modulation of macrophage activity by pulsed electromagnetic fields in the context of fracture healing

ID: 1093

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Background:

Fractures are a major cause of disability, morbidity, and even mortality, particularly in elderly patients, therefore leading to a high economic burden. During fracture healing, the communication between immune cells and bone cells is crucial for the healing outcome. Macrophages represent a large proportion of immune cells with high plasticity present at the site of fracture; thus, they are good target cells for modulation to support the healing process. Interestingly, extremely low-frequency pulsed electromagnet fields (ELF-PEMFs) have been shown to exert cell-specific effects depending on the field conditions. Thus, we intended to identify specific ELF-PEMFs able to modulate macrophage activity to indirectly regulate mesenchymal stem/stromal cell (SCP-1 cells) function and promote fracture healing.

Materials and methods:

In this study, a two-step blinded screening was performed to find out immunomodulatory ELF-PEMFs from 22 different ELF-PEMFs. According to the influences on monocytes activation and the indirect effects on migration of SCP-1 cells, two fields (termed A and B) were chosen for further experiments. To explore the immunomodulatory effect of field A and field B, the levels of macrophage differentiation phenotype Arginase I, phospho-Stat1, and CD86 were evaluated by Western blot; intracellular reactive oxygen species (ROS) levels were detected with DCFH-DA probe; cytokine secretion was determined with a cytokine array. To verify the indirect influences of field A and field B on fracture healing, SCP-1 cells were cultured with the conditioned medium from the ELF-PEMF exposed macrophages. SCP-1 cell migration was determined as well as expression of extracellular matrix genes.

Results:

The unblinding showed that field A and field B have similar fundamental frequencies (51.8 Hz and 52.3 Hz) but are emitted in different groups of pulses or rather send-pause intervals. Macrophages exposed to field A showed pro-inflammatory function, represented by increased levels of phospho-Stat1 and CD86, accumulation of ROS, and increased secretion of pro-inflammatory cytokines. In contrast, macrophages exposed to field B showed anti-inflammatory and pro-healing function, represented by increased levels of Arginase I, increased secretion of anti-inflammatory cytokines, and growth factors known to induce healing processes. The conditioned medium from macrophages exposed to both ELF-PEMFs favored migration of SCP-1 cells, but the effect was stronger for field B. Furthermore, the conditioned medium from macrophages exposed to field B, but not to field A, stimulated expression of extracellular matrix genes in SCP-1 cells; *i.e., COL1A1, FN1,* and *BGN*.

Conclusion:

Our data show that specific ELF-PEMFs may affect immune cell function. Thus, knowing the specific ELF-PEMF conditions and underlying mechanisms bears great potential as an adjuvant treatment to modulate immune responses to support healing outcomes.

magnesium supplementation reduces cigarette smoke-associated osteoporotic-like changes in a three-dimensional bone co-culture system

ID: 1097

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Background:

Cigarette smoke (CS) is known to have harmful effects on bone homeostasis, contributing to increased risk of fragility fractures and impaired fracture healing. CS inhibits osteoblast activity and enhances osteoclast activity. A clinic study found that heavy smokers have less magnesium (Mg) serum level compared to nonsmokers. It is shown that Mg exerts a crucial role in bone metabolism. Our aim was to investigate the influence of Mg on bone cells functionality and viability under CS exposure.

Materials and methods:

We used an *in vitro* 3D bone co-culture model consisting of the human immortalized mesenchymal stem cell line (SCP-1, osteoblast precursor cells) and human myeloid cell line (THP-1, osteoclast precursor cells), seeded in a 1:8 ratio and cultured on a gelatin (GEL)-based cryogel. 3D co-cultures were exposed to 5% cigarette smoke extract (CSE) with or without 1 - 4 mMol/L magnesium chloride ($MgCl_2$). Bone cells functionality and viability were evaluated after 3, 7, and 14 days of culture.

Results:

$MgCl_2$ at the concentration up to 4 mM was non-toxic to the 3D bone co-culture. As expected, exposure to 5% CSE significantly reduced the total DNA content in the 3D bone co-cultures. Additional supplementation with 4 mM $MgCl_2$ for 14 days significantly increased the total DNA content by 31% in the 3D bone co-cultures. Exposure to 5% CSE inhibited ostegenic function but increased osteoclastic function in the 3D bone co-cultures. Co-incubation of the CSE exposed 3D bone co-culture with 4 mM $MgCl_2$ reduced tartrate-resistant acid phosphatase 5b activity and carbonic anhydrase activity by 41% and 78%, respectively when compared to 3D bone co-cultures exposed to CSE alone. Collagen formation (PINP levels) was increased 1.7-fold and matrix metallopeptidase 9 activity was decreased 1.4-fold following treatment with 4 mM $MgCl_2$ compare to 5% CSE.

