

Methods: We investigated calcium and vitamin D serum levels of 35 postmenopausal women. Lumbar scoliosis was assessed from DEXA scans, which was available for all of the women and was defined as Cobb angle $>10^\circ$. Women were divided into two groups-with scoliosis and without scoliosis and both calcium and vitamin D serum levels were assessed for the women in each group.

Results: The mean age of all postmenopausal women was 61 years \pm 9 SD (range 40-79 years). 8 of 35 women (22.8%) had scoliosis and 27 of 35 women (77.2%) were without scoliosis. Women with scoliosis had significantly lower vitamin D serum level (12.85nmol/l) compared to those without scoliosis (30.24nmol/l), ($p=0.000$). Calcium serum level (2.18 mmol/L) was also significantly lower in the women with scoliosis than those without scoliosis (2.38 mmol/L), ($p=0.020$).

Conclusion: Postmenopausal women with scoliosis showed lower calcium and vitamin D levels as compared to those without scoliosis.

doi:10.1016/j.bonr.2020.100369

P019

Bone neoformation in calvaria bone defects after the association of PVDF membrane and photobiomodulation therapy in ovariectomized rats: microtomographic evaluation

Fernanda Cristina Toloi Rufato^a, Luiz Gustavo Sousa^a, Priscilla Hakime Scalize^a, Marcio Mateus Beloti^a, Adriana Luisa Gonçalves Almeida^a, Rossano Gimenes^b, Adalberto Luiz Rosa^a, Karina Fittipaldi Bombonato-Prado^a, Simone Cecilio Hallak Regalo^a, Selma Siessere^a

^aUniversity of São Paulo - School of Dentistry of Ribeirão Preto, Ribeirão Preto, Brazil

^bFederal University of Itajubá, Itajubá, Brazil

Politetrafluoretilene membranes (PTFE) are considered the best for guided bone regeneration, but photobiomodulation therapy (PT) and poly(vinylidene fluoride)/PVDF membranes associated to barium titanate may represent good alternatives. This study evaluated bone architecture by means of computerized microtomography after the association of PT with PTFE and P(VDF-TrFE)/BT membranes in calvaria bone defects of ovariectomized rats. The experimental protocol was approved by Ethics Committee for Animal Experimentation of the University of São Paulo (n. 2018.1.417.58.0). Twenty Wistar Hannover rats (300g) were submitted to bilateral ovariectomy (OVX) and 5 just submitted to ovary exposition (Sham). After 90 days, there were performed 5-mm calvaria bone defects in all animals. Ovariectomized rats were distributed in the following groups: 1) OVX (n=5); 2) OVX + P(VDF-TrFE)/BT membrane (n=5); 3) OVX + P(VDF-TrFE)/BT membrane + PT (n=5) and 4) OVX + PTFE membrane + PT (n=5). There were performed 12 sessions with gallium-aluminum-arsenate diode (780 nm) laser with energy density of 30 J/cm². Thirty days after euthanasia, samples were fixed and analyzed by means of micro-CT SkyScan 1172 (SkyScan, Belgium) for bone volume (BV/mm³), bone surface (BS/mm²), trabecular number (TN/1/mm), trabecular thickness (TT/mm), trabecular separation (TS/mm) and connectivity density (CD/1/mm³). There was observed a significant increase in the values of BV, BS, TN and CD when comparing the groups 2, 3 e 4 with sham and group 1 ($p<0.05$). Groups 3 and 4 showed higher values of CD when compared to group 5. Trabecular separation was decreased in groups 3, 4 and 5 when compared to sham and group 1 ($p<0.05$). There were no statistical differences either between groups 3 and 4 or for trabecular thickness parameter. It is concluded that all treatments with the membranes improved bone architecture when compared to sham and OVX animals, regardless the association with photobiomodulation therapy.

Fapesp - 2017/25683-4

doi:10.1016/j.bonr.2020.100370

P020

Mesenchymal stromal cells' secretome enhances osteoclastogenesis but reduces multinucleated giant cells formation *in vitro*

Paul Humbert, Julien De Lima, Meadhbh Á. Brennan, Frédéric Blanchard, Pierre Layrolle

UMR 1238 - Phy-OS, INSERM, Université de Nantes, Nantes, France

Human Mesenchymal Stromal Cells (hMSCs), when transplanted in combination with Calcium Phosphate materials (CaPs), lead to effective fracture healing in patients. Studies in Nude mice showed that implanted hMSCs are not contributing directly to bone-matrix deposition as they die rapidly and that efficient bone formation by host cells was associated with early osteoclast formation. We hypothesized that hMSCs paracrine effect alleviates the foreign body reaction against the biomaterial, restraining Multi-Nucleated Giant Cells (MNGCs) formation to the benefit of osteoclasts, thus initiating locally a bone remodeling cycle.

We investigated *in vitro* if hMSCs could secrete pro-osteoclastogenic and/or anti-MNGCs factors, especially under apoptotic stress using Staurosporine (STS). Osteoclasts and MNGCs were differentiated from hCD14⁺ monocytes in the presence of Conditioned Media (CM) from hMSCs' culture. CM from 4 donors of hMSCs (untreated-CM, UNT-CM) consistently increased the size of osteoclasts by 20% while having no effect on MNGCs. After STS treatment of hMSCs, the CM obtained (STS-CM) significantly decreased the number of MNGCs by 20% (p -value <0.001 by paired t-test). Further RNA expression analysis in osteoclasts revealed that three major differentiation markers (*NFATC1*, *cathepsin K* and *calcitonin receptor*) were consistently up-regulated by STS-CM compared to UNT-CM from 5 different donors (p -value <0.05 by paired t-test). A proteomic analysis by LC-MS for 3 donors of hMSCs found 90 proteins enriched and 96 proteins depleted in the CM after STS treatment. A complementary multiplex immunoassay detected the overexpression of three cytokines (GRO α , PDGF-AA and IL-8) and the reduced expression of two (Eotaxin and MCP-1, p -value <0.05 by paired t-test). Additional tests are ongoing using neutralizing antibodies directed towards key cytokines or receptors based on these results.

This novel work supports the known immunomodulatory effect of hMSCs' secretome, suggests the impact of apoptosis and reinforces the hypothetical mechanisms of hMSC-CaP based bone regeneration through osteoclast recruitment.

doi:10.1016/j.bonr.2020.100371

P021

Glucose promotes transplanted human mesenchymal stem cell paracrine function pertinent to angiogenesis

Guotian Luo, Cyprien Denoëud, Nathanael Larochette, Esther Potier, Hervé Petite

Université de Paris, CNRS, INSERM, B3OA, Paris, France

Ecole Nationale Vétérinaire d'Alfort, B3OA, Maisons-Alfort, France

Objective: Massive cell death post-implantation is a roadblock for the use of human mesenchymal stem cell (hMSC) for cell-based regeneration of large bone defects. We previously established that glucose is instrumental for hMSC survival post-implantation. Because osteogenesis and angiogenesis are tightly coupled in the process of bone repair, we hereby investigate whether glucose affects hMSC-mediated angiogenic paracrine functions.

Methods: *In vitro* experiment: Release of bioactive factors and chemo-attractive potential of conditioned media (CM) from hMSCs towards human umbilical vein endothelial cells (HUVECs) were performed. The CM was obtained by exposure of hMSC to near-anoxia in the presence of glucose at 0, 1 or 5 g/L for 3 days.

In vivo experiment: hMSC pro-angiogenic potential was investigated using hMSC-containing hydrogels loaded with either 0,