The exact mechanisms controlling the development and progression of osteoarthritis have not yet been clarified. Our aim was to investigate new pathomechanisms, with an emphasis on novel molecular targets that might regulate human chondrocytes in osteoarthritis. As a model for studying cell survival and metabolism, C-28/I2 and T/C-28a4 human chondrocytes were grown in complete medium, in dextran-coated charcoal treated medium and in serum-free medium. Healthy and osteoarthritic human cartilage samples were obtained from discarded surgical material. Cell survival, PTEN, AKT, Beclin1, AMBRA, AMPK and glucose/triglyceride metabolism were evaluated by immunoblotting and spectrophotometric assays. Starvation and steroids depletion decreased cell survival concomitantly with PTEN elevation, repression of the PI3K/AKT signaling axis and autophagy activation. These experimental conditions promoted the accumulation of glucose, decreased levels of G6PDH and resulted in differential expression of OXPHOS complexes. Furthermore, they induced the expression of AMPK, reduced triglyceride levels and increased lipase activity, which was accompanied by a change in chondrocytes toward a fibroblast-like morphology. In osteoarthritic human cartilage, increased PTEN, AMPK and autophagy reflected the chondrocyte responses observed during starvation and steroids depletion. In conclusion, we defined the metabolic phenotype of human chondrocytes, in which both starvation and steroids depletion induce the activation of PTEN, AMPK and autophagy signaling, concomitant with metabolic reprogramming. Our data may aid in the development of novel in vitro models for the discovery and design of drugs or nutraceuticals capable of ameliorating the course of osteoarthritis.
Adequate blood supply is essential for prosthesis osteointegration and bone healing as it supplies oxygen, nutrition and progenitor cells. The bone healing process and vascularization depend upon the endothelial cells, which speed up implant osteointegration. Endothelial Progenitor Cells (EPC) are a population of stem cells that can reproduce, migrate and acquire mature endothelial phenotype. Their recruitment occurs in the tissue lesion to enhance neovascularization. Trabecular Titanium™ (TT™) is a new biomaterial with very interesting biomechanical characteristics and fast osteointegration. This study has investigated adhesion, proliferation and characteristics of EPC on three types of biomaterial: unmodified trabecular titanium, trabecular titanium coated with the ECM deposited by human mesenchymal stem cells isolated from subcutaneous adipose tissue and decellularized and trabecular titanium coated with type I collagen (control scaffold). MTT assay showed similar percentages of EPCs seeded on the different kinds of scaffold: 67% on TT, 70% on decellularized scaffolds and 82% on collagen-coated scaffolds. There were no statistically significant differences between the three groups. We therefore conclude that TT™ allows EPC adhesion and proliferation and, consequently, by permitting vascularization, it favours prosthesis osteointegration.
BIOPHYSICAL STIMULATION FOR NONUNIONS

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Nonunions account for 5-10% on the total number of fractures. Biophysical stimulation is a non-surgical, conservative, frequently used therapy in nonunions and a greater efficacy has been demonstrated for pulsed electromagnetic fields (PEMF). The mechanisms of action of PEMF at cellular and molecular levels are still under debate and no dose-response study is available. Moreover, the vast majority of in vitro studies were conducted on healthy cells. The primary aim of the research was to investigate the capacity of PEMF with different exposure times to stimulate the osteogenic process in cells from the callus of a nonunion patient. Another important objective was the characterization of nonunion cells in terms of clonogenicity, cluster of differentiation expression and the tri-lineage differentiation capacity. Overall, the results indicated the presence of osteochondroprogenitor cells in the callus of a nonunion, with an impairment in the osteogenic differentiation process. PEMF may enhance cell viability, the formation of osteoid matrix and accelerate the process of osteogenic differentiation. BMP-4 production, TIMP1 and TIMP2 expression were influenced, as well as VEGFA, whose early upregulation may account for a possible improvement in both the osteogenic and vasculogenic processes. In conclusion, even with some discussed limitations, these preliminary data showed the presence of a multipotent progenitor population and suggested some hints of the effect of PEMF on nonunion cells.
