

Abstracts

7th Meeting of the European Society of Tissue Regeneration in Orthopaedics and
Traumatology (ESTROT)

FROM BENCH TO PATIENT BEDSIDE: LATEST ADVANCES AND INNOVATIONS
IN TISSUE REGENERATION AND REPAIR

July 3-5, 2023

Frankfurt am Main Germany

Congress President:
Ingo Marzi
Frankfurt am Main, Germany

Abstracts Note

Dear colleagues, dear friends,
the 7th Meeting of the European Society of Tissue Regeneration in Orthopaedics and
Traumatology (ESTROT) 2023 was to take place in Frankfurt, on July 3-5. We are very pleased
that we have been able to put together a top-class program with the following main topics:

- Bone regeneration:
Non-union, bone defects, bone voids, avascular necrosis. The role of stem cells,
Scaffolds, Growth factors, Composite grafts, Physical stimulation
- Cartilage Regeneration
Matrices, scaffolds, chondrocyte re-implantation, biological response modifiers
- Nerve and muscle regeneration
Latest advances
- Soft tissue reconstruction
VAC devices, Growth promoting factors, Artificial skin, Composite flaps
- Fracture related infection
Preventative strategies for bone infection Modern treatment of osteomyelitis
- Surgical techniques to improve outcomes

We hope that reading through the abstracts will remind you of the wonderful event and help
you in your everyday clinical or research work.

Ingo Marzi

Congress President 2023

President ESTROT:
Prof. Peter V. Giannoudis
Leeds, United Kingdom



Abstracts from the 7th European Society of Tissue Regeneration in Orthopaedics and Traumatology (ESTROT)

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MATRIX-ASSOCIATED AUTOLOGOUS CHONDROCYTE IMPLANTATION (MACI) IN THE KNEE

Clinical results of patients with and without concomitant knee pathologies

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MACI has been used effectively for the treatment of cartilage defects in the knee for several years. The application is restricted by accompanying damage to the internal knee structures, axis misalignments and osteochondral defects, which must be corrected parallel to or in temporal proximity of the MACI. A large number of the studies currently available only included patients with "optimal" knee conditions, i.e. without the need for additional interventions. The aim of this study was to determine the current status of the treated knee in patients with and without additional interventions and to determine their subjective assessment of the surgical success. In addition, we searched for possible influencing factors on subjective surgical success.

124 patients who received a MACI in the period 2011 up to and including 2019 were included in the study. Data was collected using questionnaires and patient files. Subjective surgical success was assumed when people rated the postoperative knee condition as "better". The chi-square test and Mann-Whitney U test was used for group comparisons, and correlations were calculated using the t-test or binary logistic regression.

Patients with and without additional interventions did not differ significantly in the following scores: KOOS (66.7 without vs. 66.5 with; p 0.878), Lysholm (69.8 without vs. 72 with; p 0.417), TAS (3.5 without vs. 3.6 with; p 0.949) and IKDC (63.8 without vs. 64.3 with; p 0.963). In the group with additional interventions, 32 of 70 people (46%) rated the postoperative condition as "better", in the group with no additional interventions it was 30 of 52 (58%), this difference was not significant (p 0.191). In the study population, age was weakly positively correlated with damage size (r 0.245; p 0.007). There was a negative correlation between rating the postoperative condition as "better" and the damage size, this was statistically significant (OR 0.795, 95% CI [0.646 - 0.978]), the influence of age was not (OR 0.971, 95% CI [0.941 - 1.002]). Nevertheless, the group with the greatest damage (≥ 6.0 cm²) rated the condition as "better" significantly more often than the other groups (" ≤ 3.0 cm²" p 0.016; "> 3.0 - < 6.0 cm²" p 0.022) did. A subgroup analysis showed that people ≥ 50 years rated their condition as "better" significantly more often than the groups "< 35 years" (p 0.006) and " ≥ 35 - < 50 years" (p 0.046) did.

In this study population, similarly good results were achieved in patients with and without additional surgery. Despite the limitations of this study, this coincides with the results of other studies, nevertheless future studies should compare patients with and without concomitant

pathologies in a more targeted manner. It is interesting that older people and those with greater damage rated their postoperative knee condition significantly more often as "better". A possible explanation is that greater damage may be more impairing and older people tend to have lower functional demands.

IMPACT OF TSG-6 ON THE HEALING OF CRITICAL-SIZED BONE DEFECTS IN MICE

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Introduction Critical-sized bone defects are a challenging clinical problem with a high risk of impaired regeneration and non-union development. Therefore, novel therapeutic approaches to enhance bone repair are needed. TNF-stimulated gene 6 protein (TSG-6), a protein with anti-inflammatory and pro-regenerative properties, was shown to be secreted by various cell types including mesenchymal stem cells and to improve wound healing. As wound healing and bone healing share similar features, we were interested whether TSG-6 can also stimulate bone regeneration.

Material and Methods The impact of TSG-6 on bone healing was studied in 12-weeks-old male C57BL/6J mice. A critical-sized defect (1.5mm) was created in the femur and stabilized by an external fixator. A collagen type I gel (5 mg/mL) served as a carrier to transfer 10 or 50µg recombinant human TSG-6 (rhTSG-6) into the bone defect. An unloaded collagen gel served as control. Bone regeneration was analysed using microcomputed tomography and histomorphometry on day 35 post-surgery ($n=6$ /group, ANOVA with post hoc Fisher's LSD test, $p<0.05$). Furthermore, bony bridging was evaluated in between the cortices using a scoring system, where a score of zero means no bony bridging and four a complete bony bridging of the cortices.

Results The low dosage of 10µg rhTSG-6 was insufficient to improve bone healing. Notably, the higher dosage of 50µg rhTSG-6 increased bone volume (rhTSG-6 vs. collagen gel: 0.92 mm³ vs. 0.5mm³, $p=0.079$) and significantly reduced relative fibrous tissue area (rhTSG-6 vs. collagen gel: 37% vs 63%, $p=0.040$) compared to the control group. The evaluation of the bony bridging revealed that 67 % of the fractures were healed (bony bridging score ≥ 3) in the 50µg rhTSG-6 group, compared to 33% in the control group.

Conclusion Our data showed that rhTSG-6 treatment considerably improved bone regeneration. We are currently further investigating the role of TSG-6 *in vivo* in the early phase of critical-sized bone defect healing and *in vitro* during osteoblast- and osteoclastogenesis.

Conflict of Interest No conflict of interest.

A NOVEL MARKER OF WOUND RESPONSE: THE PHOSPHORYLATED RIBOSOMAL PROTEIN S6

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The spatial boundaries of tissue response to wounding are unknown and difficult to visualise. We have identified a novel wound marker: the ribosomal protein S6 (rpS6) is phosphorylated in response to skin injury forming a zone of activation enveloping the initial insult. This p-rpS6-zone forms immediately after wounding and is present until healing is complete. The zone encapsulates markers of the healing process, including proliferation, angiogenesis and senescence. This wound response is global: using a model of skin injury in pig, mouse and human *ex vivo* skin we were able to confirm that the wound response is conserved in mammals, and shows up in response to various injuries (burns, excision wound, needle prick). A transgenic mouse model unable to phosphorylate rpS6 shows an initial acceleration of wound closure, but results in disrupted healing, confirming an important role for p-rpS6 in healing. Along with its role in healing, the zone is an ideal wound marker as it correlates with healing progress over weeks and accurately reports on the status of dermal vasculature. The p-rpS6-zone is a promising diagnostic tool which divides an otherwise homogeneous tissue into regions with different properties, clearly separating tissue undergoing a wound response from unaffected tissue.

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Titel:

One stage Masquelets technique-

Evaluation of Different Forms of Membrane Filling

Introduction: The membrane technique (Masquelet technique) is a widely used and effective procedure for the treatment of large bone defects, which is performed in two steps. Our group was able to show that the induced membrane can be replaced by a decellularised dermis (Epiflex, DIZG). Thus, the two-stage procedure can be shortened to a one-stage procedure. Until now, syngeneic cancellous bone has been used to fill the membrane defect. Our current animal study will look at other filling options besides syngeneic cancellous bone.

Methods: The focus of this work was to clarify whether syngeneic cancellous bone (SCB) can be replaced by granular (g-DBM) or fibrous (f-DBM) demineralised bone matrix. In 63 Sprague-Dawley rats, a critical femoral defect of 5 mm length was stabilised with a plate and then encased in decellularised dermis. Filling with syngeneic cancellous bone served as a control group. The defect was then filled with SCB, g-DBM or f-DBM. After a healing period of eight weeks, the femurs were harvested and subjected to histological, radiological and biomechanical analysis.

Results: The analyses showed an incipient bony bridging of the defect zone after 8 weeks in all groups. All groups showed histologically equivalent new bone formation. The biomechanical analysis of the

explanted femora also showed no significant difference between the g-DBM group and the SCB group.

Discussion: Cancellous bone as a defect filler in the one-stage membrane technique could be replaced, so patients could be relieved from discomfort due to the removal.

Disclosure statement: The authors declare that they have no conflicts of interest.

Ethical disclosure: All animal experiments were performed according to the applicable regulations of the Animal Protection and Monitoring Committee of our institution (project no. FK/1075; Regierungspräsidium, Darmstadt, Germany) in accordance with German law.

Tissue impregnated bone substitutes for the promotion of bone healing

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Background In the field of orthopaedics and traumatology we come across bone defects of a critical size the treatment of which is challenging. The gold standard on treating such defects still remains the autologous bone grafting, even though it can be associated with relevant comorbidities. In vitro studies suggest a strong osteogenic regeneration potential of surgical site released tissue. However, the clinical value and application of this tissue is discussed in controversy. In some instances, there is no solid scaffold on the surgical site allowing cell adherence and guided growth to fulfill and heal large defect zones. Therefore, additional bone substitute materials can be required. We investigated the healing process of bone defects at risk of non-healing as well as defects in risk of delayed healing using a combination of bone substitute material and surgical site release tissue. To optimize penetration of the harvested tissue into the porous bone substitute (beta tricalcium-phosphate/TCP) we applied vacuum within an innovative tissue collector (BoneFlo®).

Materials and methods In a prospective study we report on 40 non-randomized patients with critical size bone defects or osseous defects with an increased risk of non-healing (pseudarthroses, enchondroma...), treated with a bone substitute material (TCP, bone allograft), vitalized by autologous surgical-site released tissue. The minimal follow-up period for these patients was 6 months. The study concluded the safety and efficiency of the biomaterials impregnated and activated by the tissue collected intraoperatively. The healing of such defects was documented and presented in relevant time intervals. The study provides the clinical and radiological progression of the patients in the postoperative period.

Results Regarding the intraoperative use of the suction handle device, there were no complications documented with neither regular or off-label use. The suction ability of the handle was not influenced with or without the use of a bone substitute (bone allograft, β -TCP). The filter has proven to be more than sufficient for collecting a considerable amount of surgical site release tissue, that could be easily extracted intraoperatively with surgical forceps and further reintroduced in the bone defect.

All forty probands showed a remarkable bone regeneration. In 37 patient healing of the defect was documented. The cumulative radiological results show a uniform bone formation covering most of or even sometimes the whole defect. The β -TCP as well as the bone allografts were integrated into the bone and were proven to provide a very good alternative to autologous bone grafting. The newly formed bone showed to be stable under the stress applied by performing daily activities. The individual minerals released from β -TCP crystals were absorbed and slowly intergraded into the bone.

Conclusions The BoneFlo® system as a tissue collector device is proven easy and simple to operate. As an off-label application,

the system provides an opportunity to treat bone defects or delayed bone healing. We believe, that vacuum coated and bioactivated bone substitute materials have strong potential to reduce the amount of autologous bone grafting, and therefore surgery time and associated patient's risk. In our opinion autologous tissue collectors can be a useful tool for in situ tissue regeneration.

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EFFECTS OF CARTILAGE EXTRACELLULAR MATRIX COMPONENTS ON OSTEOARTHRITIS-RELEVANT CELLS

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Osteoarthritis is a degenerative disease affecting all structures in the joint. During OA progression, the cartilage extracellular matrix undergoes significant alterations: expression patterns change, matrix components are cleaved by different enzymes and are released – intact or as fragments - from the matrix. These released ECM components can then activate joint resident cells including chondrocytes but also immune cells such as macrophages.

Two proteins whose expression is known to change in OA are thrombospondin 4 (TSP-4) and cartilage oligomeric matrix protein (COMP). Both are also cleaved by various proteases present in the osteoarthritic joint including MMP13 and cleavage products were detected in synovial fluid and serum of OA patients. Here we investigated the effect of intact and MMP13-cleaved TSP-4 and COMP on chondrocytes and macrophages.

In chondrocytes, intact TSP-4 and COMP led to low-grade activation of pro-inflammatory signalling pathways including NFκB-p65 but did not significantly alter the expression of anabolic genes such as ACAN, nor were catabolic factors including ColX, MMP-3 and -13 significantly induced. In macrophages, TSP-4 and COMP reduced the expression of CD80 significantly when the proteins were present during M1-polarization. Short-term exposure of M0-macrophages to low levels of TSP-4 and COMP reduced the expression of IL-1β and TNFα upon an inflammatory stimulus, while high levels seem to increase the expression. Furthermore, high levels of COMP

significantly reduced the Bax/Bcl-2 ratio in M1 macrophages, indicating decreased apoptosis, while TSP4 had no influence.

Incubation of chondrocytes with fragments of TSP-4 and COMP led to the activation of the NFκB-p65 and p38 signalling pathways indicated by increased phosphorylation. However, MMP-13 alone also led to p65 and p38-phosphorylation to a certain degree. Interestingly, especially COMP fragments seemed to decrease the activation of ERK1/2 under the level of the control treated with medium only and to cells treated with intact COMP.

In line with previous observations, intact TSPs seem to preserve the chondrocyte phenotype, especially catabolic factors were not significantly induced. The observation that both TSP-4 and COMP also reduce the expression of the M1 marker CD80 and the expression of pro-inflammatory cytokines under inflammatory conditions hints to a protective role of both proteins. Fragments of TSP4 and COMP might contribute to a pro-inflammatory milieu in the joint as pro-inflammatory signalling pathways were activated. The contribution of MMP13 to this as well as functional consequences of the stimulation with TSP fragments will be dissected in future experiments.

THE USE OF ANTIBIOTIC-IMPREGNATED CANCELLOUS BONE GRAFTS IN ONE-STAGE SURGERY FOR LONG BONE FRACTURE RELATED INFECTIONS: A CASE SERIES

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Objectives The use of cancellous bone allografts is an established technique in bone reconstruction surgery, but its use is avoided in the presence of infection. Purified cancellous bone grafts impregnated with antibiotics (AIBG) have shown their effectiveness as a local antibiotic delivery system.

This study evaluates the clinical and radiographic outcomes of long bone fracture-related infections (FRI) with use of AIBG in a one-stage treatment.

Study design and methods

Study design: retrospective consecutive case series retrieved from the AIBG database at a level 1 trauma centre.

Recruitment period: from June 2018 till July 2021

Inclusion criteria: long bone FRI treated in a single stage and with a minimum follow up of 9 months

Population There were 24 patients in total (17 men and 7 women) with an average age of 55 years (30 to 75). 13 patients had an infected nonunion and 11 patients an osteomyelitis. 20 internal fixation implants were present preoperatively, 14 postoperatively (8 x plates, 4 x nails, 2 x screws of which 11 implants replacements, 1 DAIR and 2 first implants).

Deep tissue specimens revealed a single germ in 18 out of 24 cases: 12 *Staphylococcus* (6 *epidermidis*); one *Streptococcus*; three *Cutibacterium* and two gram-negative. Four patients had a polymicrobial infection; two had negative culture.

