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Basic Science Free Papers
14:15 – 15:45
Hall 12

175
A NOVEL ULTRA-LOW DOSE CT ALTERNATIVE TO RSA FOR MEASURING MIGRATION IN ARTHROPLASTY
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Background: Implant migration is a predictor of survival after hip arthroplasty, and thus crucial to introduce and monitor novel prostheses. Roentgen stereophotogrammetric analysis (RSA) is the gold standard method, but requires calibrated radiographs using expensive, specialist equipment and significant technical expertise. We aimed to develop an ultra-low dose CT-based spatial analysis (CTSA) method as an alternative.

Methods: A ceramic hip resurfacing arthroplasty and 20 tantalum beads were implanted into a pelvis model, mounted onto a 6 degree of freedom motion stage. The pelvis was repeatedly scanned with an ultra-low dose CT protocol, with imposed micro movements in translation (T) from 0.1mm to 1mm, and rotation (R) from 0.2° up to 1° in x, y and z axes to enable the accuracy and precision (double measurements) to be determined. Data were interrogated using a semi-automated 3D CT model-based technique with Materialise Mimics and Mathworks MATLAB software. The effective radiation dose was < 0.15mSv.

Results: For the head, worst accuracy was 0.19mm (T_y) and 0.74°(R_{ZZ}); for the cup it was 0.13mm (T_y) and 0.62 (R_{xx}). For the head, worst precision was 0.36mm (T_x) and 0.38° (R_{XX}); for the cup, it was 0.12mm (T_z) and 0.51° (R_{YY}).

Conclusions: This *in vitro* study demonstrates that ultra-low dose CTSA is similar in accuracy to standard-dose (0.3 - 0.7mSv) RSA and far safer than a conventional hip CT (5 - 10mSv). CT is ubiquitous, so this method may be a safer and inexpensive alternative to RSA. Clinical validation studies are required to estimate the effect of patient variability on accuracy.

Disclosure: Nothing to disclose.

272
ASSESSING CHROMATIN ACCESSIBILITY IN A HUMAN CHONDROCYTE CELL-LINE
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Background: Osteoarthritis (OA) is chronic, debilitating and degenerative conditions with a complex pathophysiology that includes genetic, environmental and patient factors but characterised by chronic cartilage destruction. Our aim was to employ a novel laboratory technique "Assay for Transposase-Accessible Chromatin using Sequencing" (ATAC-seq) to determine differential genomic enhancer and promoter regions regulated by the proinflammatory cytokine interleukin-1 (IL1), in chondrosarcoma cell-line SW1353.

Methods: SW1353 cells were untreated or stimulated with Interleukin-1 (IL-1) to simulate a pro-inflammatory environment and both groups assessed using ATAC-Seq. The ATAC-seq protocol has modified to reduce mitochondrial DNA contamination. Peaks were called using MACS2 and correlated to global gene expression data.

Results: In total we identified ~120,000 peaks corresponding to open chromatin. As expected the vast majority were located around transcription start sites (essentially promoters). Only 241 genomically open regions were differentially present upon IL-1 stimulation. The presence of these peaks correlated with genes the expression of genes regulated by IL-1.

Conclusions: We identified 241 genetic loci that are altered by the exposure of SW1353 to IL-1. Regions were near relevant genes such as MMP13, a collagenase important for cartilage collagen destruction. The function of several of these loci are currently being investigated using CRISPR/Cas9 genomic deletion strategies.

Implications: Understanding the genetic controls of genes involved in OA provides potential targets for pharmacological therapies that may slow down or stop the progression the disease.

Disclosure: Nothing to disclose.

292
SONOGRAPHIC BRIDGING CALLUS : AN EARLY PREDICTOR OF FRACTURE UNION
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Background: Ultrasound has the potential to detect early bridging callus prior to radiographs. There is currently a lack of agreed criteria for sonographic assessment of callus and reliability between reviewers. The primary aim of this study was to determine objective criteria and reviewer agreement for assessment of sonographic bridging callus (SBC) on ultrasound.

Methods: A prospective cohort of conservatively managed displaced midshaft clavicle fractures underwent ultrasound scanning at three, six and 12 weeks post-injury. Five nonunions were matched against a control group of 15 unions. Interpretation at the fracture site was based on the sonographic detection of fibrocartilaginous material and SBC. The echo intensity (EI) of the callus was recorded at each time point. The ultrasound scans were evaluated by two blinded reviewers and weighted kappa was used to determine intra- and inter-observer agreement.