Wnt1 is one of the several glycoproteins activating Wnt signaling, critical for normal skeletal development and bone homeostasis. Wnt1 was previously believed to solely regulate central nervous system development, in particular in midbrain and cerebellum. However, remarkable findings have recently shown that several patients affected by severe form of Osteogenesis Imperfecta (OI) display a Wnt1 mutation thereby revealing a possible role of Wnt1 in bone metabolism. Here, we show that recombinant Wnt1 (r-Wnt1) strongly increases differentiation of bone marrow stromal cells into mature osteoblasts, as demonstrated by the enhanced number of cells positively stained for alkaline phosphatase, one of the osteoblastic marker genes, whose mRNA levels are also significantly up-regulated. Furthermore, other osteogenic master genes such as Collagen I and Osteopontin are also enhanced when bone marrow precursors were differentiated toward osteoblastic phenotype in the presence of r-Wnt1. Intriguingly, by in vivo and in vitro findings, we report that in the bone marrow of mice subjected to physical activity there is a high endogenous Wnt1 synthesis compared to mice kept in resting conditions. Moreover, conditioned medium collected from ex vivo myoblasts, harvested from exercised mice, up-regulates Wnt1 expression in osteoblast cell cultures obtained from control mice. Overall our findings support the role of Wnt1 in regulating bone metabolism and suggest that this molecule could be one of the mediators through which physical activity may exert beneficial effect on bone.
Skeletal muscle injuries are common causes of severe long-term pain and physical disability, accounting for up to 55% of all sports injuries. The phases of the healing processes after direct or indirect muscle injury are complex but clearly defined and include well-coordinated steps: degeneration, inflammation, regeneration, and fibrosis. Despite this frequent occurrence and the presence of a body of data on the pathophysiology of muscle injuries, none of the current treatment strategies have shown to be really effective in strictly controlled trials. Platelet-rich plasma (PRP) is a promising alternative approach based on the ability of autologous growth factors (GFs) to accelerate tissue healing, improve muscular regeneration, increase neovascularization and reduce fibrosis. The present study is focused on the use of different concentrations of PRP as a source of GFs. Unilateral muscle lesions were created on the longissimus dorsi muscle of Wistar rats. Twenty-four h after surgical trauma, the lesion was filled with an intramuscular injection of PRP at 2 different concentrations. A group of rats were left untreated (controls). Animals were sacrificed at 3, 15 and 60 days from surgery. Histological, immunohistochemical and histomorphometric analyses were performed to evaluate muscle regeneration, neovascularization, fibrosis and inflammation. The PRP-treated muscles showed better muscle regeneration, more neovascularization and a slight reduction of fibrosis compared with the control muscles in a dose dependent manner. However, further studies also assessing pain and functional recovery are scheduled.

EFFECT OF PLATELET RICH PLASMA CONCENTRATION ON SKELETAL MUSCLE REGENERATION: AN EXPERIMENTAL STUDY

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HISTOLOGICAL FEATURES OF PSEUDOTUMOR AFTER SMALL HEAD DIAMETER METAL-ON-METAL TOTAL HIP ARTHROPLASTY

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The pathologic aspects of periprosthetic tissues in failed second-generation metal-on-metal (MoM) resurfacing hip arthroplasties have been widely described in terms of necrosis and inflammation. To our knowledge little data are reported on the association of this lesion with the use of small head diameter (<32 mm). In this study we present a small series of pseudo-tumor in small head metal-on-metal total hip arthroplasty focusing our attention on the histologic aspects of the harvested pathologic tissue. The histological examination of our cases showed a presence of lymphocytic infiltrate suggesting a delayed hypersensitivity reaction to metal of type IV (ALVAL) but different to each other in terms of the prevalence of the cellular component. If macrophages are predominant, the pathogenetic mechanism seems to be the reaction against metallic particle. On the other hand, if granulocytes are predominant, it is possible to consider a hypersensitivity reaction. Our observation suggests that the evidence of Pseudotumor in case of small-head metal-on-metal arthroplasty should be considered with the same properties of big-head and therefore these patients should be followed scrupulously.
