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SBMS – Swiss Bone and Mineral Society
17th Annual Meeting, Bern, April 7, 2011

Clinical and Preclinical Abstracts

IVA in addition to BMD can change the osteoporosis management in 25 % of clinical routine patients

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Introduction Vertebral fracture is one of the major osteoporotic fractures which are unfortunately very often undetected. In addition, it is well known that prevalent vertebral fracture increases dramatically the risk of future additional fracture. Instant Vertebral Assessment (IVA) has been introduced in DXA device a couple of years ago to ease the detection of such fracture when routine DXA are performed. To correctly use such tool, ISCD provided clinical recommendation on when and how to use it. The aim of our study was to evaluate the ISCD guidelines in clinical routine patients and see how often it may change of patient management.

Methods During two months (March and April 2010), a medical questionnaire was systematically given to our clinical routine patient to check the validity of ISCD IVA recommendations in our population. In addition, all women had BMD measurement at AP spine, femur and 1/3 radius using a Discovery A System (Hologic, Waltham, USA). When appropriate, IVA measurement had been performed on the same DXA system and had been centrally evaluated by two trained doctors for fracture status according to the semi-quantitative method of Genant. The reading had been performed when possible between L5 and T4.

Results Out of 210 women seen in the consultation, 109 (52 %) of them (mean age 68.2 ± 11.5 years) fulfilled the necessary criteria to have an IVA measurement. Out of these 109 women, 43 (incidence 39.4 %) had osteoporosis at one of the three skeletal sites and 31 (incidence 28.4 %) had at least one vertebral fracture. 14.7 % of women had both osteoporosis and at least one vertebral fracture classifying them as "severe osteoporosis" while 46.8 % did not have osteoporosis and no vertebral fracture. 24.8 % of the women had osteoporosis but no vertebral fracture while 13.8 % of women did have osteoporosis but vertebral fracture (clinical osteoporosis).

Conclusions In 52 % of our patients, IVA was needed according to ISCD criteria. In half of them the IVA test influenced of patient management either may changing the type of treatment of simply by classifying patient as "clinical osteoporosis". IVA appears to be an important tool in clinical routine but unfortunately is not yet very often use in most of the centers.

Previous fractures in healthy adolescent boys are associated with reduced bone strength as assessed by finite-element analysis at weight-bearing skeletal site

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In healthy children and adolescent boys, fractures result from trauma of various severity. Whether these fractures are characterized by an intrinsic bone biomechanical fragility remains to be demonstrated. We reported in a cohort of 176 healthy adolescent boys prospectively followed from age 7.5 ± 0.5 to 15.2 ± 0.5 yrs that fracture history (156 fractures recorded in 87/176 boys) was associated with decreased femoral neck (FN), areal (a) bone mineral density BMD (–6.0 %, p = 0.005) measured by dual-energy x-ray absorptiometry (DXA) and lower distal tibia trabecular volumetric (v) density (–5.5 %, p = 0.029) and number (–4.2 %, p = 0.040) as determined by high resolution peripheral computerized tomography (HR-pQCT). In the present study, we assessed to which extent this lower trabecular microstructure in the distal tibial among boys with previous fractures was associated with reduced bone strength variables evaluated by micro-finite element analysis (μFEA) based on HR-pQCT measurements. Associations between FN aBMD, distal tibia microstructure and bone strength estimates, and fracture status were evaluated by univariate logistic regression and expressed as odds ratio (OR [95 % CI]) per 1 SD decrease. As compared to those without fractures, boys with fractures had a 5.8 % lower bone strength of the distal tibia as estimated by stiffness (245 vs. 260 kN/mm, p = 0.024) and failure load (11706 vs. 12430 N, p = 0.016), after adjustment for age, standing height, body weight, pubertal stage, calcium and protein intakes, physical activity, and calcium supplement or calcium randomization between age 7.5 and 8.5 yrs. At the distal tibia, the adjusted ORs (95% CI) for fracture per 1 SD decrease were as follows: stiffness 1.53 (0.96–2.44, p = 0.072); failure load 1.60 (0.99–2.60, p = 0.056); trabecular density 1.46 (1.02–2.09, p = 0.038); trabecular number 1.59 (1.04–2.42, p = 0.031). The corresponding fracture OR for FN aBMD was: 1.80 (95 % CI 1.18–2.76, p = 0.006).

In conclusion, although trauma plays a non-negligeable role in the occurrence of fractures in healthy children and adolescent boys, our study provides evidence for an intrinsic component of skeletal fragility as assessed by μFEA at weight-bearing site. The measured biomechanical deficit at distal tibia corroborates indirect estimates of fracture risk by assessing macro- and microstructural components of bone mineral density and architecture.

A randomized controlled trial of music-based multitask training to improve gait, balance and reduce fall risk in the elderly

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Background Fracture prevention aims at reducing both bone loss and fall risk. Falls occur mainly while walking or performing concurrent tasks. Measures to reduce falls are often complex and of limited benefits. We aimed to determine whether a music-based multitask training could improve gait, balance, and reduce fall risk in elderly people.

Methods We conducted a randomized controlled trial involving 134 community-dwelling individuals aged 65 years or older (76 ± 7 years), who were identified at increased risk of falling. They were randomly assigned to an intervention group (n = 66) or a delayed intervention control group scheduled to start the program 6 months later (n = 68). The intervention was a structured program of weekly 1-hour group exercise classes featuring various multitask exercises performed to the rhythm of improvised piano music (i.e., Jaques-Dalcroze eurhythmics). Change in gait variability under dual-task condition from baseline to 6 months was the primary end point. Secondary end points included changes in gait, balance and functional tests performances. Both groups were assessed at baseline and months 6 and 12. Falls during follow-up were assessed using a monthly calendar method with daily records.

Results In an intent-to-treat analysis, at 6 months, there was a reduction in stride length variability (adjusted mean difference, –1.4 %; 95 %CI, –2.3 to –0.6; p = .002) under dual-task condition in the intervention group, compared with the delayed intervention control group. Usual gait speed, balance and functional tests improved compared with the control group. There were fewer falls in

the intervention group (incidence rate ratio, 0.46; 95 %CI, 0.27 to 0.79; p = .005) and a lower risk of falling (relative risk, 0.61; 95 %CI, 0.39 to 0.96; p = .02). Similar changes occurred in the delayed intervention control group during the second 6-month period with intervention. Benefit on gait variability measured in dual-task condition persisted for 6 months after the program had ended.