Bone grafts impregnated with vancomycin were used in all cases and bone grafts with tobramycin were used in 8 cases.

Result After a mean follow-up of 16 months there were 2 recurrences of infection: one persistent clavicle nonunion infected with *Cutibacterium* and one persistent distal tibia nonunion infected with *Staphylococcus epidermidis*. There were two persistent nonunions without infection (one distal femur, one distal tibia) requiring secondary stabilisation surgery.

Conclusion Elimination of infection following one stage surgical treatment of FRI with the use of AIBG was successful in 91% of cases comparing favourably with multi stage approaches.

Ex vivo pretreatment of mesenchymal stem cells with electrical stimulation as strategy to improve bone tissue engineering outcomes

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Delayed and non-healing large bone defects represent a major challenge for patients and surgeons and rise the costs for the healthcare systems. Electrical stimulation (ES) alone or in combination with bone tissue engineering (BTE) approaches was shown to promote bone healing. However, its application in clinical practice faces challenges like the need to surgically implant, monitor, and explant the ES device. ES could exert its pro-healing effect by enhancing cell migration, proliferation, alignment, differentiation and attachment to scaffolds. In previous *in vitro* studies, mesenchymal stem cells (MSC) were exposed alone or onto scaffolds (to simulate BTE treatments) to ES 1h/day and significant increases in alkaline phosphatase activity, and expression of osteogenic gene markers were observed. Importantly, these positive osteogenic effects were shown to persist after discontinuation of treatment. Based on these findings, we hypothesized that pretreating BTE constructs (MSC + scaffold) with ES can produce a sustained long-lasting increase in osteogenic activity when used to treat large bone defects.

Methods: femur critical size defects were created in 120 Sprague-Dawley rats and treated with MSC + β -TCP scaffold constructs, *in vitro* pre-exposed or not (control group) to ES for 7 days in 2D- (cells alone) or 3D-culture (MSC + scaffold). Bone healing was evaluated at 1-, 4-, and 8-weeks post-surgery via μ CT, mechanical test, histological and immunohistochemical staining and gene expression analysis.

Results and discussion: treatment was well tolerated by all the animals and bone healing increased along the time. Although bone mineral density, mechanical strength, amount of new bone, cartilage, fibrocartilage, and vascularization were slightly better in defects treated with constructs pre-exposed to ES in 3D, these differences were not statistically significant. Concerning gene expression, no differences were detected when comparing ES treated vs. control group. We speculate, that the environmental conditions during the first stage of bone healing characterized by inflammatory response and poor blood supply could counteract the long-lasting pro-osteogenic effect of pretreatment with ES.

Conclusion: Pretreating stem cells with electrical stimulation for 7 days prior to their use in BTE is not efficient to improve bone healing outcomes. Thus, ES seems to be more effective during bone healing as indicated by other studies.

Cathepsin expression in human fracture hematoma is associated with fracture healing phases and patient age

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INTRODUCTION: A fracture hematoma (fxH) is formed as soon as a fracture occurs. This fxH plays important roles in the initiation and progression of the fracture healing cascade since it is a microenvironment that contains a variety of cells that coordinate fracture healing. Cathepsins, a type of lysosomal enzymes, are involved in various cellular processes and may influence inflammatory and metabolic processes in the fxH. In particular, cathepsin D (CTSD) and cathepsin K (CTSK) have been linked to inflammatory and osteogenic processes and are therefore of interest. The aim of this study was therefore to determine the expression of CTSD and CTSK in human fxH and examine the potential influence of patient characteristics on their expression levels. **METHODS:** FxH was harvested during fracture surgery of long bones. Subsequently, mRNA was isolated, transcribed, and analysed using qPCR. Expression levels of Cathepsin D (CTSD) and Cathepsin K (CTSK) were analysed based on a broad literature study on fracture healing, osteogenesis, and the immune response after fracture. GAPDH and TBP were used as housekeeper genes. A threshold of $|\Delta Cq| > 2$ was used above which the respective mRNA was considered significantly deregulated. Potential correlations between gene expression and patient characteristics were examined using a multiple linear regression model containing patient age, sex, difference in days between trauma and surgery, smoking (in pack years), and trauma severity.

RESULTS: In total, fxH was harvested from 58 patients (mean age 52 ± 19 ; 30♀), ranging from zero to 19 days after trauma. The expression of CTSD was lower when compared to CTSK, but was detected in all fxH samples. The expression of CTSD was significantly correlated to both patient age, and the difference in days between trauma and surgery. Increased patient age significantly reduced the expression of CTSD, while a longer time interval between trauma and surgery enhanced CTSD expression. Contrarily to CTSD, CTSK expression was independent from any of the patient characteristics in the multiple linear regression model, but did show a universal upregulation among all patients.

DISCUSSION: This study revealed expression profiles of CTSD and CTSK in human fxH, linked to key processes in the fracture healing cascade. The expression levels of CTSD significantly correlated to several patient characteristics and was mainly involved in the immune response after fracture, in particular the inflammatory response. Compared to CTSD, CTSK was upregulated in all fxH samples throughout the early fracture healing cascade until 19 days after trauma. This universal expression throughout the early fracture healing cascade matches CTSK's functions, such as osteoblast functioning and osteoclast activation. These data broaden our view on the potential roles of lysosomal enzymes in fracture healing.

Surgical trauma treatment strategy influences systemic inflammation and local fracture healing mechanisms

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INTRODUCTION: Surgical treatment after multiple trauma is considered the so-called 'second hit' after the initial trauma itself. Long, invasive surgical interventions cause excessive inflammation and can be harmful to a patient's condition. The invasiveness and timing of the primary surgery after multiple trauma are important components of two main trauma-treatment strategies: Damage-Control-Orthopaedics (DCO) and Early-Total-Care (ETC). Both treatment strategies have their assets and liabilities, but exact cellular mechanisms that cause their differential effects on the fracture healing cascade are not fully understood. The fracture hematoma (fxH) is key in initiating and prolongating the fracture healing cascade, a process that is mainly regulated by protein expression. Although proteomics is increasingly applied in trauma research, it mainly focusses on circulatory proteins rather than local proteins at the site of injury. The aim of this study was therefore to investigate the proteome of the fxH in a multiple trauma model, comparing two surgical treatments.

METHODS: The porcine multiple trauma model consisted of bilateral femur fractures, liver laceration, blunt chest trauma, and controlled haemorrhagic shock. Animals were surgically and medically stabilised, followed by a 72-hour time period of ICU monitoring. Two fracture treatments were applied; external fixation (DCO; n=8), and intramedullary nailing (ETC; n=7). FxH were sampled, snap-frozen and stored at -80°C. Samples were sectioned and proteins were isolated, gel separated, followed by protein band collection and in-gel digestion. Digested samples were run on a tandem mass-spectrometry system. Proteins were identified using proteome discoverer software followed by the calculation of their respective abundance ratios and fold changes.

RESULTS: Label-free proteomics analyses identified a total of 2014 common proteins in fxH samples from both treatment groups. In total, 83 and 176 proteins were exclusively expressed in fxH samples from the DCO and ETC groups, respectively. Of these proteins, 30 proteins showed a statistically significant difference in expression ($p \leq 0.05$) between the two groups. Among those, 11 and 19 proteins showed higher abundance in the DCO and ETC groups respectively. Protein interaction networks were generated using STRING software. The significantly deregulated proteins were mainly involved in signalling pathways related to osteogenic differentiation, inflammation, and the trauma immune response.

DISCUSSION: This study is the first to describe the proteome of the fxH, showing that label-free proteomics is a suitable analytical tool for fxH proteomics. The degree of surgical invasiveness had a clear effect on the fxH proteome at the injury site, causing treatment-specific proteome changes that were linked to key processes in inflammation and fracture healing, such as the enhanced activation of the complement system, increased osteoblastogenesis, and immune cell attraction. These proteome changes indicate that DCO prompted more balanced systemic inflammatory responses, while ETC seemed to elicit more advanced fracture healing responses in the acute phase after trauma.

Rifampicin-loaded polymethylmethacrylate: is it possible to preserve mechanical properties and setting time?

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Background: Two-stage exchange with antibiotic-loaded bone cement spacers remains the gold standard for chronic periprosthetic infection. Rifampicin is highly efficient on stationary-phase staphylococci in

biofilm; however, its addition to PMMA to manufacture spacers prevents polymerization and reduces mechanical properties.

Objectives: Isolation of rifampicin during polymerization by microencapsulation, which could allow manufacturing rifampicin-loaded bone cement maintaining elution and mechanical properties.

Design and methods: Microcapsules of rifampicin with alginate, polyhydroxybutyrate (PHBV), ethylcellulose and stearic acid were synthesized. Rifampicin elution to phosphate buffer was measured by UV-visible spectroscopy. Alginate and PHBV microcapsules were added to bone cement CMW[®]1 and elution, compression, bending, hardness and setting time tests were performed. Antimicrobial activity was assessed by disk diffusion test with *S.aureus* ATCC[®]29213TM. Repeated measures ANOVA and Bonferroni post-hoc test using SPSS version 22.0 were performed, considering a $p < 0.05$ as statistical significance.

Results: Alginate microcapsules showed the highest rifampicin elution ($p = 0.0001$). Bone cement specimens containing alginate microcapsules eluted more rifampicin than PHBV microcapsules or non-encapsulated rifampicin over time ($p < 0.012$). Addition of microcapsules to PMMA did not significantly alter the bending modulus compared to control cement ($p = 1$). Cement with alginate microcapsules showed similar behavior in hardness tests to control cement over the study period ($73 \pm 1.68 H_D$), reaching the maximum hardness within 15 minutes. Microencapsulated rifampicin preserved its antimicrobial properties. Statistically significant differences in mean values of diameters of zones of inhibition between alginate-rifampicin ($p = 0.0001$) and alginate-PHBV ($p = 0.0001$) were detected.

Conclusion: Rifampicin microencapsulation with alginate is the best choice to introduce rifampicin in PMMA, preserving mechanical properties, setting time, elution and antimicrobial properties. The ability to obtain rifampicin-loaded bone cement would allow achieving high doses of rifampicin in infected tissues, increasing the successful of periprosthetic infection treatment.

CAN HUMAN MSC BE FROZEN DIRECTLY ON THE SCAFFOLD AND USED FOR BONE DEFECT GRAFTING?

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Introduction: Clinical bone tissue engineering (BTE) approaches have shown promising early results, however, a bottleneck in the process is the need of stem cell supply. The translation of BTE techniques into the clinic is hampered by the intensive time- and financial- commitment required for the isolation, characterization, and expansion of autologous mesenchymal stem cells (MSCs). In our recent in vitro study, we have shown that human (h)MSC frozen and thawed on a β -TCP scaffold maintained their metabolic activity and displayed favorable osteogenic potential after cryopreservation. The present functional animal study is focused on the evaluation of the osteoinductive potency of cryopreserved and stored at different temperature hMSC- β -TCP constructs in a large femoral defect. **Methods:** osteoinductive potency of cell seeded granules was evaluated in previously established femur critical size defect (CSD) model in nude rats. Bone defects were loaded with: β -TCP granules alone (negative control group, n=10); rat donor cancellous bone (positive control group, n=10); β -TCP granules seeded with hMSC (n=10) or β -TCP granules seeded with hMSC, cryopreserved and stored either in liquid nitrogen, or at -80°, -20° or dry ice (each n=10). After 8 weeks healing time, bones were collected and bone healing was measured and compared among groups by mean of μ CT, bone mechanical and histological analysis.

Results and discussion: Overall analysis revealed that the procedure was carried out successfully, however comparing to the cancellous

bone treatment group, bone repair was generally poor in all groups, regardless of the type of treatment. We also found significant increase of CD68+ cells in all groups except cancellous bone group. According to histological analysis, the constructs stored at -80°C or in liquid nitrogen showed the same bone growth activity as freshly prepared constructs. At the same time, the construct stored on dry ice or at -20°C showed a lower bone growth activity.

In conclusion, our *in vivo* studies, similar to previous *in vitro* studies, have shown that human MSC can be frozen on the scaffold and used for bone defect grafting. However, the storage temperature of the frozen constructs plays an important role in the bone healing effect. The reduced bone regeneration found in all groups is related to the *in vivo* model used (athymic rat); however it is currently not possible to find an alternative *in vivo* model for testing human cells.

MSCS AND BIOSCAFFOLDS IN COMPLEX ORTHOPAEDIC SURGERY: OUR RESEARCH FOR THE FUTURE

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OBJECTIVE: New methods have been developed to achieve tissue regeneration of complex bone defects and restore the healing process, which is impaired by several factors; in this contest bone tissue engineering (BTE) is an alternative to autologous gold-standard treatment. BTE combines biocompatible scaffolds with morphogenic signals and stem cells, to create a biomimetic microenvironment that provides mechanical and chemical cues. In this experimental study, we studied all key elements of BTE and evaluated Mesenchymal stem cells (MSC) with xenograft-derived bone scaffold (SmartBone® SBN) and rhBMP-2 as a growth factor.

MATERIALS AND METHODS: The experimental study, whose three cornerstones are mesenchymal stem cells (hMSCs), scaffolds and growth factors, aims to develop new tissue engineering strategies to be applied in orthopaedic surgery. MSCs are an attractive source of stem cells because of their ability to undergo self-renewal, multi-lineage differentiation (including into osteoblast lineage) and paracrine actions. We reported our experience in MSCs expansion protocols and proposed human Platelet-Rich Plasma (PRP) as a substitute for Fetal Bovine Serum (FBS) in media supplementation. After MSCs isolation from patients' bone marrow, we expanded the cells in media supplemented with FBS or PRP, obtained from a venous blood sample of the same patients. In the second part of the project, we seeded hBM-MSC on the xenograft-derived bone scaffold and cultured them in osteogenic and MSC expansion media to investigate the effects of the composition of support on cell metabolic activity and osteogenic differentiation.

RESULTS: The experimental study, currently underway, is providing excellent results.

The data suggest that PRP is a good cell culture supplement since it did not impact MSCs marker expression and differentiation potential. The scaffold retains biological properties and resembles the human bone structure. We demonstrated its biocompatibility supporting both BM-MSCs proliferation and differentiation. Moreover, new collagen deposition was revealed in both analysed conditions, suggesting a good osteoconductivity of the scaffold. Finally, we aimed to modify the scaffold by the addition of rhBMP-2 to improve its osteogenic abilities and enhance new bone formation. Future experiments will assess the impact of BMP2 modification on cellular differentiation.

CONCLUSIONS: In our opinion, MCSs are and will become more and more a valid tool to provide the surgeon with important help in cases of great complexity and therefore to obtain the tailored care that every patient needs and deserves.