Results: At three weeks post-injury fibrocartilaginous material was present in 80% of patients and was associated with union (sensitivity 93%, specificity 60%, $p = 0.03$) but inter-observer agreement was rated "fair" on kappa (0.44). By six weeks SBC was evident in 60% of patients and when present all united. In comparison, 10% of patients had radiographic bridging callus at six weeks. When SBC was absent at six weeks, 63% of patients went onto nonunion at six months (5/8) (sensitivity 80%, specificity 100%, $p = 0.002$). The reliability of SBC detection was rated "very strong" for intra- (kappa 0.92) and inter-observer agreement (kappa 0.84). The EI of SBC was not found to be predictive of union. At twelve weeks, bridging callus was present on both radiographs and ultrasound in all patients that united and in none of those who developed a nonunion (sensitivity 100%, specificity 100%, $p < 0.001$).

Conclusions: Ultrasound evaluation of bridging callus at six weeks has excellent accuracy to predict union with strong reviewer agreement.

Disclosure: Nothing to disclose.

582
THE BASIC SCIENCE OF THE BRADLEY CARBON FIBRE REINFORCED PLASTIC HIP REPLACEMENT STEM
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Background: In the early 1990's an experimental low modulus of elasticity femoral hip prosthesis stem was developed from carbon fibre reinforced poly-ether-ether-ketone (PEEK) and trialled at the Royal Gwent Hospital. The aim was to overcome the problems arising from stress shielding associated with metal stems. The new composite stem had a Young 愀 modulus profile tailored to the elasticity of an average adult femoral shaft with a hydroxyapatite coating on its proximal third and a metal neck and trunion. Both cemented and uncemented cups were implanted with the stem.

Methods: Patients were investigated for stress shielding between 1993 and 2000. The first 60 cases were assessed at one and two years post-operatively by radiographs and by metal exclusion DEXA-scanning. As a control, the density of the normal unimplanted femur was assessed and then compared with that of the implanted side. These results were compared with published figures for metal stems, and with a small series of metal stems from the Royal Gwent Hospital.

Results: Sixty of the 136 patients were analysed, 21 males and 39 females, with an average age of 61 years at operation. Bone density around the carbon composite hip was found to increase, most significantly in the calcar region, between the first and second postoperative years. In the unimplanted controls, bone density remained unchanged.

Conclusion: The results of the DEXA scanning that showed restoration of bone mineral density compared favourably with published figures for metal prostheses used at the time. This report compliments the long term clinical follow up study, providing the scientific background for this experimental stem.

Disclosure: John Bradley designed the Bradley stem and received royalties from the manufacturing company (Orthodynamics Ltd, Dorset, UK).

706

EFFICACY OF ANTIBIOTIC-LOADED CALCIUM SULFATE TO ERADICATE ESTABLISHED BIOFILMS IN AN *IN VITRO* MODEL

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Background: Formation of biofilms following periprosthetic joint surgery is a severe complication due to difficulties with diagnosis and eradication. Treatment for periprosthetic joint infection (PJI) often includes procedures such as surgical intervention and antibiotic therapy, whereby the most effective antimicrobial treatments are administered both locally to the infection and systemically. There is also evidence that releasing antibiotics directly at surgical sites can be used effectively in preventing implant colonisation and eradicating established biofilms. This investigation assesses the ability of synthetic recrystallised calcium sulfate (SRCS*) mixed to contain a combination of vancomycin and gentamicin (VG), or vancomycin and tobramycin (VT) to eradicate pre-formed biofilms *in vitro*.

Materials and methods: Biofilms of *Pseudomonas aeruginosa* or *Staphylococcus aureus* were established on polycarbonate coupons within a CDC biofilm reactor. Biofilm coupons were exposed to plates containing suspended SRCS with VG/VT beads at concentrations of 500mg/240mg per 10cc and 1g/240mg per 10cc respectively. Control coupons were tested concurrently. Challenge plates were incubated for 24 hours at 37°C ± 2°C. All testing was performed in triplicate. Data was analysed by Students T-Test to determine statistical significance.

Results: An average of 6.78 ± 0.23 $Log_{10}CFUmL^{-1}$ and of 6.60 ± 0.23 $Log_{10}CFUmL^{-1}$ of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were recovered from the negative control biofilms respectively. No viable organisms were recovered from biofilms exposed to the positive control or those exposed to SRCS beads containing VG or VT, within detection limits. This equated to an average log reduction in P. aeruginosa of >5.78 $Log_{10}CFUmL^{-1}$ and >5.60 $Log_{10}CFUmL^{-1}$ in S. aureus ($p < 0.001$).