Conclusions These findings indicate that 6-month participation in music-based multitask exercises classes once a week, can improve gait performance under both single and dual-task conditions, as well as balance, and reduce both the rate of falls and the risk of falling in at-risk community-dwelling older adults.

Areal bone mineral density at distal tibia predicts microstructure assessed by HR-pQCT

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Background The predictive value of areal bone mineral density (aBMD) at the distal tibia regarding fracture risk has been demonstrated in a population-based cohort of elderly women [Popp AW et al. Osteoporos Int 2009]. While the adjusted hazard ratio of clinical fractures was similar for the tibial diaphysis (T-DIA) and epiphysis (T-EPI), the T-scores in both sub-regions were different: –1.6 SD at T-DIA versus –2.4 SD at T-EPI. As mechanical properties of the bone are also determined by its micro-architecture, the aim of the current study was to evaluate the ability of DXA to predict microstructural parameters at the distal tibia.

Methods Cadaveric tibiae from female donors were scanned with DXA (Hologic QDR 4500 A™, Hologic, Bedford, MA, USA) and high-resolution peripheral quantitative computed tomography (HR-pQCT, XtremeCT™ Scanco Medical, Brüttisellen, Switzerland). T-DIA and T-EPI were considered and parameters of interest were aBMD, volumetric BMD, mean cortical thickness (CtTh), trabecular number (TbN) and trabecular spacing (TbSp) assessed by the distance-transform method, inner trabecular density (Dinn) defined as the inner 60 % and meta trabecular density (Dmeta) defined as the outer 40 % of the trabecular region, and bone volume to total volume ratio (BV/TV).

Results Sixty tibias from 35 female donors were included in this study, corresponding to 25 pairs and 10 single tibias. The mean (± SD) age of the donors was 78.5 ± 9.3 yr with mean height of 160.1 ± 7.8 cm and mean weight of 57.2 ± 11.4 kg. T-scores for T-DIA and T-EPI were –2.0 SD and –2.3 SD, respectively. No side-to-side differences were found for the paired tibias (p > 0.05). aBMD correlated highly with CtTh at T-DIA and substantially with trabecular parameters at T-EPI according to the **Table 1**.

Conclusions At the distal tibia, aBMD seems to reflect the microarchitecture assessed by HR-pQCT with respect to cortical and trabecular bone. Based on these results, T-DIA could apply as a region of interest to investigate the relation between cortex and fracture risk.

A single high dose of oral vitamin D3 is insufficient to correct a deficiency in a rheumatologic population

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Introduction Vitamin D plays a major role in bone metabolism and neuromuscular function. Supplementation with vitamin D is effective to reduce the risk of fall and of fracture. However adherence to oral daily vitamin D is low. Screening and correcting vitamin D insufficiency in a rheumatologic population could improve both morbidity and quality of life. After determining the prevalence of vitamin D deficiency in this population, we evaluated if supplementation with a single high dose of oral 25-OH vitamin D3 was sufficient to correct this abnormality.

Methods During one month (November 2009), levels of 25-OH vitamin D were systematically determined in our rheumatology outpatient clinic and classified in: vitamin D deficiency (< 10 µg/l), vitamin D insufficiency (10 to 30 µg/l) or normal vitamin D (> 30 µg/l). Patients with insufficiency or deficiency received respectively a single high dose of 300'000 IU or 600'000 IU oral vitamin D3. In addition, all patients with osteoporosis were prescribed daily supplement of calcium (1 g) and vitamin D (800 IU). 25-OH vitamin D levels were reevaluated after 3 months.

Results Vitamin D levels were initially determined in 292 patients (mean age 53, 211 women, 87 % Caucasian). 77 % had inflammatory rheumatologic disease (IRD), 20 % osteoporosis (OP) and 12 % degenerative disease (DD). Vitamin D deficiency was present in 20 (6.8 %), while 225 (77.1 %) had insufficiency. Of the 245 patients with levels < 30µg/l, a new determination of vitamin D level was available in 173 (71 %) at 3 months.

Conclusion Vitamin D insufficiency is highly prevalent in our rheumatologic population (84 %), and is not adequately corrected by a single high dose of oral vitamin D3 in > 50 % of the patients with IRD and DD. In patients with OP, despite association of a single high dose with daily oral vitamin D supplementation, 40 % of patients are still deficient when reevaluated at 3 months.

Systemic treatment with strontium ranelate markedly improves the healing of critical bone defect

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Rapid bone defect filling with normal bone is a challenge in orthopedics and dentistry. Systemic treatment with antiosteoporotic agent able to stimulate bone formation may be potentially useful. Healing of a critical bone defect is a model associating a phase of bone resorption and of bone formation. Strontium ranelate which has been shown to decrease bone resorption and to positively influence bone formation, represents a potential agent able to stimulate bone defect filling. To further explore this question, we set up a model of critical bone defect performed at the level of the rat proximal tibia. A drilling of 2.5 mm in diameter was created in the secondary spongiosa in 6 month-old female rats which were given strontium ranelate (625 mg/kg/d, 5/7 days) or vehicle for 4, 8 or 12 weeks (10 rats per group and per time point) starting at the moment of the surgery. The tibias were removed for micro-tomographic histomorphometry at the level of the healing bone defect at each time point. All results are expressed as means ± SEM. One-way ANOVA with a Fisher post-test was used to analyze the data (**Table 2**).

Strontium ranelate treatment induced an early increase of trabecular bone mass already visible by 4 weeks. This was associated with improvement of the microarchitecture with a significant thickening of the trabeculae visible after 4 weeks of treatment and increasing

Table 1. Results, Popp A W et al.

R^2	vBMD	CtTh	TbN	TbSp	Dinn	Dmeta	BV/TV
aBMD/T-DIA	0.846[a]	0.922a	0.090	0.073	0.029	0.001	0.001
aBMD/T-EPI	0.846[a]	0.462[a]	0.504[a]	0.410[a]	0.593[a]	0.672[a]	0.656[a]

[a]p < 0.01

Table 2. Results, Zacchetti G et al.

Trabecular	Weeks	4	8	12
Bone volume/ total volume (%)	Controls	10.3 ± 3.0	4.8 ± 1.3	7.8 ± 2.0
	Strontium ranelate	17.7 ± 3.5	16.3 ± 2.4**	20.9 ± 2.8**
Thickness (mm)	Controls	0.066 ± 0.005	0.062 ± 0.003	0.064 ± 0.005
	Strontium ranelate	0.075 ± 0.005	0.081 ± 0.002**	0.082 ± 0.002**
SMI	Controls	2.78 ± 0.27	3.30 ± 0.24	2.88 ± 0.30
	Strontium ranelate	2.25 ± 0.28	2.14 ± 0.13**	1.91 ± 0.23**

** p < 0.01 vs time-control

progressively, illustrating the potential benefit of strontium ranelate on bone formation. Finally as evaluated by SMI (3 = rod like, 1 = plate like) trabeculae are more plate-like (optimal structure for mechanical resistance) in strontium ranelate treated rats than in control. Strontium ranelate represents a potential intervention to accelerate and enhance the filling of a bone defect, with potential advantages in dental or orthopedic surgery for bone healing after tooth extraction and for implant osseointegration.