ACHILLES TENOCYTES FROM DIABETIC AND NON DIABETIC DONORS EXPOSED TO HIGH- OR NORMOGLYCEMIC CONDITIONS RESPOND DIFFERENTIALLY TO INFLAMMATORY STIMULUS

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Diabetes mellitus type 2 (DMT2) is well known to predispose the Achilles tendon to degeneration and subsequent rupture. Nevertheless, the shared targets of DMT2's pathogenesis and tendon degeneration remain unclear. Oxidative stress, cytokine and complement dysregulation as well as myofibroblast transition could be involved. The aim of this study was to understand the specific responses of Achilles tendon (AS) tenocytes isolated from diabetic and non diabetic rats after exposure to normo- (NG) and highglycemic (HG) conditions in the presence of the proinflammatory cytokine TNF α . AS tenocytes were isolated either from adult diabetic (fa/fa) or lean (fa/+) Zucker Diabetic Fatty (ZDF) rats and exposed to 10 ng/mL TNF α either under NG or HG conditions (1 g/L *versus* 4.5 g/L glucose, 4 or 24 hours). Tenocyte survival and metabolic activity were assessed. Gene expression of the main tendon extracellular matrix (ECM) component collagen type I, suppressors of cytokine signaling (SOCS)1, -3 which are acting as negative feedback regulators of cytokine expression and the antioxidant defense enzyme hemoxygenase-1 (HMOX1) was analyzed by real time PCR. Synthesis of the myofibroblast marker α -smooth muscle actin (α SMA), the anaphylatoxin receptor C3aR and the complement regulatory protein CD46 were visualized by immunolabeling (24 hours) and the volumes of tenocyte cell nuclei were calculated based on 4',6-diamidin-2-phenylindol (DAPI) stain (24 hours). Tenocyte vitality was not affected by the treatment regime but the metabolic activity was slightly impaired by TNF α in tenocytes under NG conditions and was generally higher in cells of diabetic rats. Higher amounts of α SMA protein were visualized in tenocytes exposed to TNF α , irrespectively of their donor origin and culture conditions. The anaphylatoxin receptor C3aR protein was higher expressed in tenocytes from diabetic animals. CD46 was suppressed by TNF α (NG) in cells of diabetic rats. The areas of cell nuclei in tenocytes of diabetic rats were higher compared to those of lean animals. Collagen type I gene expression was suppressed by TNF α under NG condition in tenocytes of diabetic and lean animals after 24 hours. In tenocytes from lean donors, HMOX1 was induced by TNF α under both conditions (NG, HG, 24 hours), but not in those from diabetic rats at the same time. At 4 hours SOCS1 was induced by TNF α in cells of diabetic rats under both, LG and HG conditions, but not in those of leans. Under NG conditions SOCS3 gene transcription was slightly induced by TNF α after 4 hours and suppressed after 24 hours in animals of both genotypes (NG). The response of tenocytes to TNF α varies depending on the glucose supply and non/diabetic origin in regard to the regulation of mediators involved in tendon homeostasis suggesting irreversible impairment of tenocytes in DMT2.

The potential of scaffolds loaded with mouse iPSC and iPSC-derived extracellular matrix in treating critical size bone defects

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In certain situations, bone does not heal completely after fracturing. One of these situations are critical size bone defects where bone cannot heal spontaneously. In such a case, complex fracture treatment over a long time is required which bears a relevant risk of complications. Current methods used such as autologous and allogeneic grafts do not always lead to successful treatment results. Current approaches to increase bone formation to bridge the gap include application of stem cells in the fracture side. While most studies investigated the use of mesenchymal stem cells less evidence exists about induced pluripotent stem cells (iPSC). In this study, we investigated the potential of mouse iPSC-loaded scaffolds and decellularised scaffolds containing extracellular matrix from iPSCs for treating critical size bone defects in a mouse model.

In vitro differentiation followed by Alizarin Red staining and quantitative reverse transcription polymerase chain reaction confirmed the osteogenic differentiation potential of the iPSCs lines. Subsequently, an *in vivo* trial using a mouse model (n = 18) for critical size bone defect was conducted, in which a PLGA/aCaP osteoconductive scaffold was transplanted into the bone defect for 9 weeks. Three groups (each n = 6) were defined as 1) osteoconductive scaffold only (control), 2) iPSC-derived extracellular matrix seeded on scaffold, 3) iPSC seeded on scaffold.

Micro-CT and histological analysis show that iPSCs grafted onto an osteoconductive scaffold followed by induction of osteogenic differentiation resulted in significantly higher bone volume 9 weeks after implantation than an osteoconductive scaffold alone, suggesting an improvement in bone regeneration by the application of iPSCs.

CLINICAL, HISTOLOGICAL AND RADIOGRAPHIC EVIDENCE OF NEW BONE FORMATION AT THE PERIPHERY OF BONE DEFECTS USING ANTIBIOTIC-LOADED CALCIUM SULFATE BEADS IN BONE TRANSPORTS

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Purpose: The use of polymethylmethacrylate (PMMA) or absorbable antibiotic carriers in the form of cylindrical blocks has been described to fill the resection area in bone transports, in cases of trauma or osteomyelitis. The aim of this study was to describe the use of an absorbable antibiotic carrier in the form of beads, to improve biological and mechanical environment.

Methods: Five patients with post-traumatic osteomyelitis (type IV according to Cierny-Mader classification) underwent bone transport surgery at our Institution, using an absorbable calcium sulfate carrier in the form of beads, mixed with vancomycin and gentamycin, to fill the bone defect area.

Results: A bone-like tissue envelope progressively formed at the periphery of the defect area, along with the progressive absorption of the antibiotic carrier. Histological analysis showed newly formed bone tissue with high vascularization, high presence of osteoblasts, few osteoclasts, and no necrotic areas. This biologically active rigid envelope avoided the collapse of the soft tissues on the transport path, and also provided a biological stimulus to the docking site. The absorbability

of calcium sulfate avoided the need to surgically remove it, in order to clear the way for the transported bone segment.

Conclusion: The use of antibiotic-loaded calcium sulfate beads in bone transports allows better biological and mechanical support to the docking site and reduces the number of surgeries required.

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The Role of N1-N2 Neutrophil Phenotypes in Bone Regeneration: A Systematic Review

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Introduction: Neutrophils are the first cells to invade the fracture hematoma (FH). Neutrophils influence fracture healing both positively and negatively. Recent studies identified different neutrophil phenotypes and described their inflammatory (N1) and regenerative (N2) roles in other tissues than bone. We hypothesise that N1 and N2 neutrophils have different roles in bone regeneration, thus explaining the dual role of neutrophils.

Method: A search was performed in the PubMed database using (*neutrophil*) AND ((*bone regeneration*) OR (*fracture healing*)). Exclusion criteria were: no English language, published before 2012, review articles, case-reports, meta-analyses, the content about the regeneration of other tissues than bone, no description of the role of neutrophils in the bone regeneration field, subject limited to the interaction between materials and neutrophils. The article quality was evaluated using the ARRIVE 2.0 Checklist and NIH Quality Assessment of Case-Control Studies Tool.

Results: Twenty-one studies were included in this review: 17 pre-clinical *in vivo* studies, two *in vitro* studies, and two retrospective clinical studies. The 17 *in vivo* studies consisted of 13 rodent models, two rabbit models, one porcine model, and one study which used human samples. The two *in vitro* studies all used human samples. Two retrospective clinical studies investigated the relationship between the number of circulating neutrophils and the outcome of fracture healing. These studies illustrated that the number of neutrophils is important for a balanced inflammatory response as well as subsequent initiation and prolongation of fracture healing. An excessive presence of neutrophils, both locally at the fracture site and in the systemic circulation, can be detrimental for the initiation of the fracture healing cascade. On the other hand, neutrophil depletion at the fracture site or in the systemic circulation can lead to impaired fracture healing as well. Interleukin-8 and tumour necrosis factor- α are two cytokines that mainly participate in regulating the activity of neutrophils, which subsequently have either a stimulatory or an inhibitory effect on bone regeneration. Neutrophils can impact the downstream cascade of M1-M2 macrophages, but the exact mechanism is still unclear. Identifying the response of neutrophils to cytokines and their interactions with other cell types, particularly the contribution of N1-N2 neutrophil phenotype to the macrophage converting to M2 phenotype, is crucial for better understanding the crosslinking between immune response and fracture healing.

Conclusion: This systematic review identified several roles of neutrophils in relation to fracture healing. The identification of N1 and N2 neutrophil phenotypes may provide new insights into the temporal changes that occur in the fracture hematoma, with cytokine profiles and cell populations that change rapidly over time. In this way, both inflammatory and regenerative neutrophil functions in fracture healing meet.

THE INDUCED MEMBRANE TECHNIQUE IMPROVES THE HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH A POST-TRAUMATIC LONG BONE NON-UNION

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Background: The optimal treatment strategy for post-traumatic long bone non-unions is the subject of an ongoing discussion. At the Maastricht University Medical Center (MUMC+) the induced membrane technique is used to treat post-traumatic long bone non-unions. This technique uses a multimodal treatment algorithm involving the use of bone marrow aspirate concentrate (BMAC), the reamer-irrigator-aspirator (RIA) and P-15 bioactive peptide (iFactor, Cerapedics) during definitive treatment. Bioactive glass (S53P4 BAG, Bonalife) is added when infection is suspected. The objective of this study was to evaluate the effect of this treatment algorithm on the health-related quality of life (HRQoL) of patients with post-traumatic long bone non-unions. We hypothesized that HRQoL would improve following treatment.

Method: From January 2020 to March 2023, consecutive patients who were referred to a multidisciplinary (trauma, orthopedic and plastic surgery) non-union clinic at the MUMC+, The Netherlands, were evaluated using the Non-Union Scoring System (NUSS). The EQ-5D-5L questionnaire and the Lower Extremity Functional Scale (LEFS) were employed to obtain reported HRQoL outcomes both prior to and subsequent to surgery, with follow-up being conducted at three time points (7, 18 and 35 weeks).

Results: Seventy-five patients were assessed at baseline (T0), with a mean NUSS of 39.63 (\pm 12.9 SD). Thirty-one patients had their first follow-up 7 weeks after surgery (T1). Twenty-five patients had a second follow-up at 18 weeks (T2), and fifteen patients had the third follow up at 35 weeks (T3). The EQ-5D index mean at baseline was 0.487, followed by an index of 0.610 at T1, 0.610 at T2, and 0.677 at T3. Statistical tests showed a significant difference in the HRQoL score between T0 and T1, as well as T2 and T3 ($p=0.002$; $p=0.044$). The mean LEFS significantly increased from 26 before intervention to 34, 38, and 43 after treatment ($p<0.001$; $p=0.033$; $p=0.016$).

Conclusion: This study demonstrated a significant improvement in the health-related quality of life of patients with post-traumatic long bone non-unions during standardized treatment algorithm following the induced membrane technique. In conjunction with the increase in LEFS scores, patients seemed to regain their mobility and might be able to reintegrate into society.

CD4⁺/CD8⁺ T-CELLS AS PROGNOSTIC BIOMARKER TO EARLY IDENTIFY PATIENTS WITH RISK FOR IMPAIRED ACHILLES TENDON HEALING

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Introduction: The low endogenous regeneration potential of tendons is resulting in high numbers of unsatisfactory healing outcomes. Therefore, there is an urgent medical need to identify patients with an increased risk of impaired healing at an early stage and to identify targets for therapeutic intervention. The aim of the study was to elucidate the role of T-cells and their inflammatory mediators involved in acute Achilles tendon healing.

Methods: Peripheral blood and hematoma aspirate was taken from 26 patients pre-operatively, and at follow up 6 weeks, 6 and 12 months after Achilles tendon reconstruction. T-cell subsets were analyzed by flow cytometry using CD3, CD4, CD8, CD11a, CD57 and CD28 antibodies to evaluate the adaptive immunity of the patient. A clinical outcome analysis using subjective questionnaires, functional tests and MRI assessment was done pre-operatively and at each follow up time point. *In vitro*, the functional behavior of patient-derived tenocytes was investigated in co-culture with autologous unpolarized CD4⁺ or CD8⁺ T-cells, or IFN γ -polarized CD8⁺ or IL17-polarized CD4⁺ T-cells (n=5-6). This included alterations in gene expression (qPCR), MMP secretion (ELISA), migration rate (scratch wound healing assay) or contractility (collagen gels).

Results: Higher CD4⁺ T-cell levels and reduced CD8⁺ T-cell levels (increased CD4/CD8 ratio) in hematoma aspirate and peripheral blood at the time of surgery was associated with a worse clinical outcome regarding pain and function 6 and 12 months after Achilles tendon surgery. Increased levels of CD8⁺-memory T-cell subsets in peripheral blood analyzed 6 weeks after surgery was associated with less tendon elongation. ROC analysis identified CD4⁺ and CD8⁺ T-cells as early prognostic biomarker for Achilles tendon healing. *In vitro*, tenocytes showed increased MMP1/2/3 levels and collagen III/I ratio in co-culture with unpolarized and/or IL17-polarized CD4⁺ T-cells compared to unpolarized CD8⁺ T-cells. This coincided with increased IL17 receptor expression in tenocytes co-cultured with CD4⁺ T-cells. Exposure of tenocytes to IL17-polarized CD4⁺ T-cells decreased their migration rate and increased their matrix contractility, especially compared to IFN γ -polarized CD8⁺ T-cells.

Conclusion: CD4⁺/CD8⁺ T-cells could serve as prognostic biomarker for the early identification of patients with impaired Achilles tendon healing. The local reduction of CD4⁺ T-cell levels or their IL17 secretion represent a potential therapeutic approach to improve Achilles tendon healing. This could decrease the susceptibility of tenocytes towards IL17 and thus prevent weakening of the tendon ECM.

2 YEAR CLINICAL AND RADIOLOGICAL FOLLOW-UP OF A MAIOREGEN BIOMIMETIC OSTEOCHONDRAL SCAFFOLD FOR A FEMORAL HEAD FRACTURE

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Purpose: The intention of the study is to present clinical and radiological outcome of a hip fracture-dislocation of the femoral head treated with biomimetic osteochondral scaffold (MaioRegen).

Methods: An 18-year-old male, after a motorcycle accident, sustained an obturator hip dislocation with a fracture of the femoral head and an intra-articular bony fragment. After closed reduction of the hip joint, a CT scan was performed and showed a type IV A femoral head fracture (Brumback classification) that involved the anterosuperior aspect of the femoral head in a weight-bearing area, with few fragments displaced anteriorly.

The surgery consisted of anterior approach with hip dislocation, reduction with 2 screws of the biggest osteochondral fragment, while the rest of fragments were removed. The osteochondral lesion (31mm x 28mm), after preparation, was filled with a biomimetic nanostructured osteochondral scaffold (MaioRegen), adequately shaped. The MaioRegen osteochondral scaffold consists of a nanostructured biomimetic material with a porous three-dimensional tri-layer composite architecture, mimicking the anatomy of the osteochondral unit.

Results: Passive mobilization was allowed immediately. Weight-bearing was forbidden for 8 weeks. Full weight bearing without crutches was restored 16 weeks after surgery. At one-year follow-up the patient had a normal gait and complete ROM in all the planes. At two-year follow-up the patient was able to run without pain and he returned to practice sports. X-Rays at the last follow-up visit showed a complete healing of the femoral head fracture with no signs of osteoarthritis or avascular necrosis MRI exams showed that the implant remained in site and a restoration, at 24 months, of a hyaline-like signal of the articular surfacet. without any signs of subchondral edema.

Conclusion: The MaioRegen biomimetic osteochondral scaffold could represent a safe and effective option for the treatment of traumatic osteochondral lesions of the femoral head, providing optimal clinical and radiological outcomes at 2-year follow-up.

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TITLE: POROUS TUBE LIKE STRUCTURES MADE BY 3D-PRINTED POLYLACTIC ACID IMPROVES LARGE BONE DEFECT HEALING IN A FEMUR DEFECT MODEL OF THE RAT - IMPACT OF LUMEN DIAMETER

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INTRODUCTION: The treatment of large bone defects is a major challenge for trauma surgeons and orthopedists. In order to avoid the complicated transplantation of autologous bone material into the defect, various classes of mostly granular bone substitute materials have been developed. However, these often only yield significant bone growth when combined with regenerative cells, bone marrow, or growth factors. 3D printing enables the realization of new scaffold structures. We have developed porous tubular structures (length 6 mm, diameter

5 mm) characterized by a freely accessible and continuous lumen and by pores (0.7 mm) in the walls, but differing in wall thicknesses and, with identical external dimensions, in the diameter of the free lumen (4 mm=A1; 2 mm=A2). The hypothesis that the diameter of the lumen is a critical parameter for bone defect healing was investigated.