RELEASE OF GENTAMICIN FROM CEMENT SPACERS IN TWO-STAGE PROCEDURES FOR HIP AND KNEE PROSTHETIC INFECTION: AN IN VIVO PHARMACOKINETIC STUDY WITH CLINICAL FOLLOW-UP

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Eighteen patients undergoing two-stage exchange arthroplasty for infected total hip or knee arthroplasty using gentamicin-loaded bone cement spacers (80g bone cement, 2 g gentamicin and 2 g clindamycin) were studied. The concentration of gentamicin eluted from the spacers was assessed on samples of blood, urine, and drainage fluid that were collected from each patient at set intervals during the 48 hours following the first-stage surgery. The hip and knee cement spacers showed similar curve of release over the first postoperative hours (early peak followed by slow release), but the mean gentamicin concentration in the drainage fluid was higher in patients with hip spacers compared to patients with knee spacers (30.61 ± 19.47 mg/L vs. 17.43 ± 13.63 mg/L, p<0.05). In patients with hip spacers, the mean, maximum, and minimum concentration of gentamicin was higher with respect to the minimum inhibitory concentration (MIC) break point for Staphylococcus spp, Pseudomonas Aeruginosa and Enterobacteriaceae throughout the first postoperative 48 h. Conversely, in 25% of patients with a knee spacer a drug concentration below the MIC break point for Gram negative bacteria was found in the drainage fluid after 12 h. Gentamicin levels in the blood samples were negligible over the entire time interval and were steadily well below the renal toxicity reference. The highest urinary concentration of gentamicin was observed between 4 and 9 h postoperatively. Subsequently, it gradually declined until 48 h. Clinically, the rate of cure was 100% at a mean follow-up of 113 weeks (range 90-182). Gentamicin-loaded cement spacers offer the advantage of achieving early high concentrations of the antibiotic directly at the site of infection but especially in the knee a systemic antibiotic therapy must be given as a complement to the spacer implantation to eradicate periprosthetic joint infection (PJI).
Bone metastases from carcinomas are epidemiologically rising because of the increased survival rate of oncologic patients, related to several factors such as improvement of primary and secondary screening, advancement of medical research and technology and the better understanding of mechanisms underlying bone metastases origin from primary tumor. Skeletal Related Events (SREs) can seriously affect quality of life in patients with metastatic disease. These events include the necessity of radiotherapy or bone surgery, malignant hypercalcemia, pathologic fractures and spinal cord compression. Among the SREs, pathologic fractures are the most disabling events and represent an emergency in these delicate patients. A pathologic fracture is defined as a fracture that occurs at the level of a pre-existing bone lesion (that is often a tumor), spontaneously or as the result of low-energy trauma (1). The pre-existence of the metastatic lesion in the bone, its evaluation and the assessment of progression can make these complications predictable and preventable. Pathologic fractures imply several severe consequences, including patient immobilization (in the case of fractures involving the lower limbs), loss of autonomy, anaemia, need of blood transfusion, discontinuation of medical therapies or radiotherapy and protracted hospitalization. Secondary effects of prolonged immobilization and loss of autonomy further lengthen this list of complications in patients who are already significantly limited in their activities. In the present paper, the authors present a review on the main aspects involved in bone metastatic disease: biology, quality of life, economic impact and survival.
The purpose of this study was to assess bone mineral density in a cystic fibrosis (CF) outpatient clinic population and to investigate the relationship between BMD and forced expiratory volume in one second (FEV1), DEXA T-scores and 25-hidroxivitamin D (25-OHD) serum levels. We examined a consecutive series of 44 CF patients. Bone mass density was measured by dual-photon X-ray absorptiometry of lumbar spine and femur (total and neck) and lung function was performed in all patients. Medication data were obtained from medical records. A correlation analysis was performed to determine the relationship between BMD and forced expiratory volume in one second (FEV1), DEXA T-scores and 25-hidroxivitamin D (25-OHD) serum levels. In the results, age showed a significant inverse correlation indicating that as the age increases, bone density decreases and we concluded that most CF patients have low BMD and that there is a positive correlation with lung function and an inverse correlation with age.