Basic Science

TNFα mediated osteoblastic inhibition of osteoclast development is caused by a block in monocyte differentiation

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Background Osteoclasts are bone resorbing multinucleated giant cells. Previously, we demonstrated that TNFα inhibits the development of osteoclasts in vitro through osteoblast-mediated mechanisms, which were found to include Granulocyte-Macrophage-Colony-Stimulating-Factor (GM-CSF). Within this study, the molecular mechanism(s) of the inhibition of osteoclastogenesis mediated by TNFα and the differentiation of haematopoietic progenitor cells were investigated.

Methods Expression of haematopoietic cell surface markers (RANK, c-kit, c-fms, CD11c, F4/80) during the development of osteoclasts was investigated by FACS analysis in cultures of CSF-1 dependent non-adherent osteoclast precursor cells (OPC) grown with CSF-1 (30 ng/ml) and RANKL (20 ng/ml). Levels of transcripts encoding RANK, c-fms and NHA2 in cultures of OPC were assessed by RT-PCR. Conditioned media (CM) were generated from primary osteoblasts by collecting cell supernatants from cultures of cells treated with TNFα/1,25$(OH)_2D_3$ after 72h.

Results FACS analysis revealed that in cultures of OPC, grown with 10 % CM from wt osteoblasts treated with TNFα/1,25$(OH)_2D_3$, RANK and CD11c were no longer expressed. CM from GM-$CSF^{-/-}$ osteoblasts did not block the expression of RANK, the cultures, however, were negative for CD11c. Levels of F4/80 and c-fms increased over time and no differences between CM from control and treated cell cultures were observed. Levels of transcripts encoding RANK were reduced by 50 % after 24 h and 75 % after 48 h when the cells were grown with CM from wt osteoblasts, but only by 20 % and 40 %, respectively, when cells were grown with CM from GM-$CSF^{-/-}$ cells. Levels of transcripts encoding c-fms were not changed in these cultures.

Conclusion The reduction of RANK expression demonstrates that the prevention of RANK-RANKL signalling is, at least in part, responsible for the inhibition of osteoclastogenesis by TNFα and that the cells are maintained in an undifferentiated state rather than being diverted to become dendritic cells or granulocytes.

IL-17 alone in vitro supports osteoclastogenesis but inhibits 1-25$(OH)_2$ D_3 mediated development of osteoclasts

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Background IL-17 is a cytokine secreted by the Th17 subset of T-cells. Suda et al. [J Clin Invest 1999; 103: 1345] reported increased IL-17 levels in the synovial fluids of patients suffering from Rheumatoid Arthritis. In vitro IL-17 was shown to increase osteoclast formation in a dose-dependent manner. The detailed role of IL-17 in osteoclast development, however, remains to be elucidated. In the present study the regulation of osteoclastogenesis by IL-17 was investigated.

Methods Osteoclast development was studied in co-cultures of murine osteoblasts (ddY mice) and bone marrow cells (BMC) from C57Bl/6J mice and in cultures of CSF-1 dependent non-adherent osteoclast progenitor cells (OPC). Cells were maintained in 48-well plates with 10 nM 1-25$(OH)_2$ D_3 and IL-17 (0.1 ng/ml to 10 ng/ml) alone and in combination both in low-density co-cultures (4×10^3 osteoblasts and 6×10^4 BMC) and high-density co-cultures (2×10^4 osteoblasts and 5×10^5 BMC). Levels of mRNAs encoding CSF-1, RANKL, OPG, RANK, c-fms, calcitonin receptor (CTR) and sodium-hydrogen exchanger NHA2 were determined in co-cultures.

Results IL-17 alone induced osteoclast formation in the absence of 1-25$(OH)_2$ D_3 only in high density co-cultures but did not affect the formation of osteoclasts in cultures of OPC. However, in the presence of 1-25$(OH)_2$ D_3 IL-17 caused a decrease in the number of TRAP-positive cells in low and high density co-cultures. At 50 ng/ml IL-17 abrogated 1-25$(OH)_2$ D_3 mediated osteoclastogenesis by 80 %. Furthermore, gene-expression studies revealed a decrease in the expression of RANK, CTR and NHA2 mRNA levels in high and low density co-cultures in the presence of IL-17 and 1-25$(OH)_2$ D_3.

Conclusions The present data shows that IL-17 supports osteoclastogenesis by replacing 1-25$(OH)_2$ D_3 in high-density co-cultures. IL-17 does not exert its effects directly on hematopoietic precursors of osteoclasts but mediates its effects via osteoblasts. IL-17 may be capable of modulating the culture conditions favouring osteoclastogenesis in the absence of 1-25$(OH)_2$ D_3 and inhibiting the development of osteoclasts in osteoclastogenic conditions.

PPAR beta-deficiency impairs muscle and skeletal response to exercise

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Background PPARβ is expressed in skeletal muscle and bone, and promotes fatty acid oxidation in response to exercise. We found that PPARβ-deficient mice have reduced muscle and bone mass and altered bone microarchitecture, which worsen with age. We investigated the influence of PPARβ on muscle and skeletal responses to exercise.

Methods Bone mass and microarchitecture were monitored in female $PPAR\beta^{-/-}$ and $PPAR\beta^{+/+}$ mice from 16 to 21 weeks of age. Mice were subjected to moderate treadmill exercise (EXE) or untrained (UN). Relative expression of PPARβ and γ mRNA (normalized for GAPDH) was evaluated in gastrocnemius and femurs by qRT-PCR.