MATERIALS AND METHODS: In 30 male SD rats (300g), a 6 mm plate-stabilized bone defect was created at the femur and filled with either syngeneic bone material from donor rats (positive control, n=10), scaffold A1, (group A1, n=10), or scaffold A2, (group A2, n=10). After 8 weeks of healing, the harvested femora were analyzed by μ CT (osseous bridging, bone mineral density, BMD) and subsequently three-point bending test in relation to the healthy femur. Kruskal-Wallis test, $p < 0.05$ is significant.

RESULTS: Radiologically, there was a continuous osseous coverage of the defect in 10 of 10 femora in the control group, in 6 of 10 femora in group A1, and in 2 of 10 femora in group A2. 3D reconstruction revealed primarily in group A1 the formation of an osseous tube, often bridging the defect, which formed primarily on the inner side of the graft presumably starting from the fracture ends. In part a filling of the wall pores with bone material was also detectable. In group A2, this was also observed to some extent, but fusion of the bony growth cones occurred in only 2 cases. BMD was significantly decreased in group A2 compared to controls. Bending stiffness was significantly increased in group A1 (12.4%) compared with controls (1.5%) and group A2 (4.0%). This finding is interesting because native scaffold A1 has a lower bending stiffness (factor 3) than thicker-walled scaffold A2 *in vitro*.

CONCLUSIONS: The present data demonstrate that a porous PLA-based tubular structure can result in a continuous osseous and biomechanically stable bridging of a large bone defect without additional loading of biological enhancers such as bone material or growth factors. The tubular shape of the scaffold might be beneficial, as it provides a channel for a hematoma to form, which is essential for the induction of the bone healing response. The results suggest that a larger diameter of the free lumen facilitates the formation of the fracture hematoma and thus leads to the better bone defect healing when using the thin-walled scaffold A1.

TITLE

FUNCTIONAL AND ARTHROSCOPIC OUTCOMES IN INDIAN PATIENTS WITH OSTEOCHONDRAL LESIONS OF THE KNEE TREATED BY STANDALONE MICROFRACTURE TECHNIQUE.

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ABSTRACT

Introduction Osteochondral lesions of the knee are seen routinely in knee arthroscopy in Indian patients. Majority of the patients are treated by debridement and Microfracture technique due to poor socio-economic status which prohibits expensive cartilage restoration procedures. This study aims to analyze the functional and arthroscopic outcomes after standalone microfracture surgery.

Material and methods 50 patients who underwent arthroscopic microfracture surgery for osteochondral defects in a stable knee and who underwent a repeat arthroscopy surgery for which the previous defect could be evaluated were included in the study. The demographic profile, MRI and arthroscopic findings, lesion size, location and intra-operative findings were recorded and analyzed with relook arthroscopy findings and functional outcome calculated using the Lysholm-Gillquist Knee Scoring Scale.

Results Patients with lesions less than 2cm square (26 patients) demonstrated a better functional outcome and regenerate when compared to lesions more than 2cm square statically by using the Pearson Coefficient test. Patients with higher BMI and those with Grade 3 to Grade 4 lesions classified by Outerbridge classification had worse outcomes and inferior regenerate which was statistically significant. Patients who were involved in high demand activities had inferior functional and arthroscopic outcomes when compared to low demand activities and was statistically significant by using the ANOVA test.

Conclusions Microfracture surgery demonstrated good functional outcome and regenerate in patients with lesions less than 2cm square. Patients involved in high demand activity and high BMI had inferior outcomes and regenerate suggesting that other cartilage restorative procedures must be considered. Standalone microfracture surgery should be considered only in selected patients with less BMI and lesion size less than 2cm square size.

SEVERE INTRAOPERATIVE VASCULAR BLEEDING AS MAIN COMPLICATION OF ACETABULAR FRACTURES TREATED WITH PLATE OSTEOSYNTHESIS VIA THE MODIFIED STOPPA APPROACH

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Acetabular fractures are challenging fractures and finding the best supportive treatment is complex. Many operative treatment options exist – one of them is the plate osteosynthesis via the modified Stoppa Approach gaining popularity over the last decades. The purpose of this study is to give an overview of this surgical techniques and its main complications.

Patients ≥ 18 years between 2016 and 2022 with acetabular fractures in our department received a surgical intervention with plate fixation via the modified Stoppa Approach. All protocols and documents during a patient's hospital stay were analysed to find relevant perioperative complications concerning this operative technique.

Between 01/2016 und 12/2022 75 patients with acetabular fractures were treated surgically in the author's institution with a plate osteosynthesis via the modified Stoppa Approach. In 26.7 % ($n = 20$) of all cases, patients were confronted with one or more perioperative complications typical for this operation technique. Intraoperative venous bleedings were the main complication with 10.6 % ($n = 8$). Postoperative injuries of the Obturator Nerve and deep vein thrombosis occurred with 2.7 % ($n = 2$) and 9.3 % ($n = 7$).

This retrospective study shows that plate fixation via the modified Stoppa Approach is a good treatment option because of the excellent intraoperative overview of the fracture but has its pitfalls and complications. Especially severe vascular bleedings under fracture reduction must be taken into account.

TLR1/2 STIMULATION OF HUMAN PRIMARY CHONDROCYTES HAS LITTLE IMPACT ON AUTOPHAGY

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Abstract

Objective Autophagy plays a crucial role in regulating the balance between cartilage-anabolic and -catabolic processes and promoting chondrocyte survival. Our previous studies have shown that the activation of Toll-like receptors (TLRs) 1 and 2, which are involved in recognizing pathogens, increased the production of pro-inflammatory cytokines and matrix degradation enzymes, and inhibited mitochondrial respiration in chondrocytes derived from osteoarthritis (OA) patients. Therefore, we here aimed to identify whether autophagy plays an important role in mediating the stimulatory effects of TLR1/2 in OA chondrocytes.

Methods Human cartilage was collected from OA patients who underwent knee replacement surgery. Primary chondrocytes were isolated from cartilage by collagenase II digestion and were then cultured in a three-dimensional spheroid structure. Chondrocyte spheroids were treated with Pam3CSK4, an agonist of TLR1/2, for 24 hours. The lysosome inhibitor chloroquine was used to assist in the assessment of the autophagy status. The expression of autophagy-related genes was detected through RNAseq assay, qPCR, Western blot, and immunofluorescence staining. Autophagy flux was detected by flow cytometry.

Results In our RNAseq data sets of TLR1/2-stimulated and unstimulated chondrocytes, autophagy-related genes were found to be positively enriched in TLR1/2-stimulated conditions by a gene set enrichment analysis. In particular, the autophagy-related gene (*ATG13* and *ATG101*, which are important in the initiation phase of autophagy, were increased, while *VPS34*, which is involved in the nucleation phase, was decreased. Some genes with key roles in the elongation phase (*ATG5*, *ATG16L1*, *LC3B*, and *P62*) were upregulated, while others were downregulated like *ATG10*, or remained unchanged in the treatment of TLR1/2. The increase of *VPS34*, *BECN1*, *LC3B*, and *P62* expression was further confirmed by qPCR assay. However, chloroquine-enforced protein retention of LC3-II, which was detected by Western blot and fluorescence microscopy, tended to show a slight decrease upon TLR1/2 stimulation. Furthermore, TLR1/2-stimulated chondrocytes exhibited a slightly reduced autophagy flux.

Conclusion Despite an increased mRNA expression of autophagy-related genes in TLR1/2-stimulated chondrocytes, the slight decrease in LC3-II protein retention and autophagy flux in TLR1/2-stimulated conditions indicate that TLR1/2 stimulation only slightly inhibited autophagic activity. Thus, it is unlikely that autophagy changes play an important role in mediating the stimulatory effects of TLR1/2 in OA chondrocytes.

Bone defects greater than 6cm in the lower extremity: Is the induced membrane technique associated with favourable outcomes?

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Background Bone defects exceeding 2–2.5 times the diameter of the bone are considered critical and challenging to manage. Twenty years ago, the induced membrane (Masquelet) technique was introduced as

another option for management of bone defects and since then several reports have been published reporting on its effectiveness. Reports focusing on bone defects that ≥ 6 cm remain scarce.

Objectives The aim of this study was to report on outcomes, complications and re-intervention rates of patients treated in our institution (Leeds General Infirmary) with the Masquelet technique for lower extremity bone defects ≥ 6 cm.

Study Design & Methods Between March 2015 - March 2021, all patients presenting with defects of the femur or tibia due to trauma or infection requiring radical bone debridement were eligible. Inclusion criteria were acute fracture with bone loss, septic non-union and chronic osteomyelitis with bone loss due to radical bone debridement. Patients were excluded with pathological fracture, defects < 6 cm, or if treated with other procedures. Prospective data collected included patient demographics, mechanism/ type of injury, surgery type, time between the 2 stages, graft material, re-interventions and complications. All patients were managed via protocol designed by the senior author. Infected cases, were prescribed broad spectrum antibiotics after obtaining tissue samples, which was altered based on tissue sensitivities (minimum 6-week course). Post-operatively all patients received thromboprophylaxis (Tinzaparin 4.500 IU) for 6 weeks. Patients mobilized toe touch weight bearing for 6–8 weeks then progressed to full weight bearing. Follow-up with clinical and radiographic assessment was done at 2 weeks (wound inspection) and 6, followed by 3, 5, 6, 9, 12 months or until radiological signs of union and pain free mobilization. Radiologically union was defined if callus formation was present in 3 of 4 cortices.

Results 37 patients (24 males) with a mean age of 38.3 (range 22–80 years) met inclusion criteria. Eighteen patients had tibial defects, mean defect length 7.7 cm (range 6–13 cm) whereas, 19 patients, had femoral defects mean length 8.1 cm (range 6–14). 12 cases were infected non-unions while the rest were acute bone loss following open fractures. External fixators were used in 10 cases with the fixation revised in the second stage to IM nailing or plating. Mean time from the first stage to the second stage was 9 weeks (range 8–14). After the second stage there were 2 failures of fixation, one Ilizarov for tibia and the other one a distal femoral locking plate that required revision. Two cases during the second stage required returning to first stage of technique due to compromised induced membrane due to a residual infection (confirmed by tissue cultures). One case (open tibia) after the first stage, due to flap failure was converted to Ilizarov with acute shortening and subsequent bone transport. All the rest of the cases (36 in total) during the second stage were grafted with RIA graft which was augmented with bone marrow aspirate and platelet rich plasma or BMP-2. One patient required re-grafting due to healing failure in the proximal femoral defect side. Mean time to radiological union ($n=36$) was 7.4 months (range 6–12). The average time of healing of 1 cm bone defect was 1.2 months. There were 2 cases of leg length discrepancy (1 femur – 2 cm; 1 tibia 1.5 cm). All patients regained full function without residual pain during last follow up.

Conclusions The Masquelet technique appears a safe option for management of defects > 6 cm. Following a standardised protocol reduces the risk of reinterventions and improved outcomes.

MECHANICALLY INDUCED WNT1 PROMOTES OSTEOBLAST DIFFERENTIATION THROUGH PLAT

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Introduction: Bone is a dynamic tissue that constantly undergoes remodeling through the coordinated activity of bone resorbing osteoclasts and bone forming osteoblasts. Mechanical stimulation of bone enhances osteoblast differentiation and bone formation. Although various molecular mechanisms have been identified, the precise mechanotransduction pathways remain poorly understood.

Purpose: To explore the involvement of *Wnt1* in mechanically induced signaling cascades that control osteogenesis.

Methods: Primary murine osteoblast isolation, mechanical stimulation using laminar fluid flow, siRNA transfection, qPCR, western blotting, ovariectomy (OVX) of C57BL6 mice, RNAseq, statistical differences by one-way ANOVA and Tukey's test.

Results: Our results showed that mechanical stimulation induced *Wnt1* expression in murine primary osteoblasts, which subsequently provoked the expression of key osteogenic genes such as *Runx2* and *Sp7*. siRNA knockdown of *Wnt1* blocked the mechanically induced effects when compared with siNT control. To identify genes affected by *Wnt1* depletion and by mechanical stimulation, we performed RNAseq analysis and identified plasminogen activator (Plat) as one of the major factors that showed *Wnt1*- and mechanical stimulation-dependence. By depleting *Plat* in osteoblasts, we confirmed its positive role in promoting osteoblast differentiation. Intriguingly, we demonstrated that *Wnt1* controls *Plat* expression through activation of β -Catenin. In contrast, *Wnt1* silencing reduced mechanically induced activation of β -Catenin, which subsequently diminished *Plat* expression. These results demonstrated that osteoblasts required *Wnt1* to respond to mechanical stimulation, thereby inducing *Runx2* and *Sp7* expression, partly through the *Wnt1*/ β -Catenin/*Plat* signaling pathway. Furthermore, bone from OVX-induced and age-related osteoporotic mouse models revealed significant reduction of *Wnt1* and *Plat* expression compared with non-OVX and young mice, respectively.

Conclusion: Our data suggest that both *Wnt1* and *Plat* play a major role in mechanically induced osteogenesis. Furthermore, their reduced expression in bones from OVX and aged mice highlights their potential role in post-menopausal and age-related osteoporosis.

Title: OSTEOARTHRITIS PATIENTS EXHIBIT AN SYMPATHOVAGAL IMBALANCE

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Introduction: Osteoarthritis (OA) is the most common degenerative joint disorder worldwide and has multiple risk factors including age, sex, obesity, joint alignment, lifestyle, genetic disposition and nervous influences. The autonomic nervous system (ANS) in particular is gaining increasing attention in OA research during the past few years. The antagonistic effects of its two branches, the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS), are well-balanced under healthy conditions and maintain the body's homeostasis. Recent studies suggested that OA patients are prone to an autonomic dysfunction and therefore, we analyzed the autonomic status of early and late-stage OA patients and its associations with clinical OA symptoms. **Method:** More than 170 participants with early and late stage knee OA as well as 40 healthy probands (all age-matched) were included in this study. Late OA patients received total knee replacement (TKR) and were screened both before and one year after TKR. Heart rate variability (HRV) was measured via electrocardiogram being an established measure for long-term sympathetic (low frequency (LF) power) and parasympathetic (high frequency (HF) balance as well as parasympathetic activity (pNN50 (%), SDRR (ms) RMSSD (ms)).

Concentrations of stress-related hormones in serum and synovial fluid (cortisol, aldosterone, DHEA-S) were measured via ELISA. Perceived chronic stress (PSQ) and pain (WOMAC) were assessed via questionnaires. Furthermore, the medication of patients was considered in all analyses.

Results: Early OA patients exhibited a slightly increased LF/HF value compared to healthy controls. Moreover, LF/HF was significantly higher in early compared to late OA patients before ($p>0.05$) and after TKR ($p>0.01$). Additionally, HF in late OA patients before TKR was significantly decreased compared to late OA patients after TKR ($p>0.001$) and healthy controls ($p>0.05$). PNS activity parameters decreased with the progression of the disease: healthy probands exhibited the highest SDRR values, early OA patients had slightly lower levels and late OA patients before TKR displayed significantly reduced SDRR compared to healthy controls ($p>0.001$). Compared to late OA patients before TKR, patients after TKR displayed a significantly higher SDRR level ($p>0.01$). The same tendency was observed in pRR50 and RMSSD measurements. Late OA patients before TKR exhibited elevated serum cortisol concentrations as well as increased perceived stress levels. At the time point of TKR, women with beta blocker medication had significantly higher age (71 ± 9 years) than those without (63 ± 12 years) ($p>0.01$).

