

changes of valve interstitial cells (VIC). The mechanisms that trigger osteoblastic transformation in the heart valve are not well understood and only available treatment of CAVD is surgery.

Our previous data show higher sensitivity of VICs from the CAVD patients to osteogenic differentiation (OD) compared to healthy VICs. Here we analyzed VICs from healthy and diseased valves for their differences in osteogenic potential at (1) transcriptomics level at the early stages of differentiation (n=12) and (2) proteomics level at later stages (n=15). VICs were isolated from aortic valves of CAVD patients or from healthy hearts at transplantation, OD was induced by osteogenic medium; transcripts were analyzed 48h after the OD induction while proteomes were analyzed at the 10th day. Total RNA and total protein extracts were analyzed using RNA-seq and shotgun proteomics respectively.

All four comparison groups (control and differentiated VICs from healthy or diseased valves) formed distinct clusters in 3D PLS-DA based on both data (38% and 37% of explained variance for transcriptomics and proteomics data respectively). We identified key molecular markers of osteogenic differentiation in both VICs from healthy and diseased valves after the induction of OD ($P < 0.05$, $FC > 2$). Nevertheless, we observed differential patterns of protein and transcript changes during OD of VICs from diseased or healthy donors. The difference included proteins involved in OD: IGFBP-5 and components of HIF-1 signal pathway (HIF1A, VEGFA, EGLN3; $p < 0.05$).

The data suggest intrinsic differences in sensitivity to proosteogenic stimuli in the aortic valve interstitial cells of healthy persons and of the patients with CAVD.

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ND-P01

Microstructural effects of collagen membrane from bovine pericardium in bone defects

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Techgraft® is a membrane of collagen derived from bovine pericardium produced by Baumer® SA and could be a great alternative biomaterial to application in guided bone regeneration. Therefore, the purpose of this study was to evaluate its microstructural effects in regeneration of bone defect in rat tibia.

Methods: It was an experimental in vivo study approved by the Ethics Committee on Animal Experiments of the University of São Paulo (188/2017). Eighty male Sprague Dawley rats were submitted to surgical procedure to create a bone defect on tibia. Thus, animals were assigned into four groups according treatments: without intervention (CONT), autograft (AG), collagen membrane (CM) and collagen membrane and autograft (CM+AG). Groups were subdivided in two subgroups (n=10) according experimental time (21 and 42 days). In sequence, tibiae were collected and micro-ct analysis was carried out. Data were analyzed using ANOVA test, adopting a 5% significance level.

Results: The membrane induced bone formation in proximity of membrane, in top levels, and increased trabecular appearance of newly formed bone, while autograft groups showed more bone in bottom of defect. BV/TV was lower in CM group compared to other treated groups ($p < 0.001$), but all treated groups, including CM, had higher BV/TV than control group ($p < 0.005$) at 21 days. At 42 days just CM+AG group showed difference of CONT ($p = 0.021$). At 21 days AG group had increased trabecular thickness compared to other groups ($p < 0.05$); trabecular number was higher in CM+AG

group than other groups ($p < 0.05$) and higher in CM group than CONT ($p < 0.001$); trabecular separation was higher in CONT group than treated groups ($p < 0.05$). At 42 days only trabecular number showed significance difference between CM+AG and CONT group ($p = 0.014$).

Conclusion: The treatment of defects with membrane was positive, but more effective when associated to autograft.

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ND-P02

Coated latex membrane with calcium β-triphosphate in bone healing of tibia of osteopenic rats

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Bone repair in osteoporotic bones is complicated due to the poor bone quality. Therefore, it is essential to investigate strategies to improve this process. The aim of this study was to evaluate the influence of the latex membrane coated with calcium β-triphosphate in the tissue repair of bone defect performed in the tibia of osteopenic rats, using analysis of bone mineral density (BMD) and mechanical tests. This study was approved by the ethics committee on animal testing of the Medical School of Ribeirão Preto under the Protocol 094/2017. In this study, 40 Sprague-Dawley rats were used. Osteopenia was induced in all animals through ovariectomy and a vitamin D-deficient diet, except in animals of the sham surgery group. After 90 days of ovariectomy, a bone defect of 2.5 mm in diameter was made in the distal third of the animals' left tibia. The defects received the following treatments per group (n = 8): C and SHAM: without specific treatment; L: covered with latex membrane; CBT: filled with calcium β-triphosphate; CBTL: covered with latex membrane coated with calcium β-triphosphate. After 14 days, the animals were killed, and the left tibiae were designated for analysis. BMD was evaluated at the defect site, and the mechanical properties of maximum strength and stiffness were obtained through the mechanical shear test. There was a statistically significant difference for BMD with $p = 0.005$. The CBT (0.141 ± 0.027), L (0.147 ± 0.0306) and SHAM (0.178 ± 0.017) groups, were the same as each other and presented the highest values of BMD, being different from C (0.113 ± 0.030). The CBTL (0.137 ± 0.023) group had intermediate values. For maximum strength and relative stiffness, there were no statistical differences between groups. In conclusion, natural latex and β-TCP improved BMD at the site of the bone defect, but more analysis is needed to obtain conclusive results.