Results In UN $PPAR\beta^{+/+}$ mice, PPARβ mRNA was more abundantly expressed than PPARγ in muscle (+65 % $p < 0.001$), but less than PPARγ in bone (–49 %, $p < 0.001$). Exercise modestly increased PPARβ expression (+16 %, ns and +38 % $p < 0.05$ vs UN, respectively in muscle and bone) in $PPAR\beta^{+/+}$ mice, whereas PPARγ mRNA remained unchanged. In contrast, in $PPAR\beta^{-/-}$, exercise significantly increased PPARγ expression in muscle and bone (+90 % and +40 %, respectively, vs UN, both $p < 0.05$). Compared to $PPAR\beta^{+/+}$, $PPAR\beta^{-/-}$ mice had lower maximal speed and capacity to run a long distance. However, $PPAR\beta^{-/-}$ were able to perform the moderate exercise in full. In $PPAR\beta^{+/+}$ mice, EXE significantly increased total body (TB) lean mass (21.7 ± 0.8 vs 19.3 ± 0.3 g in UN, $p < 0.05$), femoral BMD (75 ± 1 vs 70 ± 0.4 mg/cm² in UN, $p < 0.01$), and tibial BMD (54 ± 1 vs 50 ± 0.5 mg/cm² in UN, $p < 0.01$). TB fat did not change significantly in response to exercise. In these mice, EXE increased trabecular BV/TV and number (TbN) at the distal femur (+121 % and +12.6 %, respectively vs UN, all $p < 0.05$), and similarly at the tibia. It also increased cortical bone volume (Ct.BV, 0.40 ± 0.01 vs 0.36 ± 0.01 mm^3 in UN, $p < 0.05$) and thickness (Ct.Th, 239 ± 9 vs 216 ± 3 µm in UN, $p < 0.05$) at the tibia midshaft. In contrast, in $PPAR\beta^{-/-}$ mice, EXE had no effect on TB lean mass, BMD, TbN, Ct.BV or Ct.Th either in the femur or tibia. Hence we observed a significant interaction (Pinter < 0.05 by 2F_ANOVA) between genotype and EXE/UN on these parameters.

Conclusions These results identify PPARβ as an important factor for the muscle and skeletal response to exercise. In absence of PPARβ, upregulation of PPARγ, particularly in bone, could further contribute to the lack of skeletal anabolic response to exercise. Whether PPARβ regulates bone modeling/remodeling through its effects on muscle and/or on bone cells is currently being investigated.

Pharmacological inhibition of interleukin-15 prevents colitis and associated bone loss in IL-10 knockout mice

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Bone loss secondary to inflammatory bowel diseases (IBD) is largely explained by activated T cells producing cytokines that trigger osteoclastogenesis and accelerate bone resorption while inhibiting bone formation. In IBD, elevated expression of interleukin (IL) 15, a T cell growth factor, plays a central role in T cell activation, pro-inflammatory cytokine production and the development of colitis. We previously reported that IL-15 enhances RANKL-induced osteoclastogenesis and that an IL-15 antagonist, CRB-15, prevents weight and bone loss in a mouse model of dextran sulfate sodium-induced colitis. We hypothesized that inhibition of IL-15 signaling might prevent bone loss in IL-10 deficient mice, that develop spontaneous bowel inflammation associated with osteopenia when they are no longer raised under germ-free conditions. Mice received an IL-15 antagonist (CRB-15,5 µg/day, n = 5) or IgG2a (5 µg/day, n = 4) from week 10 to 14 of age. The severity of colitis was assessed by histology and bowel cytokines gene expression by real time PCR. Bone mass and architecture were evaluated by ex vivo DXA on femur and micro-computed tomography on femur and vertebra. Body weight gain was similar in the two groups. After 4 weeks, colon was 29 % shorter in CRB-15 treated mice ($p < 0.006$), a sign of reduced inflammation. Histological analysis indicated a transmural infiltration of inflammatory cells, lymphoepithelial lesions and increased size of villi (histological score = 4/6) in IgG2a treated mice, whereas colon from CRB-15 treated mice exhibited mild infiltration of inflammatory cells of the lamina propria, no mucosal damages and a minimal increased size of villi (histological score = 1.6/6). Levels of TNFα, IL-17 and IL-6 mRNA in the colon were significantly reduced in CRB-15 treated mice ($p < 0.04$ vs IgG2), indicating a decrease in colon inflammation. CRB-15 improved femur BMD (+10.6% vs IgG2a, $p < 0.002$), vertebral trabecular bone volume fraction (BV/TV, +19.7% vs IgG2a, $p < 0.05$) and thickness (+11.6 % vs IgG2a, $p < 0.02$). A modest but not significant increase in trabecular BV/TV was observed at the distal femur. Cortical thickness was also higher at the midshaft femur in CRB-15 treated mice (+8.3 % vs IgG2a, $p < 0.02$). In conclusion, we confirm and extend our results about the effects of CRB-15 in colitis. Antagonizing IL-15 may exert favorable effects on intestinal inflammation and prevent bone loss and microarchitecture alterations induced by colitis.

Characterization of osteoprogenitors functionally isolated from human mesenchymal stem cells by a Runx2 reporter adenovirus

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Background Mesenchymal stem cells (MSC) are a heterogeneous cell population characterized by their self-renewal capability and their multidifferentiation potential. Current isolation methods of MSCs are still rudimentary due to the lack of a unique marker. Here, we report a novel method for the identification and isolation of osteoprogenitors from human MSCs, along with the characterization of the resulting cell populations. A subpopulation of MSCs was functionally identified and isolated by coupling the expression of the key osteogenic transcription factor Runx2 to the expression of enhanced green fluorescent protein (EGFP) via a Runx2 reporter adenovirus. On that basis, fluorescing cells can be selected by means of fluorescence activated cell sorting (FACS).

Materials and methods MSCs were obtained from bone marrow aspirates by Ficoll separation and cell attachment to plastic. MSCs were expanded in the presence of bFGF. Cells were infected with the Runx2 reporter adenovirus. High efficiency transduction of MSCs was achieved using lanthofection at 100 MOI. Cells were then subjected to osteogenic induction for 3 days and sorted by means of FACS. The resulting cell populations, namely Runx2 GFP^+, Runx2 GFP^-, and the unsorted cells, were separately expanded in the presence of bFGF, and thereafter subjected to comparative in vitro investigation for their ability to differentiate into the osteogenic lineage. To substantiate the characterization of the cell populations, proliferative capacity of the cell populations was also assessed. Osteogenic differentiation was evaluated by alkaline phosphatase (ALP) activity at d7, 14, and 21, as well as ^{45}Ca incorporation at d21.

Results Colony forming unit (CFU) analysis at d14 revealed that Runx2 GFP^+ cells proliferate at a slower rate than the other two groups. This suggests that Runx2 GFP^+ cells show a more committed/differentiated phenotype than the other two cell populations. ALP activity of Runx2 GFP^+ cells was shifted towards earlier timepoints, showing highest ALP activity at d7 as opposed to d14 for the other two groups. ^{45}Ca incorporation was massively higher for osteogenically differentiated Runx2 GFP^+ cells than for the other cell populations treated with the same medium. Results of both assays are in accordance with each other, indicating that Runx2 GFP^+ cells are more osteogenic than Runx2 GFP^- and unsorted cells.