Vitamin D is the main hormone regulating calcium phosphate homeostasis and mineral bone metabolism. Vitamin D deficiency is indeed extremely frequent in musculoskeletal diseases. Recent studies have shown that the treatment of osteoporosis needs to have an optimal vitamin D and calcium supplementation for its efficacy. Actually no agreement exists on the established dose of vitamin D to administer in deficiency states. We conducted a prospective study to develop a practical cholecalciferol loading dose regimen that would enable rapid correction of vitamin D deficiency. Sixty post-menopausal age woman were enrolled secondary to a fragility fracture (hip, vertebral, wrist) and screened for 25-hydroxyvitamin D (25(OH)D), calcium, and PTH at baseline (T0), after one month (T1), two months (T2), three months (T3) and six months (T4). Secondary to initial blood values of vitamin D patients were divided into 2 groups; the first group (group A, n=30) included patients with 25(OH)D values between 10-30 ng/ml and the second group (group B, n=30) with values under 10 ng/ml. Each group was then divided in 3 subgroups secondary to the randomized administered dose of 25(OH)D. By this, patients can alternatively receive 25000 UI two times monthly, 100000 UI monthly, 10000 UI (25 drops) weekly. The highest values of mean increase of 25(OH)D were observed in patients treated with 100000 UI. Patients treated with 10000 UI weekly did never achieve the target value. Additionally, as vitamin levels increased, pain intensity decreased. Vitamin D supplementation of 100000 UI monthly seems to be adequate to ensure that serum 25(OH)D values reach the threshold level; by this, it will confer the expected effects without risks of toxicity.
OPTIMAL IMPROVEMENT IN FUNCTION AFTER TOTAL HIP AND KNEE REPLACEMENT: HOW DEEP DO YOU KNOW YOUR PATIENT’S MIND?

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Osteoarthritis (OA) of the hip and knee causes pain and loss of joint mobility, leading to limitations in physical function. When conservative treatment fails total hip and knee replacement is a cost-effective surgical option. Patients have high expectations regarding functional outcome after these procedures. If such expectations are not met, they may still be dissatisfied with the outcome of a technically successful procedure. Recently, numerous studies reported that psychological factors can influence the outcome of total knee replacement (tkr) and total hip arthroplasty with total hip replacement (thr). We conducted a prospective study on a consecutive sample of 280 patients affected by hip or knee OA who underwent total joint replacement. At patients’ admission, Harris Hip Score (HHS) and Knee Society Score (KSS) were used to assess pain and function. Furthermore, SF-36, Mini-Mental Status Examination (MMSE), Symptom Checklist-90-R (SCL-90-R), Coping Orientation to Problems Experienced (BRIEF-COPE) and the Amsterdam Preoperative Anxiety and Information Scale (APAIS) were administered. Patients had clinical and radio graphical follow up at 1, 3 and 6 months post-operatively. The HHS and KSS values before surgery showed a linear correlation with both SCL-90-R and MMSE. None of the investigated variables influenced post-operative HHS and KSS scores; however, the improvement of functional scores resulted conditioned by SCL-90-R values, VAS score, schooling and MMSE. Psychological factors and mental status in primary total hip and knee replacement can affect outcome and patient satisfaction. Strategies focused on identification and facing of these conditions must be considered to improve outcome of total replacement.
Antibiotic-Loaded RegenOss for the Treatment of Septic Bone Defects: In Vitro Study and Preliminary Clinical Experience

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Bone and joint infections are a difficult to treat condition, often associated with bone loss. Although the management of septic bone defects may currently be achieved through various treatment modalities, there is a continuous need for bone substitutes able at the same time to favor bone repair and to provide local antibacterial protection. RegenOss, a biomimetic and resorbable bone substitute, has been previously shown to be highly biocompatible and osteoconductive. Aims of the present study were to test the in vitro ability of RegenOss to act as a local carrier of antibiotics and to investigate its clinical safety and efficacy in a continuous series of patients, affected by bone loss in active or previous infection. In vitro study was performed by adding vancomycin, levofloxacin or meropenem and assessing elution properties of RegenOss at fixed time intervals by means of a microbiological assay. At 48 hours, 98.5% of meropenem, 94.1% of levofloxacin and 76.3% of vancomycin were recovered in the medium, while all antibiotics were completely eluted at seven days. Clinical safety and efficacy of vancomycin- or vancomycin and meropenem-loaded RegenOss had been tested in 13 consecutive patients. After the surgical procedure, each patient underwent clinical, laboratory and radiographic evaluation at 3, 6, 12, 18 and 24 months. No adverse events associated with the use of RegenOss were observed. Twelve patients showed no infection recurrence and ten satisfactory bone healing at follow-up. In conclusion, this study shows the ability of RegenOss to act as local carrier when loaded with three different antibiotics with a complete elution in one week. The clinical use of antibiotic-loaded RegenOss appears safe in this preliminary clinical series, while larger studies are needed to confirm the efficacy of the intra-operative combination of this biomimetic bone substitute with various antibacterials in the treatment of septic bone defects.