Conclusion: Early and late OA patients exhibit a decreased parasympathetic tone and in addition, early OA patients display an autonomic shift towards a more pronounced sympathetic activity. Moreover, late OA patients presented increased chronic stress levels as indicated by enhanced serum cortisol levels. The fact that beta blocker medication in women delayed the need of a TKR indicates that SNS inhibition might slow down OA progression. Our findings suggest that modulation of autonomic nervous system activity may be a potential therapeutic target for OA.

RESPONDINS ARE PROHYPERTROPHIC STIMULATORS OF CHONDROCYTE MINERALIZATION

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Background: Osteoarthritic chondrocytes undergo a pathologic phenotype change in form of a strong cell enlargement (hypertrophy) along with upregulation of tissue mineralizing factors. A similar chondrocyte phenotype is also the result of the *in vitro* chondrogenesis of mesenchymal stromal cells (MSCs). Inhibition of endogenous WNT/ β -catenin signals can antagonize this development and reduce typical aspects of chondrocyte hypertrophy (*COL10A1*-, *IBSP*-mRNAs, alkaline phosphatase, ALP). Intriguingly, however, the source of endogenous WNT/ β -catenin signals remains elusive, as no prohypertrophic WNT ligands could be identified during MSC chondrogenesis. However, the known modulators of WNT signalling, SFRP1 and RSPO2/3, were detected in MSCs and we hypothesized that they could amplify the signalling of lowly expressed prohypertrophic WNT ligands. The aim of this study was therefore to investigate, whether SFRP1 and RSPO2/3 could enhance hypertrophy during MSC chondrogenesis. Deciphering the proarthrotic WNT activity can open novel approaches to develop WNT-based pharmacological anti-arthritis therapies.

Methods: Expanded human bone marrow MSCs were cultured as pellets in chondrogenic medium with 10 ng/mL TGF β . Pellets of the treatment groups additionally received 100 ng/mL of either SFRP1, RSPO2, or RSPO3, with or without the WNT inhibitor DKK1 (200 ng/mL). After 35 days, differentiation and chondrocyte hypertrophy were analysed via histology, proteoglycan quantification (DMMB), qPCR, and ALP activity assay.

Results: Homogenous deposition of proteoglycans and type II collagen, high GAG/DNA levels in the pellets and an unaltered increase

of *COL2A1* mRNA demonstrated that chondrogenesis was successful and independent of treatment with SFRP1 or RSPO2/3. Unaltered *COL10A1* and *IBSP* mRNA levels indicated no general increase of chondrocyte hypertrophy. However, RSPO3 increased mRNA expression of the mineralizing enzyme ALP significantly (1.6-fold over non-treated controls, $n=5$, $p<0.05$), in line with an increased ALP activity in culture supernatants (2.3-fold, $n=6$, $p<0.05$). Treatment with RSPO2 but not SFRP1 induced a similar trend (ALP mRNA 1.5-fold increased, $n=3$; ALP activity increased by 1.6-fold in 2 out of 3 experiments). In presence of the WNT inhibitor DKK1 neither RSPO2 nor RSPO3 could increase ALP activity, indicating that RSPO2/3 acted by enhancing endogenous WNT activity.

Conclusion: While WNT/ β -catenin signals are known to broadly enhance several aspects of chondrocyte hypertrophy, RSPOs were here demonstrated to be highly selective stimulators of the mineralizing enzyme ALP. Thus, as initially hypothesized, endogenous RSPOs appear capable to amplify signalling of low level WNT ligands that are below the detection limit. However, part of the broad WNT function seems inhibited, indicating that RSPO-WNT interactions are complex and may involve further modulators.

Title

Increasing bioactivity and compatibility of PLA/BG composites for bone tissue engineering- high Bioglass content makes the difference

Introduction The treatment of large bone defects is of great interest in orthopedic and trauma research. Up to now, the treatment has been complex, lengthy and expensive. Especially for large bone defects, established methods using autologous bone grafting reach their limits. One solution strategy for this problem is the development of a novel bone substitute based on the so-called Bone Tissue Engineering (BTE) concept. Ideally, a bone graft substitute (scaffold), once introduced into the bone defect, recruits osteogenic and angiogenic stem cells (osteochonductive), controls cell differentiation, and ultimately stimulates bone and vascular formation (osteoinductive and angiogenic properties). The design and material of the scaffold have a significant impact on the bone regenerative potential.

In a previous work, we have already developed a 3D-printable, osteoconductive, hierarchically organized scaffold system. By choosing the appropriate material, osteoinductive properties should now be further integrated. Composites of polylactide (PLA) (polymer)/bioglass (BG) (mineral/ion source) are promising. Previous studies on PLA/BG composites have never exceeded a BG content of 10%, since increasing the bioactive BG component negatively affects the compressibility of the composite. We have now succeeded in developing and characterizing *in vitro* a novel, 3D-printable PLA/BG composite with BG fractions of up to 20%.

Method Using extruders, filament strands with BG contents of 5%, 10% and 20% could be produced. Their influence was subsequently investigated *in vitro* on MSC (pooled MSC from 7 donors) *in vitro*. The focus was on cell adhesion (MTT assay, fluorescence microscopy), gene expression (osteogenic differentiation, inflammation; ELISA) and immunostimulatory potential (whole blood stimulation assay).

Results The material is shown to have high cytocompatibility. With increasing BG content, the colonization density increases. Metabolic activity is not affected by the bioglass. The gene expression analyses indicate a stimulation of osteogenic differentiation. At the same time, suppression of inflammatory genes occurs. An interception experiment using a chelator identified calcium ions released by the filament as the cause of suppression. The results of the whole blood stimulation assay showed no significant inflammatory response.

Conclusion Higher BG content in PLA/BG composites can improve bioactivity and tolerability.

CHRONIC STRESS ACCELERATES OSTEOARTHRITIS PROGRESSION *IN VIVO*

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Osteoarthritis (OA) pathogenesis involves the entire joint and is characterized by cartilage degeneration, synovial inflammation, subchondral bone sclerosis, and osteophyte formation leading to chronic pain. Our recent study indicated the contribution of the sympathetic nervous system (SNS) to OA progression, since sympathectomy resulted in increased calcified cartilage and subchondral bone plate thickness as well as increased subchondral bone volume in the knee joint during OA progression in mice. We hypothesize that chronic stress results in opposite effects and accelerates bone loss during OA manifestation due to increased sympathetic tone. Therefore, we analyzed experimental OA progression in mice exposed to chronic stress.

OA was induced in male C57BL/6J mice by surgical destabilization of the medial meniscus (DMM) and Sham-operated mice served as controls. As additional control, non-operated mice (Healthy) were used. In one-half of these groups chronic stress started the next day of OA induction and comprised 3 different stressors per day in an unpredictable order according to established published protocol called CUMS (chronic unpredictable mild stress). The effectivity of the chronic stress model was verified on the behavioral level by open field test, light/dark box test (anxiety), and sucrose preferences test. After 2, 4, 8 and 12 weeks, body weight and length of the mice were determined and OA related changes in subchondral bone were analysed by μ CT. The genesis of OA-related pain was monitored using the Dynamic Weight Bearing (DWB) system. We will further investigate the severity of OA by histological scoring of cartilage degeneration and synovial inflammation.

Mice exposed to CUMS protocol exhibited clear signs of chronic stress indicated by increased anxiety and significantly decreased body weight gain in all CUMS groups compared to the respective non-CUMS groups. CUMS led also to significantly increased serum corticosterone levels in Healthy mice. Corticosterone increase was even higher in CUMS mice after DMM surgery. The μ CT analyses revealed a slight thickening of subchondral and subarticular trabecular bone due to CUMS. Moreover, CUMS resulted in potentiation of DMM-associated pain, with significantly higher front paws posed duration and significantly higher rear left to rear right paws posed duration. In addition, shorter rearing duration was observed within the CUMS DMM mice.

Our results suggest that the chronic stress-induced autonomic imbalance with increased sympathetic nervous activity exacerbates the severity of OA associated changes in subchondral bone and also leads to increased pain perception.. We expect significantly increased cartilage degeneration as well as more severe synovial inflammation in CUMS DMM mice compared to DMM mice . Taken together, sympathetic dominance seems to accelerate OA progression. Therefore, the autonomic nervous system could be an attractive target for novel preventive or causal interventions that could change the clinical practice in the future.

TITLE

EVALUATION OF EFFICACY OF POOLED HUMAN PLATELET LYSATE (pHPL) AS A GROWTH SUPPLEMENT FOR CLINICAL GRADE CHONDROCYTES CULTURE

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INTRODUCTION

The increasing prevalence of orthopaedic trauma and the ageing related orthopaedic indications have not only become an economic burden to patients but also pose a significant clinical challenge in orthopaedics worldwide. In the past decades, in addition to existing standard of care therapies various emerging and promising treatment modalities such as use of cells and cell-based products (orthobiologics) have demonstrated the measurable outcomes in improving quality of patient’s life by reducing the pain and discomfort of the musculoskeletal system and augmenting the healing of orthopaedic indications. The state-of-the-art procedure for treatment of chondral defect, autologous chondrocyte implantation (ACI) involves isolation and expansion of chondrocytes from the patient’s articular cartilage biopsy in the laboratory using cell culture media supplemented with growth factors. However, the use of xenogeneic (fetal bovine serum, FBS) source of growth factors for *in-vitro* culture and expansion of clinical grade chondrocytes is of great concern due to limitations, uncertainties, ethical issue, and risk of zoonosis and immunological reactions of FBS. The media used for culture and expansion of cells in the laboratory should ensure the maintenance of all key cellular and therapy relevant features of cells and cell-based products. In spite of tremendous efforts, the development of chemically defined media for clinical grade cell production is still in its beginning and is extremely expensive.

METHODS

Consequently, the human platelet lysate (HPL), rich in growth factors and produced in most cases from expired platelets, is emerging as an efficient, xenofree and human compatible growth medium supplement for laboratory production of clinical grade cells and cell-based products. In this relevance, we have been working on the establishment of a cost-effective in-house protocol for preparation of human platelet lysate and its usage as a growth supplement in clinical grade chondrocytes production. We have prepared the pooled human platelet lysate (pHPL) from the expired platelet concentrates obtained from the university hospital blood bank. The chondrocytes are isolated from the cartilage biopsies of patients supplied by the department of orthopaedics. The study is being carried out with prior approval of the institutional ethics committee and donor consents.

RESULTS

The results demonstrated the similarity in the effect of various concentrations of pHPL and FBS with respect to chondrocytes adherence, morphology, viability and mucopolysaccharide content by microscopic observation and safranin-O, respectively. However, the augmented proliferation of chondrocytes was observed in the media

supplemented with pHPL compared to FBS. Further, the study is in progress to understand the effect of pHPL on culture, expansion and quality of chondrocytes in comparison to FBS through systematic analysis of specific gene expression, sequence analysis, chromosomes stability and cell surface marker expression patterns.

CONCLUSION

Our preliminary study demonstrated the potential of pHPL to replace the FBS for culture of cells.

AGGRECAN 32-MER IMPAIRS MITOCHONDRIAL RESPIRATION CAPACITY OF HUMAN CHONDROCYTES VIA TLR2

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Purpose: Previous studies from our group illustrated that human primary chondrocytes express various Toll-like-receptor (TLR) family members. Among these TLRs, TLR2-mediated signaling had the strongest impact on chondrocytes: Stimulation with the TLR1/2 agonist Pam3CSK4 increased the expression of cartilage-catabolic factors and inflammatory cytokines, and impaired the expression of anabolic factors and the mitochondrial respiration capacity of human chondrocytes. In parallel, other studies have described that cartilage matrix degradation generates peptides such as aggrecan 32-mer, which could activate macrophages and neurons via TLR2. Here, we aim to explore the effect of aggrecan 32-mer in primary human chondrocytes and its dependency on TLR2.

Methods: Human osteoarthritis cartilage resections were collected during arthroplasty surgery. Chondrocytes were isolated by collagenase II digestion and subjected to 3D spheroid generation. Human chondrocyte spheroids were then stimulated with aggrecan 32-mer for 4 days followed by Mito stress seahorse assays and cell lysis for mRNA isolation. In further chondrocyte spheroid cultures, monoclonal anti-hTLR2 was added 3 hours prior to 32-mer stimulation to neutralize the biological activity of TLR2. Gene expression of cartilage-anabolic and -catabolic factors as well as inflammatory factors was determined by quantitative RT-PCR, and intracellular glycosaminoglycans (GAGs) and DNA measurements. Mitochondrial respiration capacity was evaluated by Agilent Seahorse XFe96 analyzer.

Results: Aggrecan 32-mer stimulation strongly impaired mitochondrial oxidative phosphorylation (OXPHOS) activity – as shown by the reduction of both basal and maximum oxygen consumption rate (OCR) and ATP-linked oxygen consumption. Importantly, TLR2 blockade restored the OXPHOS capacity of chondrocytes stimulated with aggrecan 32-mer. In addition, 32-mer stimulation tended to increase the expression of the inflammatory cytokines *IL6*, *IL8*, and *GCSF*. However, here we did not observe significant changes when TLR2 was blocked. 32-mer stimulation did not affect the expression of cartilage-anabolic factors (*COL2A1* and *ACAN*) or cartilage-catabolic enzymes (*ADAMTS5* and *MMP3*), suggesting that 32-mer stimulation had little effect on cartilage matrix homeostasis. The GAGs/DNA ratio that was measured in each chondrocyte spheroid after a 14-day culture with or without 32-mer stimulation further confirmed this observation.

Conclusion: Aggrecan 32-mer stimulation impairs the mitochondrial OXPHOS capacity of chondrocytes via TLR2. Aggrecan 32-mer stimulation has no effect on the cartilage extracellular matrix homeostasis but it tends to increase the inflammatory status of the chondrocytes.

TREATMENT PROTOCOL FOR HIP OSTEOARTHRITIS BASED ON STEM CELLS FROM ADIPOSE TISSUE:

COMPARISON WITH PRP-BASED ANALOGUE ABSTRACT

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INTRODUCTION

Osteoarthritis (also indicated as OA) is a widespread degenerative joint disease affecting articular cartilage and subchondral bone. Hip, knee and shoulder are most affected from this kind of disease. In the common clinical activity, the X-ray image often doesn't adequately reflect the level of the symptoms. For many years now, the hip arthroplasty has represented the main treatment option when the situation became unbearable for the patient. The Total Hip Arthroplasty (or THA) represents a good option for this kind of disease. It is well-tolerated, with a very low level of post-operative pain and a high grade of functional recovery after the surgery.

Numerous alternatives have been proposed to delay or even deny the necessity for a THA surgery in mild-to-moderate grade hip osteoarthritis (Kellgren-Lawrence grade II to III). Among them, the intra-articular use of Hyaluronic Acid (HA), injected corticosteroids or Platelet-Rich Plasma (PRP) has been reported to be effective to some degree for the treatment of OA. There is no actual gold standard in terms of efficacy and cost-effectiveness.

Another treatment involves the intra-articular use of stem cells from adipose tissue (or ADSC, adipose tissue-derived stem cells), harvested in a small surgical procedure from the subcutaneous periumbilical abdominal fat. This tissue is rich in "medicinal cells", as the stem cells are also called.

MATERIALS AND METHODS

We performed a retrospective single-center study on 20 patients with a diagnosis of hip osteoarthritis with a single control group (15 patients). Of the two groups, the first received a single-dose intra-articular autologous MFAT injection between October 2020 and February 2023; the second one received a single-dose PRP intra-articular injection, roughly in the same period of time. All the patients were followed and assessed with repeated clinical scoring systems (HHS test, VAS scale, range of motion) at baseline, six months, and one year after surgery. The maximum follow-up time was one year. Primary outcome was the efficacy of M-FAT treatment, if confronted with baseline (statistically significant increase in HHS, decrease in VAS pain scale). Secondary outcome was the superiority of M-FAT treatment protocol over PRP correspondent one. Main failure criteria was the necessity for further treatment (THA, new injection treatments) or the persistence of baseline symptomatology.