Conclusion We have made use of a Runx2 reporter adenovirus to sub-divide human MSCs. Reporter-positive subpopulation displays characteristics appropriate for osteoprogenitors: (1) a slower proliferation rate, and (2) a more osteoblast-like phenotype upon in vitro osteogenic differentiation, as compared with reporter-negative as well as original cell population.

In-vitro prevascularisation of a 3D scaffold using autologous endothelial progenitor- and mesenchymal stem cells

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Background Angiogenesis is a key factor in early stages of wound healing and is also crucial for tissue regeneration. In cases of large bone defects, to date most of the efforts have been focused on the filling of the gap with autologous bone grafts, or various bio-active materials associated or not with bone forming cells. However, the neo-vascularisation of such implants is still a limiting factor. The aim of the present study is to develop a pre-vascularised hybrid bone implant made of a polyurethane scaffold seeded with autologous cells; Endothelial Progenitor Cells (EPC) and Bone Marrow Mesenchymal Stem Cells (BMSC).

Methods BMSCs were isolated by Ficoll-Paque density-gradient centrifugation from human bone marrow (KEK_Bern126/03). EPCs ($CD133^+/CD34^+$) were isolated from BMSC fractions using magnetic-activated cell sorting (MACS®). After cell fluorescent staining using PKH67-green for EPC and PKH26-red for BMSC, EPCs were seeded on 2D growth-factor-reduced-Matrigel coating alone or together with BMSC. Cellular network formation was observed using epifluorescence microscopy. In 3D co-culture set-ups, cells were seeded in different proportions in a polyurethane scaffold in presence of Platelet Rich Plasma. After 7 days of culture in different media (osteogenic, angiogenic, or mixed), cryosections were performed and stained with toluidine blue and endothelial-cell specific antibodies.

Results On Matrigel assay, EPCs showed the capacity to re-organize themselves in typical endothelial-like cellular networks and demonstrated improved tubular-like formation when co-cultured with BMSCs. In 3D scaffolds, we showed that the association EPC-BMSC within a polyurethane scaffold promoted the formation of Laminin, von-Willebrand-factor and PECAM positive tubular structures formation.

Conclusion EPC-BMSC co-culture in 2D- and 3D-environment enhanced the formation of early tubular structures within the scaffolds with both cell types contributing to this cellular re-organization. In vitro pre-culture for 7 days with 50–50 cell proportion in osteogenic media containing PRGF seems to be optimal.

Silica improves cell viability and modulates growth factor release in platelet rich plasma-alginate-hydrogels

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Background Platelet-rich plasma (PRP) is an autologous source of growth factors derived from blood plasma that holds great promise in various clinical applications (maxillofacial surgery, spinal fusion, bone augmentation, treatment of soft tissues). In typical preparations, the release kinetics of pure PRP is very fast (more than 95 % released in the first hour). In recent years, it has been shown that combined growth factor delivery is more effective than delivery of a single factor as growth factors play a different role in a time dependent manner. The aim of this study was to validate for the first time the use of a novel PRP-alginate-silica composite gel for combined controllable PRP release and cell-based therapy.

Materials and methods Sodium metasilicate nonahydrate (Na_2SiO_3, $9H_2O$) (5, 25 and 50 mM) was added to a 0.5 % sodium alginate solution in Iscoves Modified Dulbecco's medium and then mixed with an equal volume of a PRP/thrombin solution. Macro-beads were formed by adding the mixture dropwise into a $CaCl_2$ solution (pH 7) containing pentaethylenehexamine (PEHA). Gel beads were characterized by FT-IR and scanning electron microscopy (SEM). Total protein and TGF-β1 release kinetics were also quantified. Once the pH and ionic strength had been adjusted (pH = 7.4 and 330 mOsm) for the different solutions, mesenchymal stem cell encapsulation (hMSCs) could be encapsulated and cultured for 3 days. After dissolution of the beads, the proportion of viable cells was measured by the trypan blue exclusion assay.

Results Silica polycondensation occurred in PRP-alginate-silica as shown by additional vibrational bands in FT-IR spectra (1000 cm^{-1}, 950 cm^{-1} and 450 cm^{-1}) and aggregated silica particles in fractured and calcined PRP-alginate-silica beads examined by SEM. For the PRP-alginate-silica beads, the amount of protein initially released was approximately 80 % after 2 hr and slow protein release continued over the next 78 hr. All the sodium metasilicate containing beads showed a slower and significantly higher release of TGF-β1 at 48 h than the PRP-alginate. After one day of culture, the addition of silicate significantly increased the proportion of viable cells, both in alginate and PRP-alginate samples as compared to alginate and PRP-alginate without silicate, suggesting that the presence of the silicate in the preparation reduces any negative effect of the alginate gel formation on cell viability. After three days of culture, the proportion of viable hMSC in the PRP-alginate-silica samples was high (92 %), independent of the silicate concentration.

Conclusions In summary, we have reported a novel method for combined controlled PRP release and mesenchymal stem cell encapsulation. The synthetic approach described in this contribution combining silica with alginate could offer for the first time the opportunity to tune PRP growth factor release. Furthermore, this composite system is injectable and preserves the viability of hMSCs and therefore holds promise for combined tissue engineering and drug delivery applications.

Modulation of Matrix Metalloproteinase-1 (MMP-1) expression in human osteosarcoma cells directly affects intratibial tumor formation and lung metastases in mice

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Background Osteosarcoma (OS) is the most frequent primary malignant tumor of bone, predominantly affecting children and young adults. Patients with metastatic disease at diagnosis have a poor prognosis. Metastatic progression is a complex process in which tumor cells colonize distant target organs. Several steps during this process require extracellular proteolytic enzymes. Overexpression of MMP-1, a member of the matrix metalloproteinase family, has been associated with poor prognosis in a variety of human cancers.

Methods LacZ-tagged HOS (HOS/LacZ) cells stably overexpressing MMP-1, 143B (143B/LacZ) cells downregulated in MMP-1 expression by siRNA and control cell lines were generated by retroviral infection. These cell lines were used in different in vitro assays and in vivo tumor models to investigate the functional relevance of MMP-1 in OS metastasis.