Conclusion:

Our data suggest that Mg supplementation improves bone metabolism under smoking conditions, therefore, Mg supplementation may be an effective supplement for heavy smokers with osteoporotic bones.

Cellular evaluation of bone metabolism in autistic mouse model
ID: 1107

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Background:
Autism spectrum disorder (ASD) is a developmental disorder with a major impact on health care and finance systems. A possible co-regulation of the disease through bone cells can have potential therapeutic impact. Therefore, this study aims to characterize bone tissue in mouse models of ASD.

Materials and methods:
To assess the effect of autism on bone metabolism, knockout mouse models of Neuroligin 3 (*NL3*$^{-/-}$, n=5) and Neuroligin 4 (*NL4*$^{-/-}$, n=15) were compared with wild type (WT, n=12). After the establishment of autistic behaviour, whole body Dual X-ray Absortiometry (DXA) was carried out. After euthanasia, long bones were collected followed by radiological, biomechanical, and histological analysis of hind limbs.

Results:
Whole body DXA showed slightly higher Bone Mineral Density (BMD) in *NL4*$^{-/-}$ (Mean±SD: 0.077 ± 0.007) compared with WT (0.074 ± 0.0121). Fat% was significantly lower in *NL4*$^{-/-}$ compared with the WT (p=0.005). BV/TV of the tibia was higher in *NL3*$^{-/-}$ and *NL4*$^{-/-}$ (59.90 ± 4.669; 58.70 ± 5.895, respectively) compared with WT (55.31 ± 4.075). Biomechanical testing revealed lower bending stiffness in *NL3*$^{-/-}$ (59.26 ± 10.850), and higher in *NL4*$^{-/-}$ (67.39 ± 8.800) compared with WT (63.36 ± 11.938). Movat Pentachrome stain of femora showed significantly lower non-mineralized matrix in *NL3*$^{-/-}$ when compared with *NL4*$^{-/-}$ (p = 0.011) and WT (p = 0.019). Higher trend of cartilage tissue area was seen in *NL3*$^{-/-}$ when compared with both WT and *NL4*$^{-/-}$. Whereas, no difference in the calcified tissue area was seen between the groups. Although lower in both knockouts than WT, osteoclast activity assessment using TRAP enzymehistochemistry showed no significant difference between the groups.

Conclusion:
ASD is a group of behavioural disorders clinically defined by significant function impairment. Neumeyer et al. 2015 reported the higher frequency of fractures with lower BMD among young adults with ASD. Moreover, the only approved drugs (risperidone and aripiprazole) are steroid-based and were reported to increase fracture risk. Yet, no previous study has addressed bone quality in autism preclinical models or patients. This study shows discrepancies in fat accumulation, bone density and cellular imbalance suggesting a regulatory role for bone cells in autism. Correlation between bone and neurodegenerative diseases is well established. In diseases such as neurofibromatosis and Alzheimer's, bone tissue management and mesenchymal cell therapy have shown promising effects. Currently we are addressing further serum and molecular bone parameters, and investigating monocytes populations in bone marrow to further analysis the correlation between autism and bone.

Intraoperative cone beam CT in hybrid operating room set-up as an alternative to postoperative CT for pedicle screw breach detection

ID: 1112

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Background:

CT is considered the gold standard for detecting pedicle breach. However, CBCT may be a viable and low radiation dose alternative to provide intraoperative feedback to surgeons to permit in-room revisions of misplaced screws.

Methods*:*

To assess the ability and reliability of intraoperative cone-beam CT (CBCT) from a robotic C-arm in a hybrid operating room (OR) two hundred forty-one pedicle screws were inserted in cervical, thoracic and lumbar spine of 7 cadavers followed by CBCT and CT imaging. The CT images served as the standard of reference. Agreement on screw placement between both imaging systems was assessed using Cohen's Kappa coefficient (κ). Sensitivity, specificity, receiver operating characteristic (ROC), area under the empirical and fitted ROC curves (AUC) were computed to assess CBCT as a diagnostic tool compared to CT. The patient effective radiation dose (ED) was calculated for comparison. A systematic literature review was performed to provide perspective to the obtained results.