OSTEOARTICULAR ALLOGRAFTS
IN PAEDIATRIC BONE TUMOR RECONSTRUCTION OF THE KNEE

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Osteoarticular allografts represent a reconstructive option after bone tumor resection around the knee in growing children. The major advantage is the chance to preserve the growth plate of the remaining bone, but the disadvantage is the high failure rate eventually requiring definitive prosthetic replacement at skeletal maturity. We retrospectively reviewed 22 patients who underwent osteoarticular allograft reconstructions of the distal femur (16) or proximal tibia (6). There were 12 females and 10 males with an average age at surgery of 11 years (7-15). The diagnosis was osteosarcoma in 19 cases and Ewing sarcoma in 3. All patients underwent pre- and post-operative chemotherapy. At an average follow-up of 103 months (12-167), 18 patients (82%) were alive and 4 had died (18%). We observed 10 allograft failures requiring prosthetic replacement, 6 in distal femur and 4 in proximal tibia reconstructions. At last follow-up 8 allografts (36%) were still in place. Overall allograft survival was 79.6% at five and 45.8% at ten years. In distal femur, allograft survival was 86.2% at five and 59.1% at ten years. In proximal tibia, allograft survival was 62.5% at 5 years and 31.2% at 67 months. Average limb shortening was 3 cm (0-5) in 8 patients with the allograft still in situ and 2 cm (0-4) in 10 patients after prosthetic replacement. Average MSTS functional score of the whole series was 25 (83.7%). The MSTS score of patients after revision with prosthetic replacement was 24 (80%) while patients who still had the allograft retained had an average MSTS scores of 26.8 (89.3%). In conclusion, osteoarticular allograft reconstruction of the knee after bone tumor resection in pediatric age can be considered a temporary solution with the aim to limit limb length discrepancy before definitive prosthetic replacement after skeletal maturity.
The optimal reference for rotational positioning of femoral component in total knee replacement (TKR) is debated. Navigation has been suggested for intra-op acquisition of patient’s specific kinematics and functional flexion axis (FFA). The main purpose of the present study is to prospectively investigate whether pre-operative FFA in patients with osteoarthritis (OA) and varus alignment changes after TKR and whether a correlation exists between post-op FFA and pre-op alignment. A navigated TKR was performed in 108 patients using a specific software to acquire passive joint kinematics before and after TKR. The knee was cycled through three passive range of motions (PROM), from 0° to 120°. FFA was computed using the mean helical axis algorithm. The angle between FFA and surgical TEA was determined on frontal ($\alpha_f$) and axial ($\alpha_a$) plane. The pre- and post-op hip-knee-ankle angle (HKA) was determined. Post-op FFA was different from pre-op FFA only on frontal plane. No significant difference was found on axial plane. No correlation was found between HKA-pre and $\alpha_A$-pre. A significant correlation was found between HKA-pre and $\alpha_F$-pre. The study concluded that TKR modifies FFA only on frontal plane. No difference was found on axial plane. No correlation was found between HKA-pre and $\alpha^A$-pre. A significant correlation was found between HKA-pre and $\alpha^F$-pre. The study concluded that TKR modifies FFA only on frontal plane. No difference was found on axial plane. Pre-op FFA is in a more varus position respect to TEA. The position of FFA on frontal plane is dependent on limb alignment. The present study has demonstrated TKR modifies the position of FFA only on frontal plane. The position of FFA on axial plane is not dependent on the amount of varus deformity and is not influenced by TKR. Level of evidence, IV, case series.