Results MMP-1 was found upregulated in the highly metastatic 143B OS cells in comparison to its parental, non-metastatic HOS cells. The biological relevance of this finding was further investigated in vitro and in vivo. Overexpression of MMP-1 in HOS/LacZ cells enhanced adhesion to collagen type I compared to control cells and facilitated anchorage-independent growth. Conversely, siRNA-mediated downregulation of MMP-1 expression in 143B/LacZ cells inhibited the adhesion to collagen type I and reduced the number of fast-growing cell colonies in soft agar. These findings in vitro suggested that robust expression of MMP-1 in 143B/LacZ cells and in stably MMP-1 infected HOS/LacZ cells may have a significant impact on the metastatic activity of these cell lines in vivo. This was confirmed in SCID mice upon intratibial injection of MMP-1 expression modified HOS/LacZ and 143B/LacZ cells, respectively, and of the corresponding control cells. MMP-1 overexpressing HOS/LacZ cells, unlike the control cells, formed intratibial, osteolytic primary tumors and numerous micrometastases in the lung. Conversely, 143B/LacZ cells with siRNA-downregulated MMP-1 expression formed smaller intratibial primary tumors and a significantly lower number of lung macrometastases than the control 143B/LacZ cells.

Conclusions In conclusion, MMP-1 is a key modulator of intratibial primary tumor growth and of lung metastases of human 143B and its parental HOS OS cells in mice.

Osteogenic potential of total BMC (bone marrow mononucleated cells) versus EPC-depleted-BMC fraction (endothelial progenitor cells)

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Introduction Endothelial cells-osteoblast co-cultures are known to induce a synergy of cell differentiation and activity [1, 2]. Bone marrow is a rich source of mesenchymal stem cells (MSC), but EPC are also present. MSC can develop an osteogenic phenotype while EPC will differentiate into endothelial cells. The aim of our study was investigate the effect of the EPC present within the whole BMC population on the MSC osteogenic differentiation.

Methods Human BMC of 5 donors (KEK Bern 126/03) were isolated by density gradient centrifugation (Ficoll). $CD133^+$ and $CD34^+$ cells were further depleted from the BMC using magnetic beads (Miltenyi), the resulting population was named MM. Identical numbers of BMC and MM cells were seeded and culture over 28 days

in classical osteogenic medium (10 nM Dexamethasone), or in autologous growth factor medium (PRGF). PRGF was prepared from thrombocyte concentrates resuspended in PBS (2×10^6 platelets/mL). Cell growth was assessed by DNA quantification, osteogenic differentiation by real-time (RT) PCR, and ALP activity. Matrix mineralization was estimated by $^{45}Ca^{2+}$ incorporation.

Results In both culture media, the full BMC grew faster than MM. However, if PRGF showed an overall superiority for both populations' cell growth, cell differentiation was much higher in Dex^+ medium, for both BMC and MM. MM showed high up-regulation of all tested osteogenic marker genes in both media. Cell differentiation was confirmed by ALP activity that was found higher in MM compared to BMC in both media, with higher values for Dex medium. Matrix mineralization analyses confirmed these results.

Discussion The EPC present in full BMC may grow faster than the MSC (especially in PRGF3) and impair the proportion of cell with osteogenic potential. These 2-cell populations also might be in too early stages of differentiation to promote co-differentiation at this point.

References:
1. Villars et al., 2002.
2. Hofmann et al., 2008.
3. Lippross et al., 2011.

PPARγ-null mice have increased cancellous bone volume but low bone mass

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The nuclear receptor PPAR gamma (PPARγ) positively regulates adipogenesis and negatively osteoblastogenesis. $PPAR\gamma^{+/-}$ mice were characterized by high bone mass and increased cancellous bone volume (BV/TV). However, the skeletal effects of homozygous $PPAR\gamma^{-/-}$ deletion are unknown due to the lethality of $PPAR\gamma^{-/-}$ mice. Using novel recombinant technology, we were able to generate living adult $PPAR\gamma^{-/-}$ mice. Bone mass, architecture and turnover were assessed in young and mature adult (3 and 6 months) and old (12 months) $PPAR\gamma^{+/+}$, $PPAR\gamma^{+/-}$ and $PPAR\gamma^{-/-}$ mice. Six and 12 month-old $PPAR\gamma^{-/-}$ mice had significantly increased lean mass (28.8 ± 0.6 vs 20.8 ± 1.8 g, $p < 0.009$), but lower % fat and low leptin levels (0.23 ± 0.01 vs 8.22 ± 2.54 ng/mL, $p < 0.05$) compared to $PPAR\gamma^{+/+}$ and $PPAR\gamma^{+/-}$. Whereas in young $PPAR\gamma^{-/-}$ mice, femoral length and bone mass were decreased, there were trends for increased BV/TV and decreased cortical bone area compared to $PPAR\gamma^{+/+}$ and $PPAR\gamma^{+/-}$. With age (6 months), $PPAR\gamma^{-/-}$ developed a greater (BV/TV) and trabecular number compared to $PPAR\gamma^{+/+}$ and $PPAR\gamma^{+/-}$, but lower bone density and cortical area and width compared to $PPAR\gamma^{+/-}$. Similar results were found in old $PPAR\gamma^{-/-}$ with more prominent cortical and cancellous differences (**Table 1**).

Osteocalcin was decreased and markers of bone resorption were increased in 6 and 12 month-old $PPAR\gamma^{-/-}$ compared to $PPAR\gamma^{+/-}$ and $PPAR\gamma^{+/+}$. These results indicate that PPARg played an important role on bone acquisition and maintenance with age and that PPARγ deletion leading to lipodystrophy was associated with high BV/TV but low bone mass, possibly due to loss of positive peripheral leptin effects on cortical bone.

Table 1. Pierroz D D et al.

6 months	$PPAR\gamma^{+/+}$ (n = 3)	$PPAR\gamma^{+/-}$ (n = 9)	$PPAR\gamma^{-/-}$ (n = 6)
Fem BMD (mg/cm²)	79.0 ± 6.8	87.5 ± 2.1	77.9 ± 3.9[b]
Dist fem Tb N (/mm)	3.20 ± 0.11	3.23 ± 0.20	3.91 ± 0.26[a, b]
Vert BV/TV (%)	13.3 ± 1.9	11.6 ± 1.8	20.2 ± 2.7[a, b]
Vert TbN (/mm)	3.48 ± 0.11	3.62 ± 0.15	4.65 ± 0.21[a, b]
Midfemur Bone Area (mm²)	0.83 ± 0.03	0.97 ± 0.02[a]	0.81 ± 0.04[b]
Midfemur cort width (µm)	202.6 ± 4.4	221.2 ± 3.8[a]	202.2 ± 4.9[b]

[a]$p < 0.05$ vs $PPAR\gamma^{+/+}$; [b]$p < 0.05$ vs $PPAR\gamma^{+/-}$

Short period of delayed loading can increase the final bone volume inside tissue engineering scaffold

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Background In our previous study, we showed that early cyclic loading following the implantation of bone scaffold increases the rate of bone formation in polymeric scaffolds in a period of 13 weeks compared to the control group. We also found out that there is an initial decrease in bone volume in the loaded group. The goal of this study was to investigate the effect of a delayed loading following the implantation of bone scaffold in a longer time period.

