed in 24 hours. To visualize the MSCs upon the mesoporous films on the basis of TiO_2 , the method of confocal laser scanning microscopy was used. Our research showed the MSCs kept the viability upon the TNT, TMS, HA-TNT, HA-TMS during a cultivation. Moreover, a specific cell density was higher upon the TNT, TMS, HA-TNT, HA-TMS than upon the smooth surfaces of cultured plastic and initial titanium. The proliferation of MSCs was determined higher upon the order TNT and HA-TNT compared with the disorder TMS and HA-TMS. Thus, for a MSCs expansion, the films having a symmetry surface nanotopography are more favorable. The deposition of nanoscale HA allowed to increase a MSCs number on the TNT and TMS. In conclusion, we proved that the strategy of surface nanostructuring to guide the MSCs behavior on the TiO_2 was attractive to improve orthopaedic implants and a lab-on-a-chip system development.

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Litvin Y.[1], Kovac M.[2], Aliev R.[2], Zakirova E.[1], Rutland C.[3], Kiyasov A.[1], Rizvanov A.[1]

[1] Kazan Federal University
[2] Moscow State Academy of Veterinary Medicine and Biotechnology
[3] School of Veterinary Medicine and Science, Faculty of Medicine, University of Nottingham
litvin108@yandex.ru

GENE THERAPY USING PLASMID DNA ENCODING VEGF164 AND FGF2 GENES FOR THE TREATMENT OF HORSE TENDINITIS AND DESMITIS: CASE REPORTS

Tendon or ligament injuries are one of the most common causes of orthopedic disorders in horses (Equus caballus) of any age and breed. Injuries of the digital flexor tendons (superficial and deep digital flexors) and the suspensory ligament are of utmost clinical importance in the horse resulting in more than 98% of all cases observed in practice. We have designed a plasmid DNA-based genetic construct (named pBUDK-ecVEGF164-ecFGF2) to restore damaged connective tissue of the tendon and ligament. Recombinant plasmid contains coding sequences of Equus caballus protein growth factors VEGF164 (also known as VEGFA164) and FGF2 (also known as bFGF). The generation of plasmid DNA pBUDK-ecVEGF164-ecFGF2 has been previously described in «doi:10.1007/s12668-016-0273-2». In this clinical study, for the first time we successfully used the direct gene therapy to restore severe injuries of the suspensory ligament branch and superficial digital flexor tendon in horses (Equus caballus). We injected the plasmid DNA encoding two therapeutic species-specific growth factors: vascular endothelial growth factor (VEGF164) and fibroblast growth factor 2 (FGF2) at the site of injury in the suspensory ligament branch and tendon. Treatment effects were evaluated with the use of clinical observation and ultrasound imaging during a period of a few months. We showed that gene therapy used within a period of 2-3 months after the injury resulted in the complete recovery of functions and a full restoration of the severely damaged suspensory ligament and superficial digital flexor tendon.

Lopatina T.[1], Grange C.[1], Cedrino M.[1], Ranghino A.[1], Favaro E.[1], Fallo S.[1], Tetta C.[1,2], Camussi G.[1]

[1] University of Turin
[2] UNICYTE
tatiana.lopatina@unito.it

PDGF ENHANCES THE PRO-REGENERATIVE PROPERTIES OF EXTRACELLULAR VESICLES RELEASED FORM ADIPOSE MESENCHYMAL STEM CELLS

INTRODUCTION: Adipose mesenchymal stem cells (ASCs) promote angiogenesis and tissue regeneration through paracrine mechanisms. We have previously shown that platelet derived growth factor (PDGF) stimulate ASCs to secret extracellular vesicles (PDGF-EVs) with a stronger pro-angiogenic potential than EVs secreted in basic conditions (EVs). The aim of the present study was to investigate the molecular mechanism involved in angiogenic, regenerative and immunomodulatory activity of PDGF-EVs. METHODS: For this purpose we studied in vitro the effects of PDGF-EVs on the secretion of inflammatory factors by peripheral blood mononuclear cells (PBMCs) as well as their influence on PBMC adhesion on endothelial cells. EVs were used for comparison. In vivo we have also studied the effects of EVs and PDGF-EVs in an acute limb ischemia pre-clinical model. The molecular differences between EVs and PDGF-EVs were also investigated. RESULTS: In vivo results demonstrate that PDGF-EVs was significantly more effective in restoring large vessel reperfusion and muscle tissue regeneration. More of these, PDGF-EVs inhibited inflammatory cell recruitment in injured tissue, then EVs stimulate immune cell infiltration. In vitro control EVs but not PDGF-EVs enhanced PBMC adhesion on endothelium, confirming our in vivo results. In addition, PDGF-EVs were able to stimulate nitric oxide production in endothelium cells that could be implicated in PBMC adhesion. Direct stimulation of PBMC with control EVs induced secre-