Results:

Almost perfect agreement in assessing pedicle screw grading between CBCT and CT was observed (κ=0.84). The sensitivity and specificity of CBCT were 0.84 and 0.98 respectively. The AUC derived from the empirical and fitted ROC curves were 0.95 and 0.96.

Conclusion:

Intraoperative CBCT by C-arm in a hybrid OR is highly reliable in identification of screw placement at significant dose reduction.

Keywords:

Hybrid operating room, cone beam computed tomography, pedicle screw placement, pedicle breach, minimally invasive spine surgery, dose reduction

Institutional Review Board Statement:

The study was conducted according to the guidelines of the Declaration of Helsinki. Ethics approval was obtained for a cadaver study from the ethics committee of the Christian-Albrecht-University Kiel, Germany.

Informed Consent Statement:

Informed consent was obtained from all subjects involved in the study before decease.

Outcome of ankle fractures with fracture to the posterior malleolus
ID: 47

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Background:
Complex ankle fractures with fractures of the posterior malleolus (PM) appear to have poor prognosis. The type of care the PM is given could have an impact on the treatment outcome. The aim of this systematic literature review was to investigate the influence of the different treatment strategies for PM on clinical outcomes.

Materials and methods:
Systematic literature research (MEDLINE (PubMed), CINAHL, Scopus, Central and EMBASE) was performed according to the PICOS and PRISMA guidelines. Studies that examined acute, unilateral ankle fractures in patients> 16 years of age and compared different treatment strategies for the management of PM fractures were included. One of the groups had to been treated with open reduction and internal fixation (ORIF). The studies had to show patient-reported outcome measure (PROM) separately for each individual treatment group. The review was registered in PROSPERO (International prospective register of systematic reviews). Study selection and data extraction was carried out by 2 independent investigators (RCM, VH).

Results:
The search strategy resulted in 8682 publications, of which 13 studies met the inclusion and exclusion criteria. 2 studies were prospectively randomized, 3 prospective cohort studies and 8 retrospective cohort studies. In 4 studies only patients with PM fractures >25% of the articular surface were included, in the other 9 studies the PM was treated regardless of its size. The mean number of patients in the studies was 59.77 (range 23-116) and the follow-up period across all studies was between 12 and 160 months. The studies had between 2 to 3 arms in total. 7 studies compared ORIF with untreated PM. Of these, 2 study showed significantly better AOFAS values for the ORIF group. The remaining studies could not show any difference. 8 studies compared ORIF with closed reduction with screws from anterior to posterior (CRIF). Of these, 3 showed significantly better results for the AOFAS for ORIF.

Conclusion:
In summary, the results of the existing studies do not allow a clear recommendation. This is due to the small study collection with heterogenous groups and the heterogenous follow-up examinations. High quality randomized controlled trials (RCTs) are necessary to develop clear treatment recommendations.

Artificial Intelligence in classification of acetabular fractures - Performance of Deep neural networks using the Letournel classification

ID: 732

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Background:

Fracture detection by Deep Convolutional Neural Networks (DCNNs) is a topic of growing interest in current orthopedic and radiological research. Acetabular fractures (AF) pose a particular challenge, because of its rareness and complexity in anatomy. We trained a DCNN to detect and classify AF using computer tomography (CT) scans. Therefore, a multicentric approach was established to collect and assess high-quality data.

Materials and methods:

The study was approved by the local Ethics Committee. All processed datasets were anonymized by a self-programmed tool. Letournel classification was used as a reference. CT scans from 1273 patients were collected from 11 German hospitals. 991 scans remained after data curation and preprocessing. Manual labeling of all scans was performed with one out of the ten Letournel classes. Four different neural network architectures (I3D, ResNet, ResNeXt, EfficientNet) were tested and evaluated via an exhaustive nested five-fold cross-validation with hyperparameter tuning in the inner loop. Performance was primarily evaluated via Matthews' correlation coefficient (MCC).

Results:

Comparing four different neural network architectures, I3D showed the best performance. I3D uses a network architecture originally proposed for object recognition in videos, performed best, yielding an average MCC of 0.5 (other three architectures: average MCC 0.35-0.4). Average Top-1 accuracy of I3D around 0.6 was identified (average top-3 accuracy around 0.8.). Classification mistakes often pertain very similar fracture types.

Conclusion:

DCNNs are able to detect and classify AFs. Performance of the I3D model is currently comparable to clinicians with relatively little experience. More training data is likely to boost the performance of the model in the future.