Methods Both femoral condyles of 8 female Wistar rats of weight 245–250 gr. were drilled (Veterinary Authority from the Canton of Vaud, authorizations No. 2140) and PLA + 5 % wt β-TCP scaffolds of the same size were implanted inside the drilled holes. No cells or growth factors were added in the scaffold. In the previous study, the loading started 3 days after the surgery. In the present study, the loading started two weeks after the surgery. The right knee joints of all animals were loaded and the left leg was kept as control. Compressive load of 10 N at 4 Hz for 5 minutes was applied by a custom-made compression machine. The animals were loaded 5 times every other day. Both knee joints of all animals were scanned at 8 time points using SkyScan 1076 in vivo scanner (SkyScan, Belgium) at 2, 4, 6, 7, 11, 15, 22 and 35 weeks after surgery. Bone volume (BV) and BMD of bone inside scaffold were measured. Non-linear mixed-effect modeling was used to model the evolution of BV and BMD as a function of time. Repeated measures analysis of covariances (ANCOVA) was used to evaluate the differences between the two groups.

Results Statistical test reveals that loading increases the rate of bone formation by 8 % and the final bone volume by 18 %. No difference in BMD between the control and loaded groups was observed. Histological observations revealed that two distinct patterns of bone formation were observed inside the scaffold. Close to the exterior part, i.e. in the cortical region of bone, pores are completely filled with bone, as if cortical bone is forming. In the trabecular region, the bone is formed mainly on the walls of the scaffold pores, as if trabecular bone is forming.

Conclusions We had previously shown that early cyclic loading increases rate of bone formation inside scaffold on the long run, but at the cost of an initial decrease in the bone formation compared to the control group. In this study, we delayed the loading period by two weeks, and we saw that the initial decrease in bone formation disappeared. However, the rate of bone formation was decreased by half on the long run compared to the previous study using early loading. Nevertheless, the effect of this short period of loading was long lasting and it increased the final bone volume by 18 % compared to the control group.

The surgical preparation of the bone-scaffold interface is critical for bone regeneration

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Background The goal of this study was to investigate if the preparation of implantation site has an impact on bone formation inside tissue engineering scaffold. Two drilling techniques were used to create a hole in rat distal femur before the insertion of a bone scaffold. The first drilling technique used a manually driven wood drill bit and the second technique used an electrically driven metal drill bit.

Methods Sixteen female Wistar rats (weight 245–250 gr) were randomly separated in two groups of eight, A and B, based on the

drilling method: A) Wood drill bit used, B) metal drill bit. Note that the cutting geometry is essentially different between groups A and B. Left distal femurs were operated (Veterinary Authority from the Canton of Vaud, authorization No. 2140) following a protocol already used in our laboratory. The scaffold was a biocomposite made of PLA/β-TCP. In vivo prospective micro-CT scanning was done in order to investigate bone regeneration inside scaffolds (Skyscan 1076, Skyscan, Belgium) at six time points between 2 and 21 weeks after the surgery. The BMD of each sample was quantified based on the calibrated values of the phantoms. Linear mixed-effect modeling was used to model the evolution of BV as a function of time according to our previous study. Repeated measures analysis of covariances (ANCOVA) was used to evaluate the differences between the two groups.

Results The amount of bleeding due to drilling was remarkably higher in group A compared to group B (based on visual observations). The ANCOVA test shows that the group A has significantly higher BV (p-value = 0.0005) and BMD (p-value = 0.0004) compared to group B. We observed that group A is almost three weeks ahead of group B in terms of bone regeneration. The structure of the bone at the two surfaces is clearly different; the metal drill (group B) has crushed and sheared the bone and the interface is partly clogged. On the other hand, the wood drill (group A) has resulted in a clear cut and the pores at the surface are more open.

Conclusions The major finding of this study was to demonstrate that the drilling technique strongly affects bone formation in scaffold. Indeed we found that depending on the technique used, bone healing process can be accelerated by almost three weeks in this in vivo rat study. Thermal damage in group B is unlikely because the duration of drilling and thickness of cortical bone are well below critical values. The probable explanation is the difference between amounts of blood extravasation which is due to the different cutting geometry. In conclusion, by using a "wood" type drill, a faster bone healing is obtained compared to a "metal" type drill, the latter being usually used in clinical practice.

Caprin-1 expression promotes intratibial xenograft growth and lung metastasis in mice and indicates poor prognosis of patients with osteosarcoma

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Background Osteosarcoma is the most frequent primary malignant bone tumor in children and adolescents with a high propensity for lung metastasis, the major cause of disease-related death. Reliable outcome-predictive markers and targets for osteosarcoma metastasis-suppressing drugs are urgently needed. Recently, we demonstrated that overexpression of the extracellular matrix protein Cyr61 promotes primary tumour growth and lung metastasis in an intratibial xenograft model in mice and indicates poor prognosis of patients with osteosarcoma [Sabile A et al., submitted]. In the present study, we investigated the putative Cyr61-interacting protein, Caprin-1 (cytoplasmic activation/proliferation-associated protein-1), as a novel osteosarcoma-promoting protein.

Methods We have immunoprecipitated endogenous Cyr61 with a specific antibody and performed mass spectrometric analysis to identify Cyr61-interacting proteins. The effect of stable overexpression of human Caprin-1 on primary tumor growth and metastasis was assessed in vitro and in vivo in an orthotopic mouse osteosarcoma model.

Results We identified Caprin-1 as a novel Cyr61-interacting protein. Furthermore, we showed that Caprin-1 overexpression in osteosarcoma cell lines enhanced their migration and invasion rates in vitro, reflecting enhanced metastatic potential. Finally, we demonstrated that Caprin-1 overexpression accelerated primary tumor growth in the tibia, increased the number of lung metastatic lesions, and consequently significantly decreased mouse survival.

Conclusions Using a proteomics approach, we identified Caprin-1 as a novel Cyr61-interacting protein. Furthermore, we demonstrate that Caprin-1 overexpression promotes primary tumor growth and enhances lung metastasis in vivo. Currently, we are investigating in detail the interplay between Cyr61 and Caprin-1 and their functions in the context of osteosarcoma metastasis.