RESULTS

In the M-FAT group, a significant increase of Harris Hip score from baseline value was identified at one year after surgery. An analogous decrease of visual analogue scale (VAS) pain score from baseline value at final follow-up was evident, too. ROM improved. A small number of patients were considered treatment failures because they required THA, new injections or because of the persistence of the symptomatology. No major complications were reported during follow-up period. The M-FAT group resulted superior, both in terms of efficacy and effect lasting, to the control one.

CONCLUSION

ADSC-rich M-FAT extract injection is a safe procedure with positive effect at 1-year follow-up in patients with mild-to-moderate hip OA. The superiority of M-FAT over PRP in terms of efficacy and duration of the effect is evident. The M-FAT treatment could be the long-hoped answer to the diffusion of mild early-stage symptomatic hip OA.

Evaluation of the Local Effects of PMMA Spacers Loaded with Antimicrobial Drugs on the Osteogenic Response of hMSCs

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Bone infections are a major challenge in modern orthopaedics. Their treatment consists of a radical debridement of all affected tissue followed by systemic and local antimicrobial treatment using antibiotics. For the latter, PMMA spacers loaded with antimicrobial drugs are frequently used. Different in-vitro studies show that antibiotics used in loaded PMMA spacers (e.g. Gentamicin) have a cytotoxic effect, thus inhibiting the formation of bone mineral (Hydroxyapatite). However, clinical experience shows no negative effects on bone healing when loaded PMMA spacers are used. This study assesses the effect of antibiotic loaded PMMA spacers on the proliferation and osteogenic potential (amount of hydroxyapatite (HA) formed) of mesenchymal stem cells in vitro to archive clarification for this highly important topic. 35mm petri dishes were prepared with a 1.5 cm circle of 0.5 g PMMA in 6 different groups. Group 1: Agarose blank, 2: PMMA with no additive, 3: PMMA+0.01 g Gentamicin (G) [resulting in 80 µg/ml Gentamicin in cell culture media], 4: PMMA+0.01 g Vancomycin (V), 5: PMMA+0.01g G+0.025 Clindamycin, 6: PMMA+0.01g G+0.01 g V). Bone marrow mesenchymal stem cells (BM-MSCs) (n=6) were then seeded in duplicates (10.000 cells/cm²) in these dishes. One half of the duplicates received osteogenic supplements (OM); the other half served as negative controls (CTRL). After 21 days, the amount of HA was assessed by incubation with 5 MBq of the radioactive tracer ^{99m}Tc-HDP, which binds to HA, for 30 min. The cell cultures were then washed and the remaining activity measured using an activimeter. Two duplicates (osteogenic/control) per group were DAPI stained and then counted under a microscope. Statistical analysis was performed using one-factorial ANOVA analysis (significance p≤0.05). The DAPI cell count revealed a lower cell number for groups 3, 4 and 6 (400,000 cells or less) compared to groups 1 and 2 (600,000 cells or more). However, only the cell number in group 5 (OM: 21,252 cells; CTRL: 23,020) was statistically different (p<0.001) from the osteogenic groups 1 and 2. Despite the difference in cell count, the ^{99m}Tc uptake as a marker for produced HA was very similar between the osteogenic groups 1 (2.15 MBq), 2 (2.32 MBq), 3 (2.48 MBq), 4 (2.55 MBq) and 6 (2.51 MBq). The uptake of group 5 (0.225 MBq) was significantly lower compared to all other groups (p<0.001). The uptake of all osteogenic groups was significantly higher than the uptake of their corresponding negative controls (p<0.001), indicating a successful osteogenic differentiation. In conclusion, our data show that most antibiotics frequently used in loaded PMMA spacers show a moderate negative effect on the cell number in-vitro, although this effect was not statistically significant. The ^{99m}Tc-HDP uptake as a marker of HA formation and thus of osteogenic potential was very similar between most groups, indicating that the antibiotics used had no negative effect on the osteogenic potential of MSCs, except Clindamycin. Any negative effect on the cell number seems to be compensated by the remaining cells. Clindamycin on the other hand shows a profoundly negative effect on the cell number which was not compensated in did result in a much lower osteogenic potential. We therefore showed that antibiotics eluted from PMMA may have a cytotoxic effect in-vitro, but the stem cell's ability to form bone is not inhibited in most cases, which aligns with clinical experience. The cells seem to be able to compensate a lower cell number and secrete HA at a rate comparable to antibiotic-free environments. Clindamycin however massively reduces the cell count and the osteogenic potential in vitro. Further studies are necessary to determine, whether this effect can be observed in vivo.

Aims and challenges in the management of severe open fractures. How promising is an interdisciplinary approach in a German Trauma Level I Centre?

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Background: About 3% of all fractures are open fractures. It is estimated that open fractures occur in Germany with an incidence of 11.5 per 100,000 inhabitants annually. The risk of developing osteomyelitis after an open fracture is particularly feared. Especially the extent of the trauma, accompanying vascular or nerve trauma, the degree of pollution/contamination, involved bone (e.g. tibia greater risk than hand), age, comorbidities (diabetes mellitus, obesity, circulatory disorder, nicotine abuse, immunosuppression) have a huge impact. In addition to professional osteosynthesis, soft tissue management plays a decisive role in outcome.

Materials and methods: In 2018, an interdisciplinary board for reconstructive microsurgery was established at the Unfallkrankenhaus Berlin for the treatment of complex injuries of the lower extremity, among many other localizations. We would like to give an overview of the supply algorithms in the years 2020-2022. Flap indications, coagulation diagnostics and preoperative imaging are illuminated.

Results: Presentation of flap indications and complication rate. Special features in the care were accompanying vascular and nerve injuries. In the context of the polytraumatized patient, there were in a few cases significant changes in the coagulation situation with partly clinically evident of pulmonary embolism or relevant bleeding, which required the involvement of a haematologist. Global coagulation test, ROTEM, PFA 100 (platelet function assay), multiplate and plasmatic coagulation factors usually provide adequate indications of the presence of a genetic or temporary coagulation disorder in synopsis.

CTA (CT-Angiography) has become the leading diagnostic agent in preoperative vascular imaging in our clinic. DSA (Digital subtraction angiography) was only used in cases of concomitant vascular reconstruction, necessary intervention, or very distal vascular connections. AV loops were necessary in a few cases to be able to perform a free flap.

Conclusion: The treatment of an open fracture of the lower extremity represents an interdisciplinary challenge. The open communication and professional coordination of the departments involved is a central element for successful and timely treatment. The involvement of laboratory medicine and microbiology/infectiology has become a must in this injury entity.

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Guiding nasal chondrocytes through 3D bioprinted design to generate an osteochondral tissue

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Introduction: Osteochondral defects due to trauma or osteoarthritis affect the cartilage layer and the underlying subchondral bone. Cartilage is a heterogeneous tissue divided into the articular and hypertrophic zones. Recent work has shown that the phenotype of chondrocytes within the different zones can be controlled by regulating oxygen concentration.¹ This study investigates the role of hypoxia in cartilage formation, using human nasal chondrocytes (hNCs) embedded in a collagen/tyramine hyaluronic acid-based (Col/THA) hydrogel, by controlling oxygen concentration using a 3D-bioprinting approach.

Methods: Primary hNCs were isolated from the nasal septum cartilage of patients undergoing septoplasty and were cultured in 2D and 3D aggregates (i.e., pellets) under normoxic conditions (21%-O₂), hypoxic conditions (2%-O₂), or supplemented with hypoxia-inducing compound (21%-O₂ + 1mM-DMOG). hNCs were embedded into the Col/THA (2.5%/15%) hydrogel with ruthenium/sodium persulfate (0.2 mM/2 mM) as a photoinitiator. Constructs were printed using a pneumatic bioprinter (REGENHU, Villaz-St-Pierre, Switzerland) and crosslinked using visible light for 10 min. Printed constructs were analyzed for viability using calcein-AM/ethidium-homodimer-1 and assessed for quality using Alcian Blue (AB) and Safranin-O. Gene expression on COL2A1/ACAN/SOX9/MMP13/RUNX2/ALPL/SMADs was performed on days 7/14. Protein validation for HIF-1 α /BMP2/Smad1/5/9 using western blot was carried out 0.5/1/2/24 hours after oxygen control treatment.

Results: 2D cultured hNCs in hypoxia and with DMOG maintained the native spheroidal morphology, showing positive AB staining. Conversely, the normoxic conditions showed an elongated fibroblastic morphology and a limited amount of AB-positivity. Hypoxia-conditioned hNCs exhibited statistically significant upregulation of the chondrogenic markers COL2A1, ACAN, and SOX9, and a lower expression of the hypertrophic cartilage markers MMP13, RUNX2, and ALPL. Similar results were obtained in 3D pellet cultures, where protein analysis revealed the high content of hypoxia-related factors (HIF-1 α) in DMOG and hypoxia-cultured cells compared to the normoxic condition. The viability study at 28 days in the bioconstruct showed high survival of hNCs in the Col/THA-based hydrogel. On the histological level, safranin-O staining showed new extracellular matrix formation which was further confirmed by positive immunofluorescence staining for collagen type I/II.

Conclusion: Hypoxia condition positively supports the generation of articular-like cartilage tissue using hNCs. Furthermore, topological studies on the role of hydrogel architecture and oxygen gradients diffusion are undergoing using 3D-bioprinting in the direction of stable osteochondral construct formation. The integration of the heterogeneous cartilage layer with a subchondral bone scaffold for the cartilage-bone interface will be the next challenge addressed in this project.

CAN ADIPOSE DERIVED MESENCHYMAL STEM CELLS INJECTION IMPROVE FUNCTIONAL OUTCOME AND DELAY SURGERY IN PATIENTS WITH HIP OSTEOARTHRITIS? A CASE CONTROL STUDY WITH 36 MONTHS FOLLOW UP.

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Abstract

Aim of the study. Recently, intra-articular injection of mesenchymal stem cells (MSCs) has been proposed as a valuable conservative treatment for hip osteoarthritis (HOA) after promising results in knee OA. Adipose tissue is a viable source of MSCs because of the high concentration of cells and the easy access to the donor site. The purpose of this study was to evaluate the results of a single intra-articular injection of autologous adipose derived stem cells (AD-MSC) in a series of patients with HOA.

Methods. Between September 2018 and October 2022, 30 patients with HOA, underwent a single intra-articular injection of AD-MSC. Patients were divided into two groups (Total Hip Replacement, no Total Hip Replacement) depending on the subsequent need to be treated with THR surgery. Inclusion criteria were pain and functional impairment for at least six months, ineffective conservative treatment, and age > 35 years. Exclusion criteria were: hip trauma occurred in the previous 3 months; recent hip arthroscopy; chondromatosis or infection of the hip; malignancy; BMI < 18. The Oxford Hip Score, the 12-item Short Form Survey and Visual Analogue Scale were used to evaluate the results of the proposed treatment.

Results. After a mean period of 22 months, 5 of the 30 patients underwent total hip replacement surgery. These 5 patients were affected by HOA \geq 3. The two groups differed in terms of OHS, SF-PCS, and Kallgren Lawrence grade at the final follow up. In the no THR group a constant improvement in pain relief, hip function and quality of life was observed during the entire follow up period of 27 months.

Conclusion. The single injection of AD-MSC seems to be a valuable treatment for early HOA with a subsequent constant improvement of hip function and patient's quality of life delaying hip replacement.

The application of Negative pressure wound therapy as a multi-staged protocol in septic distal tibia nonunion.

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BACKGROUND: Open fractures caused by high-energy trauma are often associated with significant soft tissue damage and may have concomitant bacterial contamination. Fracture related infection (FRI) can

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be one of the main factors opposing fracture healing, therefore, infection control and soft tissue coverage may play a decisive role. Since its introduction, the negative pressure wound therapy (NPWT) has also been used in trauma setting with valuable outcomes. The occurrence of fracture nonunion could be associated with severe soft tissue loss that has to be addressed to achieve fracture healing. The purpose of our study is to specify the role of NPWT in distal tibia septic nonunion.

METHODS: we retrospectively analyzed the results associated with the use of NPWT in the context of a multi-stage protocol for the treatment of distal tibia septic nonunion. From May 2022 to July 2023, three patients (2 Man and 1 Woman) with mean age of 57 years (50-64), with distal tibial fracture septic nonunion were treated through a standardized multi-stage protocol, based on initial debridement of the wound, resection of the infected bone and placement of an antibiotic loaded cement spacer and NPWT. Subsequently, an Ilizarov frame was used during the second stage to replace bone defect when it was over 3 cm. Finally, an retrograde intramedullary nail was used to achieve tibiotalar fusion. Specific antibiotic therapy was administered throughout the entire treatment protocol. Functional outcomes were evaluated using the ASAMI score.

RESULTS: Mean follow-up was 7,6 months (from 4 to 14 months). Time elapsed between the first two stages was on average 3.6 weeks. The bone results of the ASAMI score system, were excellent in two patients and good in one. Regarding the function results, these were excellent in one patient and good in two.

CONCLUSION: NPWT leads to a fast development of granulation tissue and reduction of the wound size. Moreover, the use of NPWT promotes the reduction of the bacterial load at surgical site, helps the removal of secretions and can promote bone regrowth. As a final result, in our experience, treatment of distal tibia septic nonunion with our proposed staged protocol seems able to reduce the need of plastic surgery. NPWT-assisted closure is a promising aid in the management of infected non unions since it reduces the need for additional procedures.

EFFECTIVENESS OF THE ‘DIAMOND CONCEPT’ IN THE TREATMENT OF MUTIFOCAL NON-UNION OF THE FOREARM. A SMALL SERIES AND A REVIEW OF THE LITERATURE

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Background Forearm non-union of the is one of the worst complications of upper limb fractures. Multiple strategies are available for the treatment of fracture non-union with variable outcomes.

We report two cases of forearm’s non-union, treated by applying the principles of the "Diamond Concept" and restoring the bone stock using intercalary allograft.

Methods

Our two patients (A.T. and T.B.) were affected by forearm non-union with the involvement of both bones (radius and ulna) in one case and isolated of the ulna in the other.

For patient 1 (AT) reconstruction of the radius was performed using a long volar plate with multiple opposite strutgraft, intercalary allograft-with the addition of bone marrow derived stemm cells (BM-MS).C).

Ulna reconstruction was realized applying a compression plate and with opposite strutgraft and intercalary allograft when needed.

Patients were immobilized for 15 days, and then active and passive mobilization began in a specific brace.

All patients underwent a scheduled follow-up at 1, 3, 6 and 9 months with clinical and radiological evaluations. Fracture union was defined as bridging of at least three cortices.

Upper limb function was evaluated using the QuickDash score.

Results

Fracture union was observed at 3 months after the surgery in both patients.

At 9 months of follow up the QuickDash improved from 79 (preoperative data) to 20 for A.T., and from 68 to 16 for T.B.

The patients also reported a noticeable pain reduction.

Conclusions

The application of the principles of the "Diamond Concept", associated with the respect of radio-ulnar relationships, thanks to the use of intercalary transplants, seems to be able to ensure both the fracture healing and adequate upper arm function

Moreover, the use of opposite strutgraft ensured stability while protecting long plates from possible fatigue breakage.

Careful preoperative planning and the use of the aforementioned approach may represent a valid solution in cases of both bi-osseous and isolated forearm’s non-union.