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ND-P03

Effects of electroacupuncture on bone defect regeneration in tibiae of ovariectomized rats

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Background: Osteoporosis is an osteometabolic disease characterized by low bone mass, deterioration of bone tissue microarchitecture,

delayed bone regeneration, and increased risk of fractures. The use of electroacupuncture (EA) for postmenopausal osteoporosis treatment is recent and has been positive for bone quality improvement.

Objective: To verify the effects of EA on the regeneration process in the bone defect in the tibias of ovariectomized rats.

Methods: 48 female Sprague-Dawley rats (aged six weeks) were subdivided into four groups (n=12): OVXDEA: ovariectomy (OVX) + bone defect. + EA; OVXD: OVX + bone defect, without EA; SDEA: SHAM surgery + bone defect + EA; SD: SHAM surgery + bone defect, without EA. OVX surgery was performed. After 90 days, the tibial bone defect was performed bilaterally. EA protocol started after 24h of the bone defect, and used the Zusanli (ST36) Sanyinjiao (SP6) acupoints. Therapy occurred once a day for 20 minutes, for three cycles of 10 days, with one day intervals between them. After euthanasia, bone microarchitecture evaluation by computed bone microtomography (Micro-CT) was performed. Statistical significance between groups were tested using analysis of variance (ANOVA); (P< 0.05 was considered statistically significant). The OVXD group had lower values for micro-CT, being statistically significant.

Conclusions: It can be concluded that EA may present the improvement of bone microarchitecture in the bone defect model in osteopenic tibias. Further studies in this area are suggested.

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ND-P04

A novel mouse model to study fracture healing at the proximal femur

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The majority of fractures especially in elderly and osteoporotic patients occurs in metaphyseal bone due to the susceptibility of trabecular bone to microstructural damage. While these injuries are important from a clinical standpoint, adequate small animal models to study them are lacking. Therefore, the aim of the current study was to develop a novel mouse model to study metaphyseal fracture healing at the proximal femur. 12 weeks old female C57BL/6J mice were used for the study (n=6 per group; p< 0.05). We successfully combined an open osteotomy approach to the proximal femur with a closed approach for intramedullary stabilization. No animals were lost due to surgical issues or anesthesia. All animals displayed normal limb loading and a physiological gait pattern within the first three days after fracture. μ CT analysis revealed successful implementation of the osteotomy between the lesser and the third trochanter in all animals. Bony bridging score increased significantly between d14 and d21 (0.2 vs. 3.5). Bone volume ratio also increased significantly between d14 and d21. Total callus volume decreased significantly between d14 and d21. Histomorphometric analysis of Safranin O-stained sections revealed that all fractured healed via endochondral ossification, whereas relative amount of cartilage decreased and relative amount of bone increased between d14 and d21. All fracture calluses at d21 displayed less than 10% of cartilage tissue, indicating successful cartilage-to-bone transition between d14 and d21. TRAP staining showed high osteoclast abundance and activity at the rims of the fracture callus at d14 and throughout the whole fracture callus at d21 after fracture, indicating that fracture callus remodelling has already started at d21 after fracture. Our novel model provides a fast,

reliable and inexpensive way to study metaphyseal fracture healing in mice. Future studies using osteoporotic mice might help to unravel molecular mechanisms of delayed osteoporotic fracture healing.

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ND-P05

Effects of the escitalopram oxalate on densitometric parameters at the intact and bone callus in growing and young adult rats

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Objective: to assess the effect of the escitalopram oxalate intake on densitometric analysis at the intact femur and at the fracture callus in growing and young adult rats.

Methods: Four-week-old and 8-week-old Hannover rats (n=28) were distributed into four groups: GP: growing and placebo; AP: adult and placebo; GE: growing and escitalopram; and AE: adult and escitalopram. Daily administration of 2.0 mg/kg of escitalopram (or saline solution) were orally administered for 35 days. Additionally, a fracture at the right femur was produced in all animals on day 21. Densitometric analysis (BMD and BMC) was performed at the distal metaphysis and at the neck of the intact femur, and in the whole bone callus. Analysis of variance with Bonferroni adjustment was made for comparisons (p<0.05).

Results: both the BMD at the distal femur (p=0.039) and BMC femoral neck (p=0.043) were higher in adult than growing animals. The drug-treated growing and young adult animals showed significantly lower BMC (p=0.042) and BMD (p=0.027) at the distal femur, which infer a negative effect of the drug on bone mass. This decrease in bone density did not differ among immature and mature animals (p=0.207). Conversely, the escitalopram oxalate intake did not affect the callus density in either group (p=0.184).

Conclusion: the escitalopram oxalate administration equally impaired bone density at the intact femur both in immature and mature animals. However, bone callus density remained unchanged with the pharmacological agent.

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ND-P06

Different prostate cancer bone metastasis models respond differently to treadmill exercise

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Background: Prostate cancer (PCa) is a leading cause of death in men with a predilection to metastasize into bone, when the disease is considered to be incurable. Exercise has been suggested to improve the health of patients with PCa but no current studies on its effects on PCa bone metastasis.

Hypothesis: Treadmill exercise can prevent the progression of PCa bone metastasis.

Methods: Human xenograft PCa cell line PC3 and murine syngeneic RM1-BM cells were intracardiacally injected (~1x10 cells/injection) into BALB/c nude (n=8) and C57BL/6J mice (n=12),