PAIN MANAGEMENT AFTER TOTAL KNEE ARTHROPLASTY: THE GOOD, THE BAD AND THE UGLY

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Improvement in pain management after knee replacement surgery has made progress in the last years, improving the results of this type of operation. Among these techniques, multimodal have shown the best results. In this study we try to compare the results of a combination of intravenous analgesia (IA), oral controlled analgesia (OCA) and periarticular injection (PAI) with our traditional protocol consisting in intravenous analgesia and femoral nerve block (IA/FNB). One-hundred patients, undergoing primary unilateral total knee arthroplasty between June 2014 and June 2015 were randomized into 2 groups. Mean patient age was 69.4. The first group received the intravenous analgesia combined with continuous femoral nerve block, while the second group received the new combined protocol. We used the same technique with standard medial parapatellar approach for all patients and they all received pre-emptive analgesia and postoperative pain protocols. All patients were interviewed daily postoperatively at 3 days, at discharge and at 3 months. The 2 groups had a similar discharge period (traditional group 7.3 days, combined group 6.9 days). In both groups, the results indicated no statistical difference in regards to rest and continuous passive movement. Pain on ambulation was the only category that was statistically lower in the PAI/IA/OCA group compared to traditional group.
Over the past few decades, spine disorders have become a major health concern and the number of spinal surgical procedures has been rising significantly. Several biotechnologies and biomaterials are often used in spine surgery to increase the effectiveness of the treatment. In the degenerative spine, when conservative treatment is ineffective the most recommended surgical procedure is decompression followed by spinal fusion. Success rates of spine fusion extensively rely on bone grafts peculiar properties. Autograft has been considered the gold standard to achieve a solid fusion but current research is focused on the development of new biomaterials. Osteoporosis is the main cause of vertebral compression fractures that are significantly associated with pain and disability, especially in the aging population. Vertebral augmentation is a minimally invasive approach in which cement is injected into the vertebral body to stabilize the fracture. New cements are being developed in the clinical scenario with reabsorbable properties and biomechanical features more similar to the native bone. The development of disc regeneration strategies such as nucleus pulposus restoration and annulus fibrosus repair may represent a minimally invasive procedure towards regeneration rather than fusion. Therefore, biomaterials and tissue engineering are fields of growing interest among both surgeons and manufacturing companies, with a major involvement in spine surgery. This review discusses current and novel biotechnologies and biomaterial used in spine surgery employing fusion, augmentation and regeneration.
Silver coatings, used in many surgical devices, have demonstrated good antimicrobial activity and low toxicity. Oncological musculoskeletal surgery have a high risk of infection, so in the last decades, silver-coated mega-prostheses have been introduced and are becoming increasingly widespread. In this study, a retrospective analysis of 158 cases of bone tumors, primary or metastatic, treated between 2005-2015 with wide margins resection and tumor implants reconstruction, was performed. The average age was 59 years (range 11-78 years), the same surgeon with antibiotic prophylaxis according to a standard protocol treated all patients. Silver-coated prostheses were implanted in 58.5% of patients and uncoated tumor prostheses in the remaining 41.5%. Patients were re-evaluated annually and complications were recorded, focusing analysis on infective complications. The average follow-up was 39.7 months: 23.4% of patients died at a median time of 35.3 months after surgery; 18.4% developed complications that required new surgery, of which 12.6% of these were due to infection. Patients treated with silver-coated implants developed early infection in 2.2% of cases against the 10.7% of the patients treated with standard tumor prosthesis. This difference between the two groups was statistically significant. The percentage of late infections occurring from 6 months after surgery was similar in both groups. Silver blood level taken in a sample of patients at different times after surgery, always showed values well below the threshold of toxicity and no patient showed any sign of local or general toxicity secondary to silver. Our study demonstrates that tumor silver-coated implants have a rate of early infection significantly lower than traditional implants, while there were no differences in the rate of late infections as described also in the literature. We recommend the use of silver–coated prosthesis as primary implants for limb salvage surgery in primary or metastatic bone tumors, considering the absence of toxicity and the lower rate of early infection.