Adipose-derived mesenchymal stem cells conditioned medium: applications in diabetic tendinopathy

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Background: Tendinopathy is a common invalidating pathology, that affects the patient’s quality of life and impacts on the healthcare system.

The pathogenesis of tendinopathies is multifactorial and diabetes seems to play an important role, also affecting the levels and the activation of TGF-β1, a key mediator in wound closure. The treatment of tendinopathies still represents a challenge for the physicians. Although several treatment options are available, both conservative and operative ones are not able to assure reliable outcomes. Adipose-derived stem cells (ASCs) may play a role in this field. However, the evidence of their use in diabetic tendinopathies is still poor.

Objectives: To evaluate the effects of ASCs conditioning medium (ASCs-CM) on tenocytes exposed to high glucose level and mechanical stress.

Design and Methods: An *in vitro* model of diabetic tendinopathy was set by using immortalized tenocytes from healthy human patellar tendon and then exposing them to two different glucose concentrations (normal glucose (NG), 5 mM; high glucose (HG) 25 mM). We evaluated the tenocyte morphology, viability and wound closure at different time points (7, 14 and 21 days), in presence or absence of ASCs’ medium culture (CM). Furthermore, we evaluated the ASCs-CM

content of TGF- β 1 and thrombospondin 1 (TSP-1), able to activate TGF- β 1. These mediators were also evaluated in tenocytes.

Results: The tenocytes exposed to ASCs-CM showed a significantly increased in wound closure ability. Cells exposed to the same settings also showed a reduction in mortality rate and an improvement in glycaemic stress resistance (cell wrinkling and cytoskeleton thinning). Furthermore, the exposition to ASCs-CM significantly increased TSP-1 and latent TGF- β 1 levels in tenocytes, both highly detected in ASCs-CM, overall resulting in a higher activation of TGF- β 1 in tendon cells.

Conclusion: ASCs have a positive effect on tenocytes under glycaemic stress, with an improvement in their morphology, viability and wound closure. These effects could be related to the internalizations of mediators released by ASCs-CM, such as TSP-1 and latent TGF- β 1.

TREATMENT OF GONARTHROSIS WITH STROMAL-VASCULAR FRAKTION RESULTS AFTE TWO YEARS FOLLOW-UP

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Background

Gonarthrosis is of very high socio-medical importance due to its high prevalence (between 27 and 90% of people over 60 years of age, depending on the study). Established conservative therapies such as hyaluronic acid or PRP injections can alleviate symptoms but have no significant effect on disease progression. Mesenchymal stem cell therapy may have the potential to fill this gap in the future. This use of laboratory-expanded stem cells, classified as an ATMP (Advanced Therapy Medicinal Product), is associated with high costs and is strictly regulated in Europe. A therapy that can find widespread applicability in Germany should therefore be feasible in one step and focus on minimal cell manipulation. In the therapy we perform, subcutaneous fat tissue is removed under local anesthesia. Subsequently, the adult fat cells are destroyed by mechanical filtration and the remaining stromal vascular fraction (SVF) can be injected. In the pilot study we performed, we examined whether a clinical improvement could be observed within a follow-up period of 2 years.

Method

A total of 27 patients treated with SVF completed the 2-year follow-up. The patients were between 46-88 years old at the time of the procedure. The average age was 60 years. Per knee, 30 ml of lipoaspirate was collected from the patients and mechanically processed into SVF. Clinical outcome was measured by VAS, Sane, and KOOS before intervention and at 3, 6, and 12 months. The number of injected cells was measured using a nucleocounter.

Results and Conclusion

After 6 months, significant improvement ($p < 0.05$) was observed in all clinical scores. After one-year, significant improvements were observed in the subscales KOOS pain, activities of daily living (ADL), quality of life (QOL), sports, as well as in the Sane score. After 2 years, there was still a significant improvement in the subscales QOL and sports, as well as a trend ($p < 0.1$) in the subscale KOOS pain. The number of injected cells ranged from 2 million to 92 million cells with a mean of 40 million cells. The improvements in clinical outcome are comparable to the results of studies in which similar cell products were used to treat gonarthrosis. The clinical results of the study are promising. However, the data suggest that the effect of the therapy wears off after

approximately one year. In a follow-up study with an adequate control group, we plan to collect further data to understand the mechanism of the therapy on a molecular-biological level and to explore possibilities for therapy optimization.

3D-PRINTED POLYCAPROLACTONE/TRICALCIUM PHOSPHATE CAGES FOR POSTTRAUMATIC CRITICAL SIZE BONE DEFECTS, A REASEACH PROPOSAL FOR RANDOMISED CONTROLLED TRIAL.

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Introduction – Critical size bone defects reduce patients' mobility and can cause persistent morbidity and a significantly lower quality of life. When the defect size increases, the risk of failure to bridge this defect also increases. The current gold standard is the induced membrane technique (IMT) in combination with autografting, though higher failure rates are reported in defects larger than five centimetres. Application of an innovative, custom made, 3D-printed scaffold of poly- ϵ -caprolactone with β -tri-calcium phosphate (PCL/TCP) combined with autologous bone graft and osteogenic factors have the potential to improve outcomes in this clinically important problem.

Objectives - The primary objective of this study is to improve bone regeneration and reduce the need for secondary surgeries in patients with a large long bone defect using a custom-made 3D-printed scaffold consisting of PCL and TCP, in addition to the currently used (auto)grafting materials. The secondary objectives are quality of life one year after surgery and cost effectiveness of the procedure.

Study design - A randomised controlled trial will be conducted including patients with a posttraumatic bone defect of a minimum of five centimetres in length after debridement. Patients will receive either standard care or standard care with the addition of a custom printed PCL/TCP scaffold (*Osteopore*, Taman Jurong, Singapore) according to randomisation. Standard surgical care consists of IMT. During the first stage procedure the defect site will be debrided and filled with a cement spacer, the gap will be stabilised and the soft tissue covered with a vascularised tissue flap when needed. During the second stage procedure the cement spacer will be removed and the defect will be randomised to be filled with or without the 3D printed scaffold. Autograft harvested from the femur using the Reamer Irrigator Aspirator (RIA) system and bone marrow aspirate concentrate (BMAC) harvested from the iliac crest is used as standard treatment in both groups, as well as bone growth factor P-15 (iFactor®, *Cerapedics*, Westminster, USA). Follow-up is scheduled at regular intervals; six weeks, twelve weeks, six months, and one year after surgery.

Outcome parameters - The main outcome parameter is the volume of newly formed bone at the defect site based on CT-scans at the 6-month follow-up point. Secondary outcome parameters are quality of life (EQ-5D5L, PROMIS), lower extremity function scale, number and type of reinterventions, recurrence or persistence of infection, burden-of-disease and cost-effectiveness, including the Medical Consumption Questionnaires (MCQ) and Productivity Cost Questionnaire (PCQ). Sample size is calculated based upon the hypothesis of a 50% increase in bone formation, resulting in a total sample size of 20 patients.

THE COMPOSITION OF THE EXTRACELLULAR AND PERICELLULAR MATRIX OF ARTICULAR CARTILAGE IN RELATION TO CARTILAGE THICKNESS

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For regenerative approaches and the development of cartilage replacements, a better understanding of the molecular composition of the cartilage matrix is crucial. It has been demonstrated earlier, that the cartilage matrix can adapt to mechanical loading and as a consequence the thickness of cartilage layer strongly depends on the loading pattern in the joint. At locations where particularly high loads occur, the cartilage layer is thicker compared to less loaded areas. Within the different zones of articular cartilage (superficial, middle and deep zone), the cartilage matrix can be separated into different subcompartments (territorial, interterritorial and pericellular matrix) that might contribute differently to the mechanical properties of the tissue. However, it is currently not known, whether the protein composition and localisation of specific extracellular matrix components depends on the cartilage thickness.

Our hypothesis is that the prevailing load on the cartilage tissue cannot be compensated by the adaptation of the cartilage thickness alone, but that additional changes in the cartilage matrix take place at the molecular level to compensate for the load.

Osteochondral cylinders from porcine knee joints were used to systematically analyse the presence and localisation of individual matrix proteins. Cylinders were generated at eight anatomically defined positions and toluidine blue and DMMB were used to stain for proteoglycans. Total thickness of articular cartilage was then determined. In addition, immunohistochemical staining was performed using specific antibodies to detect collagen II, collagen VI, matrilin-3, COMP, TSP-4 and decorin.

Proteoglycan staining of tissue sections indicated the position-dependent differences in the amount of proteoglycans and the thickness of cartilage tissue. The immunohistochemical analysis of the cartilage matrix at different locations in the knee joint is ongoing, however, some site-specific effects were observed. Of particular interest is the pericellular matrix (PCM), as the PCM is involved in both the mechanical protection of the chondrocyte and cell-matrix interactions to transmit mechanical signals into a cellular response. The staining pattern for collagen VI, which is a major component of the PCM, appeared not to change dramatically suggesting that the total thickness of the cartilage is more responsive to loading than the pericellular matrix.

Understanding how the matrix is organised at the molecular level depending on the mechanical loading/localisation may have particular significance to successfully apply tissue engineering approaches to treat local defects. This knowledge, therefore opens up the possibility of producing replacement cartilage tissue with optimal mechanical resilience.

IMPLANT RETENTION WITH SERIAL DEBRIDEMENT AND USE OF ANTIBIOTIC-LOADED CALCIUM SULFATE BEADS IN ACUTE FRACTURE-RELATED INFECTION (FRI) AFTER PELVIC RING OR ACETABULAR FRACTURES: A RETROSPECTIVE CASE SERIES OF 7 CASES

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Purpose: Fracture-related infections (FRIs) after open reduction and internal fixation (ORIF) of pelvis ring and acetabular fractures are amongst the most feared complications. The choice between implants retention and removal represents a clinical dilemma and there is little literature to aid in decision-making. The purpose of this study was to describe a management protocol for acute FRIs that aims to eradicate the infection through debridement, antibiotic pearls and retention of the implant (DAPRI) and to report the results of a case series of patients treated with this technique.

Methods: Diagnosis of infection was carried out according to FRI consensus group criteria. The patients were considered eligible for DAPRI if diagnosed within 4 weeks from ORIF. Accurate tumor-like debridement and sample collection for cultural examination was followed by placement of calcium sulfate antibiotic-added beads. The aim was to remove the biofilm and allow a higher and prolonged local antibiotic concentration. The patients were then administered a 12 weeks antibiotic course. Wound status, radiological signs of bone healing, gait and functional activity of the patients were evaluated during a standardized follow-up.

Results: This protocol was administered to 7 patients who developed early FRIs after ORIF of pelvic and/or acetabular fractures. The average follow-up was 9 months (range 6-16 months). All patients achieved bony union and complete wound healing without major complications, with no need for implant removal. Average time of bony union was 4.3 months (range: 3–6 months). No clinical, laboratory or radiological evidence of infection was detected during follow-up in all cases.

Conclusion: The DAPRI technique might represent a safe and more conservative treatment for management of early FRIs of the pelvis and acetabulum.

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Physicochemical and Biological Characterisation of Chitosan Scaffolds from Crustacean and Fungal Sources

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Chitin extraction from crustacean sources, i.e., shells of crabs, shrimps and krills, is well established; however, chitin also exists within other natural sources, including squid pens and fungal sources. Despite chitin being outweighed in abundance in nature by cellulose, the fundamental comparison regarding bone regeneration between the deacetylated form of chitin, i.e., chitosan (CS) from animal and fungal sources, is overall lacking. The source, i.e., crab, squid, fungi etc. [1] and processing conditions of chitin ultimately affect the molecular weight (Mw), for example, the number of amino (-NH₂) and hydroxyl (OH) functional groups formed [1, 2] and the Degree of Deacetylation (DD) of the CS formed. The DD is the ratio between glucosamine and the sum of glucosamine and N-acetyl-glucosamine units [3]; thus, DD corresponds

to the free amino groups in the polysaccharide structure [4]. CS with a higher DD value corresponds to a higher percentage of protonated primary amino groups, thus leading to an overall higher charge density [5]. Higher charge density results in specific physicochemical and biological properties exhibited by CS derived from fungal or crustacean sources.

Our research aimed to compare CS's physicochemical and biological properties derived from crustacean and mushroom sources. We formulated highly porous CS scaffolds embedded with different concentrations (0, 10, 20 and 30wt%) of calcium phosphate minerals via lyophilisation. Characterisation of the scaffolds included Fourier Transform Infrared Spectroscopy, X-ray Diffraction, Ultraviolet-Visible Spectroscopy, Scanning Electron Microscopy and Energy Dispersive Spectroscopy. We also performed physical testing, including viscosity, zeta potential, liquid uptake, degradation, thermal and mechanical analysis. Cytotoxicity assays (XTT) and bone marrow stem cell adhesion (LIVE/DEAD, Alexa fluor and Dapi and Alamar Blue) were also investigated to understand any differences between the two CS for bone tissue engineering applications.

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INVESTIGATING THE FEASIBILITY OF UTILIZING A BIOINK CONSISTING OF SELF-EXPANDING HYDROGEL AND BONE MARROW ASPIRATE FOR TISSUE REGENERATION.

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Introduction: Bone marrow is a vital component in the field of regenerative medicine, particularly in the development of bioink. Bioink refers to the biomaterials used for 3D bioprinting, which mimic the extracellular matrix of living tissues and provide a scaffold for cell growth and differentiation. The use of bone marrow as a bioink offers several advantages, including high cell viability, a low immune response, and the ability to promote tissue growth and regeneration. As such, bone marrow bioink holds great promise for developing next-generation tissue engineering and regenerative medicine therapies. The current study develops a Bioink that combines self-expanding hydrogel with bone marrow aspirates (BMA). The purpose of this Bioink is to facilitate a personalized expandable scaffold in a single stage.

Materials and methods: To create the bioink, a self-expanding hydrogel was mixed with BMA ($0.5\text{--}1.3 \times 10^9$ cells/mL) and filled in 3-ml injections. The 3D-Bioprinter (BioX6, CELLINK, Gothenburg Sweden) was then used to print the STL Data file. The resulting samples were photographed next to a scale and incubated over time in defined chondrogenic differentiation medium (DMEM; $0.1\text{-}\mu\text{M}$ dexamethasone, $50\text{ }\mu\text{g/mL}$ of ascorbic acid, $40\text{ }\mu\text{g/mL}$ of proline, $110\text{ }\mu\text{g/mL}$ of pyruvate, $6.25\text{ }\mu\text{g/mL}$ of insulin, $6.25\text{ }\mu\text{g/mL}$ of transferrin, $6.25\text{ }\mu\text{g/mL}$ of selenious acid, 1.25 mg/mL of bovine serum albumin, $5.55\text{ }\mu\text{g/mL}$ of linoleic acid, and 10 ng/mL TGF- β 3). After 21 days, the samples were photographed again, harvested, and fixed for histological evaluation.

Results: The results showed that the scaffold size had doubled after 21 days in the medium, while the cell density was calculated as 1825 cells per square mm from H&E-stained sections. Additionally, the scaffold remained intact during the entire differentiation period.

Discussions and Conclusions: While still in its early stages, the present study is currently in progress to perform immunostaining of various collagen types and to conduct mechanical testing of scaffold stability. Nevertheless, the findings so far indicate that the utilization of expandable hydrogels and BMA as a bioink in 3D bioprinting has the potential to enhance the quality and speed of tissue regeneration. However, achieving better surgical outcomes for patients necessitates the development of appropriate testing models both in vitro and in vivo. While it is feasible to control the shape and size of the scaffold and to incorporate BMA, MSCs, and growth factors in the bioprinting process, precise control and functionalization continue to pose significant challenges.