Non-invasive monitoring of implant strength in-vivo

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Background Immediately after implantation, a dynamic process of bone formation and resorption takes place around an orthopedic implant, influencing its mechanical fixation. The delay until complete fixation depends on local bone architecture and metabolism. Despite its importance, for post-operative care, the temporal pattern of implants fixation is still unknown. The aim of this study was to evaluate the potential of micro finite-element modeling based on in vivo micro computed tomography to monitor longitudinally the evolutions of bone around an implant and of the implant strength in vivo.

Methods Titanium cancellous bone screws (ø = 1.7 mm, L = 5 mm) were inserted surgically in the proximal tibias of twelve female Wistar rats (3 months old, 240 ± 8 g). Bone growth around the implant was assessed using in-vivo micro-computed tomography at days 0, 3, 6, 9, 14, 20 and 27 at a resolution of 12 µm in 10 animals (x-ray group). Two control rats were scanned only at days 0 and 27 and served as radiation controls (control group). The bone in contact with the implant was evaluated from the scans and micro finite-element models were built from the image data to simulate the screws' pullout strength at each time point. The finite-element results were calibrated with biomechanical pullout after euthanasia.

Results Contact bone volume fraction increased from 43 ± 7 % to 55 ± 8 % between day 0 and day 27 ($p < 0.05$). The pullout stiffness increased from 137 ± 30 N/mm to 265 ± 45 N/mm ($p < 0.05$) and failure load from 105 ± 30 N to 180 ± 20 ($p < 0.05$). These increases were most prominent between day 0 and day 14. No significant differences in stiffness or failure load were measured between the monitoring group and the radiation control group.

Conclusions Limitations, such as image artifacts and radiation, still compromise the immediate clinical application of this method, but it has a promising potential in preclinical studies, as it provides very valuable data about the dynamic aspect of implant integration with considerably reduced animal resources.

P38α MAPK regulates osteoblast function and bone formation

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Various osteogenic ligands that stimulate osteoblast differentiation and function act, in part, through the p38 mitogen-activated protein kinase (MAPK) pathway. A recent in vivo investigation has highlighted the physiological role of the TAK1-MKK3/6-p38 pathway in osteoblastogenesis and bone formation. Interestingly, the authors have shown that p38β is critical for late osteoblast differentiation and that loss of p38β is not compensated by p38α, thus suggesting that p38α and p38β may have different functions in bone formation. To elucidate the in vivo role of p38α in regulating osteoblast function, we generated mice lacking p38α in mature osteoblasts. Mice expressing Cre recombinase under the control of the osteocalcin promoter (Ocn-Cre) were crossed with mice harboring floxed p38α-encoding gene ($p38\alpha^{f/f}$). The bone phenotype of control ($p38\alpha^{f/f}$) and mutant (Ocn-Cre;$p38\alpha^{f/f}$) mice was assessed by dual energy X-ray absorptiometry, micro-computed tomography and gene expression analyses at 3 months of age (n = 6 per group). Mutant mice exhibited lower bone mineral density compared to control mice (–8.2 %, p = 0.003). Ocn-Cre;$p38\alpha^{f/f}$ mice displayed an important reduction in

trabecular bone volume at the distal femoral metaphysis (–37.1 %, p = 0.002) associated with low trabecular thickness (–20.7 %, p < 0.001). A similar pattern of low trabecular bone mass was observed at the fifth lumbar vertebral body. In addition, Ocn-Cre;p38α$^{f/f}$ mice also showed decreased cortical thickness at the femoral midshaft (–20.2 %, p < 0.001). Consistent with this low bone mass phenotype, *Osx*, *Col1a1*, *Alp* and *Ocn* expressions were reduced by 34, 32, 10 and 40 % in long bones of mutant mice, respectively. Finally, primary p38α knockout osteoblasts demonstrated lower *Osx*, *Col1a1*, *Alp* and *Ocn* expressions ($p \leq 0.01$) and reduced capacity to mineralize in-vitro, indicating a defective function of osteoblasts lacking p38α. These findings indicate that p38α is an essential regulator of osteoblast function and bone formation in vivo.

External mechanical microstimuli improves osseointegration of titanium implants in rat proximal tibiae

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A poor osseointegration of endosseous implants is the cause of early implants failure compromising the outcome of a surgical intervention both in dentistry and orthopedics. The aim of this work was to measure the effect on implant osseointegration in rat proximal tibiae following the application of external mechanical microstimuli of controlled intensity. Increasing loads were selected and a dose-dependent effect on parameters of implant osseointegration was researched. 40 females rats 6 months old were operated at the right tibiae by transcutaneous insertion of two titan cylindrical implants, respectively 1 mm and 0.8 mm of diameter. The stimulated implant was fixed within the trabecular bone of the secondary spongiosa whereas the anchorage implant was inserted 8 mm distal, encompassing both cortical surfaces. After 2 weeks rats were assigned to 4 groups (non stimulated, 1N, 2N 3N), and further underwent to a daily external mechanical stimulation during 4 weeks. Ultimate Strength (US) was measured ex-vivo by a pull-out test as indicator of implant osseointegration. Both determinants of pullout strength BV/TV and the number of bone/implant contacts (BIC) were evaluated by microcomputerized tomography (micro-Ct) within the trabecular bone adjacent the proximal implant (**Table 2**). Values are ± SEM, and significant differences were identified by t-test (< 0.05).

Table 2. Results, Zacchetti G et al.

Stimulation	Non stimulated	1 N (ca 1250 με)	2 N (ca 2500 με)	3 N (ca 3750 με)
US (N)	39.57 ± 2.23	40.82 ± 3.12	46.63 ± 2.21*	43.81 ± 3.41
BV/TV (%)	44.2 ± 3.1	50.5 ± 2.3	48.5 ± 1.9	48.0 ± 3.9
BIC (%)	76.02 ± 2.66	78.73 ± 2.25	78.35 ± 2.19	76.85 ± 3.70

*p < 0.05 compared to non stimulated controls (t-test)

Higher US values were observed in stimulated animals, the group 2N displaying a significant increase of the pull-out force necessary to loosen the implant compared to non-stimulated control rats. The modest increase in BV/TV and bone/implant contacts observed in stimulated implants is not sufficient to explain the increase in US observed in 2N group. We hypothesize that ingrowths within the etched implant surface may explain it. In conclusion, application of external mechanical microstimuli of controlled intensity improves osseointegration of titanium implants in rat proximal tibiae.

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