The osteoconductive and antibacterial responses of photothermally treated biphasic Al₂O₃-TiB₂ - TiO₂/ CuO/ CeO₂/CaO Scaffolds

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Bone grafting is a surgical procedure whereby a new bone or bone substitute is used to replace damaged, fractured or infected bone. Potential bone scaffolds should exhibit comparable density and mechanical properties to natural bone whilst encouraging re-vascularisation. Porosity is essential for bone scaffolds to ensure successful integration, circulation of nutrients, removal of waste products, and the formation of blood vessels.

Biphasic bone scaffolds of Al₂O₃-TiB₂, were synthesised via a novel in-situ SHS combustion synthesis from Al, TiO₂ and B₂O₃. The SHS combustion synthesis is a low-cost and time efficient manufacturing process for producing high purity implants of varying densities, sizes and shapes.

The Al₂O₃-TiB₂ matrix, was doped with TiO₂, CuO, CeO₂ and CaO oxides, known for their antibacterial properties. The scaffolds were coated by a liquid chitosan film, and they were photothermally treated on the bone defects by a CW NIR laser which promoted sterilisation. Layer-1 of the biphasic scaffold mimics the structure of cortical bone, whilst Layer-2 mimics the structure of cancellous bone.

Characterisation of the biphasic scaffolds included XRD, SEM, EDS and UV-vis, while their osteoconductive and antibacterial responses were investigated by XTT assay and confocal microscopy imaging of Alexa-Fluor-488.

The Al₂O₃-TiB₂ composite is an ideal bone scaffold candidate, as it exhibits high compressive strength, hardness, fracture toughness and it is resistant to oxidation. The oxides significantly improved its antibacterial properties without compromising its osteoconductive ability emphasising on the potential of the composite to become a bone scaffold.

Keywords: Biphasic scaffolds, Osteoconductive, Antibacterial

Title

Vancomycin pharmacokinetics and activity in a novel *in vivo* model of orthopaedic device-related infections : comparison with *in vitro* data

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Background

Orthopaedic device-related infections represent a devastating complication, causing high rates of morbidity and mortality. Their management is complicated by the presence of bacteria forming biofilms that are recalcitrant to antibiotherapy. An option to increase therapeutic potential is to use topical route. Local antibiotic treatment of musculoskeletal infections has the advantage, compare to systemic route, of achieving high antibiotic concentrations and exceeding the minimum inhibitory concentration (MIC) without increasing systemic toxicity. *In vitro* and *in vivo* models of biofilms growing on implanted materials are needed to evaluate therapeutic strategies.

Objectives

(a) To develop a preclinical relevant model of foreign-body infection in guinea pigs, using vancomycin as an exemplative antibiotic and (b) to compare local activity with that measured in-vitro.

Study design and methods

***In vivo* model.** This model is adapted from Zimmerli et al. (PMID: 7119479). Sterile multiperforated tissue cages containing titanium beads were implanted in the back of guinea pigs. Animals were assigned to three groups: vancomycin pharmacokinetics (A), infected/non-treated (B) and infected/treated (C). Group (A): single intraperitoneal (*ip*) dose of vancomycin (15 mg/kg) then follow-up of pharmacokinetic profile over 12h in serum and tissue cage fluid. Group (B-C): cages infected by MRSA ATCC33591 (10⁷ CFU/mL). Group (C): 15 mg/kg q12h vancomycin *ip* over 4 days, with tissue cages fluid samples collected regularly in all animals and implanted material collected from euthanized animals at preset times. Tissue cage fluid and cages/beads processed for bacterial counts.

***In vitro* model.** ATCC33591 biofilms were grown on titanium coupons, exposed 24h to vancomycin at increasing concentrations and processed for bacterial counts. **2 Results**

***In vivo* model.** (A) Peak and Area under the curve (AUC) were 4.4 and 2-fold lower in tissue cage fluid than in serum. (B) No spontaneous recovery was observed 10 days after the infection. (C) Vancomycin was ineffective against both planktonic and adherent bacteria.

***In vitro* model.** Significant reduction in bacterial counts was observed for coupons exposed to ≥ 0.1 mg/mL during 24h, corresponding to an AUC_{0-24h} 10-times higher than that reached in serum and 20-times higher than that reached in the cages.

Conclusions

A reproducible guinea pig model has been successfully developed, allowing to follow drug local and systemic pharmacokinetics and activity. The fact that vancomycin was ineffective could be attributed to its insufficient concentration at the infection site to act against planktonic bacteria and biofilms. Local treatment could be an option regarding results. This model could be used to evaluate other antibiotic treatment strategies

The addiction of amino acids boosts the positive effects of hyaluronic acid injection in knee osteoarthritis. Early results from the Hyaloplus protocol.

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Introduction:

Knee Osteoarthritis (OA), is one of the main causes of disability affecting the population, and it is characterized by degradation of the cartilage, subchondral bone sclerosis and reduction of the viscoelastic properties of the synovial fluid. The treatment may be either conservative or operative. The use of intrarticular injections of hyaluronic acid is becoming extremely popular as an effective device to conservatively treat knee OA. The goal of our study was to evaluate the safety and performance of two devices: Artrocomb (High molecular weight hyaluronic acid): Sinovidol (High molecular weight hyaluronic acid associated with such amino acids proline, glycine, lysine and hydroxyproline at a concentration of 1%. In the present paper we report the early effects of both formulations in a randomized single-blinded cohort of patients.

Materials and methods:

Knee OA was classified according Kellgren-Lawrence (K-L) classification. Only patients with a K-L I-III were included in the study. Patients were randomized in two groups: Artrocomb and Sinovidol. Each patient received three intra-articular injections and constantly followed-up at regular intervals (3,6 months) evaluating knee function (through WOMAC, and Oxford Knee Score) and HA safety. The protocol started in October 2022 and is set to enroll 82 patients; therefore, we report the very early results of the present study, including only patients who completed at least the first evaluation.

Results:

27 patients completed the first evaluation and were included in the present report, 13 in the Group A (arthrocomb), 14 in the Group B (Sinovidol). The Womac at baseline was on average 41.5 in Group A, 44.9 in Group B.

At the first time point (12 weeks), both groups showed a relevant reduction in the WOMAC total score. Group B showed a more significant decrease (23 group A vs 14.85 group B). Both treatments show to be very well tolerated and no serious adverse events were recorded.

Conclusions:

This study has shown that the viscosupplementation based on hyaluronic acid and hyaluronic acid supplemented with amino acids in the treatment of mild-moderate gonarthrosis is able to reduce pain and improve function at 12 weeks after treatment. The supplementation of the HA with amino acids seems to boost this positive effects. Nevertheless, more data with a longer follow-up are needed to confirm these results.

CAN ANTIBIOTIC-IMPREGNATED BONE GRAFTS IN ASEPTIC SECONDARY BONE SURGERY PREVENT INFECTION?

A clinical case series

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Aim

Local antibiotics released through a carrier such as PMMA or collagen is a commonly used technique to prevent infection in orthopaedic procedures. Bone chips impregnated with antibiotics (AIBG) can be indicated in secondary aseptic bone reconstructive surgery in order to treat bone defects and simultaneously decrease the risk of infection. In this case series we looked at the incidence and type of infection in aseptic bone reconstructive surgery using AIBG impregnated with vancomycin and tobramycin.

Study design and methods

It is a retrospective study of patient records. Patients were consecutively retrieved from our hospital AIBG registry. Recruitment period was situated between September 2018 and January 2022. Included were patients without any clinical sign of infection who underwent secondary surgery for any reason and who had at least one year follow up postoperatively. Patients with positive cultures preoperatively were excluded.

Population

63 patients (26 men and 37 woman) who met the inclusion criteria with an average age of 66 (18-97) at surgery.

AIBG were used in a wide variety of pathologies: 21 patients had a **non-union**, 17 **fractures** of which 1 open fracture, 3 peri-implant fractures and 13 periprosthetic fractures, 9 patients had **complications after prosthetic surgery** (loosening, dysfunction or dislocation), 3 patients had a **conversion from hemi-prosthesis to total prosthesis** and one patient had a **conversion from PSOS to total prosthesis**.

Other pathologies were: bone cyste (1), bone defect (1), failure of osteosynthesis material (2), mal-union (1), osteonecrosis (1), revision osteosynthesis (5) and removal of osteosynthesis material (1).

Impregnated bone chips were used at different locations: clavicle (5), femur (28), foot (1), hip (11), humerus (13) and tibia (5).

Result

Microbiological samples were taken during surgery in 23 patients. Three patients had a positive microbiology and were excluded.

One patient developed wound problems after a revision plate and screw osteosynthesis for a non-union of the humerus. Microbiological samples taken at the location of AIBG use, 3 weeks postoperatively, were positive for *Staphylococcus aureus ssp aureus*.

Conclusion

The use of AIBG seems to diminish the rate of infection in aseptic secondary surgery. Only one in 63 study patients developed an infection. However a direct comparison of the infection rate to non-impregnated grafts has not yet been performed.

STRUGGLING WITH A CEFAZOLIN IMPREGNATION PROTOCOL OF BONECHIPS

The effect of the timing of the impregnation and gamma irradiation on the cefazoline release

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Aim

Local antibiotics released through a carrier is a commonly used technique to prevent infection in orthopaedic procedures. An interesting carrier in aseptic bone reconstructive surgery are bone chips

impregnated with AB solution. Systemically administered Cefazolin (CFZ) is commonly used for **surgical site infection prophylaxis** however our own *in vitro* study showed that fresh frozen and processed bone chips impregnated with CFZ solution completely release the CFZ within a few hours questioning their potential for local infection prophylaxis. On the other hand irradiated freeze-dried bone chips, treated with supercritical CO₂ (scCO₂) have been shown to be an efficient carrier for the antibiotics vancomycin or tobramycin.

With this pilot *in vitro* study we wanted to investigate if CFZ solution impregnation of bone chips treated with scCO₂ shows a more favourable release pattern of CFZ. We looked at the impact of the timing of impregnation within the processing protocol on the release of CFZ.

Material and methods

The bone chips were prepared using the standard scCO₂ protocol. These bone chips were impregnated with 100 mg/ml cefazolin at different timepoints during the process: before freeze drying (BC type A), after freeze drying (BC type B) and after gamma-irradiation. Each impregnation step was followed by a freeze dry step.

0.5g of the impregnated bone grafts were incubated with 5ml of fetal calf serum (FCS) at 37°C. At 2, 4, 6, 8 and 24h of incubation 200µl of eluate was taken for analysis. After 24h the remaining FCS was removed, bone grafts were washed and new FCS (5ml) was added. Consecutive eluate samples were taken at 48, 72 and 96h of incubation. The concentration of CFZ in the eluates was measured with the validated UPLC-DAD method. Analysis was performed in triplicate.

Result

The mean concentration of CFZ in the eluate obtained from BC type A incubated for 2h was higher compared to BC type B, respectively 581 mg/l and 297 mg/l. However, the elution profile is the same for both types: the CFZ concentration in the eluates drops within the first 24h from 581 mg/l to 365 mg/l (37%) for BC type A and from 297 mg/l to 132 mg/l (56%) for BC type B. After 24h no further significant CFZ release is seen.

Impregnation of the bone chips before or after gamma irradiation did not affect this elution profile.

Conclusion

Bone chips treated with scCO₂ show a comparable elution pattern compared to non-scCO₂ treated bone chips. The amount of CFZ release depends on the timing of impregnation in the processing protocol. CFZ release is not influenced by gamma irradiation of bone grafts.

AB release depends on the properties of the AB, making it impossible to copy the same impregnation protocol for different antibiotics. The stability of CFZ in solution at 37°C and its release are a major concern when establishing an impregnation protocol with CFZ.

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ANTI-OSTEOPOROTIC EFFECTS OF PERIOSTEAL STEM CELL DERIVED EXOSOMES CONTAINING BIPHOSPHONATES

INTRODUCTION

The periosteum, the connective tissue surrounding bones, can be used for tissue engineering or bone and cartilage regeneration. Due to their high regenerative capacity, Periosteum-Derived exosomes may represent an interesting alternative to current treatments used in osteoporosis. The aim of this study was to investigate whether periosteal stem cells derived exosomes containing bisphosphonates (PSECB) promote osteoblast proliferation and improve osteoporosis via inhibiting cell apoptosis.

METHODS

Periosteal stem cells were isolated and cultured, followed by the identification of surface antigens via flow cytometry. Osteoblasts and periosteal-derived exosomes were cultured in vitro, subjected to treatment with alendronate, risedronate, or ibandronate for 0 to 72 h and then assayed for IL-1 β , sRANKL, and TNF- α production by ELISA. Proliferation and viability of osteoblasts treated with PSECB were detected by cell count kit-8 assay. The effect of PSECB on cell apoptosis was evaluated by flow cytometry. Protein expression levels of apoptosis-related genes in osteoblast cells were detected by Western blot.

RESULTS

PSECB significantly enhanced the proliferation, alkaline phosphatase activity, and the Alizarin red S staining in osteoblast cells. The results of cell cycle distribution demonstrated that PSECB increased the proportion of cells in the G2 + S phase and decreased the proportion of cells in the G1 phase. Functionally, PSECB could promote the viability of osteoblasts, and significantly increase the expression level of annexin V. In addition, PSECB remarkably downregulated apoptosis-related genes and decreased apoptosis in osteoblasts. There was significant downregulation of IL-1 β and TNF- α in osteoblasts, and there was upregulation of IL-1 β and downregulation of TNF- α in osteoclasts.

CONCLUSIONS

PSECB can promote osteoblast proliferation by inhibiting cell apoptosis, and therefore may have a potential preclinical therapeutic effect on osteoporosis.

ABSTRACT ESTROT

IN VITRO CULTURE MESENCHYMAL STEM CELL-DERIVED CHONDROCYTES UNDER CYCLIC MOTION.

Objective:

Based on our work, we generate an in vitro culture model of mesenchymal stem cells (MSCs) subjected to compression and shear stimuli to obtain their differentiation and characterization of hyaline articular cartilage, exploring from a tissue engineering point of view a new pathway towards cartilage repair.

Material and methods:

We employed MSCs extracted from donor iliac crest (prior informed consent) that we embedded in a sodium alginate hydrogel generating a three-dimensional matrix. An in-house manufactured bioreactor (3D printing of the parts and servomotors controlled with arduino board), stimulated the three-dimensional culture by exerting compression and shear for 1, 3 and 6 weeks. We used chondrogenic differentiation medium for cell culture. We prepared the samples for histology in hematoxylin-eosin, toluidine blue and immunofluorescence with anti-collagen II and chondroitin sulfate antibodies as markers of extracellular matrix production and antibodies against connective tissue growth factor (CTGF) and F-actin as markers of mechanotransduction response and phenotypic maintenance.

Results:

In histological sections we observed that cultures subjected to compression and shearing maintain the cell phenotype appear and there were more isogenic groups and greater cell condensation in the central zone of the gel. Some surface cells acquire a flattened shape reminding us of the cellular distribution of hyaline cartilage. In control cultures there is less cell viability. In the immunofluorescence, the expression of collagen II, chondroitin sulfate, CTGF and F-actin increases under motion with respect to the control cultures.

Conclusion:

Mechanical stimulation favors the phenotypic maintenance of chondrocytes, their proliferation and migration within the gel. Cultures subjected to mechanical stimulus favor the production of extracellular matrix components such as collagen II and chondroitin sulfate. Mechanical stimulation activates mechanotransduction pathways increasing the expression of CTGF and F-actin, factors that favor cell proliferation and phenotypic maintenance.

Our bioreactor is able to generate tissue with hyaline cartilage characteristics.

Conflict of interest:

The authors declare that there is no conflict of interest.

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