Conclusions: Exposure of *P. aeruginosa* and *S. aureus* biofilms to SRCS containing a mixture of vancomycin and gentamicin or vancomycin and tobramycin resulted in eradication of pre-formed biofilms in the method described. Further assessment is required to confirm clinical performance.

*Stimulan Rapid Cure, Biocomposites

Disclosure: Craig Delury, Sean Aiken, Leanne Cornes and Phillip Laycock are all employees of Biocomposites Ltd.

723

3D BIOPRINTED BACTERIAL BIOFILMS: A NOVEL, 3D METHOD FOR STUDYING ORTHOPAEDIC INFECTION

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Background: Antimicrobial resistance (AMR) is projected to result in 10 million deaths annually worldwide by 2050. Without urgent action, routine orthopaedic operations could become high risk and joint infections incurable in a "post-antibiotic era". However, current methods of studying AMR processes including bacterial biofilm formation are 2D in nature, and therefore unable to recapitulate the 3D processes involved within *in vivo* infection.

Methods: Within this study, 3D printing was applied for the first time alongside a custom-developed alginate bioink to bioprint mature 3D bacterial biofilm constructs from clinically relevant species including *Staphylococcus aureus* (MSSA), *Methicillin-resistant staphylococcus aureus* (MRSA), *Escherichia coli* and *Pseudomonas aeruginosa*. Bioprinted bacterial structures were ioinically crosslinked following bioprinting with barium chloride to increase stability in culture. Biofilm formation was observed using confocal laser scanning microscopy (CSLM).

Results: Bacterial viability and biofilm formation was excellent in bioprinted constructs. Formation of mature 3D bioprinted biofilms was observed for the first time and the biofilm lifecycle observed using CLSM over 28 days *in vitro*. 3D MRSA and MSSA biofilm constructs had greater resistance to antimicrobials than corresponding two-dimensional (2D) cultures, with higher minimum inhibitory concentrations (MIC) found. Thicker 3D *E.coli* biofilms had greater resistance to tetracycline than thinner constructs over seven days of treatment. Raman spectroscopy was also adapted in a novel approach to non-invasively diagnose 3D bioprinted biofilm constructs located within a joint replacement model.

Conclusions: In conclusion, bacterial biofilm constructs were reproducibly 3D bioprinted for the first time using clinically relevant bacteria. This methodology can be applied to study antimicrobial penetration of biofilms in 3D and potentially aid in the future search for novel antimicrobials. Furthermore, by deploying Raman spectroscopy in a novel fashion, it was possible to diagnose 3D bioprinted biofilm infections within a joint replacement model.

Disclosure: Nothing to disclose

741

***IN VITRO* ERADICATION OF ESTABLISHED BIOFILMS BY LOCAL RELEASE OF ANTIBIOTICS FROM A BI-PHASIC BONE GRAFT SUBSTITUTE**

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Aims: Periprosthetic joint infection is a serious surgical complication, often compounded by the presence of biofilms. Successful treatment relies on surgical interventions coupled with antimicrobial therapies including local release at the site of infection.

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This can be achieved by mixing bone graft substitutes with a relevant antimicrobial drug for implantation at the surgical site. The aim is to achieve high doses directly targeting infection.

Genex is a fully absorbable bi-phasic bone graft substitute (BPBGS) composed of β-tricalcium phosphate and calcium sulfate; in this study mixed with either vancomycin and gentamicin (VG), or vancomycin and tobramycin (VT) to investigate the ability of antibiotic-loaded BPBGS to eradicate *in vitro* pre-established biofilms.

Methods: Biofilms of *Staphylococcus aureus* (NCTC 8325) and *Pseudomonas aeruginosa* (NCIMB 10434) were established on polycarbonate coupons within CDC biofilm reactors. Coupons were then suspended between pre-prepared antibiotic BPBGS beads at concentrations of 500mg vancomycin and 185mg gentamicin or tobramycin per 5cc, then transferred to challenge plates. Plates were incubated for 24 hours at 37+/- 2°c, then coupons were removed, washed and sonicated to recover remaining microorganisms. Viable colonies were counted, and significance was determined by Student's t-test.

Results: Negative controls retained 5.08 +/- 0.09, and 6.94 +/- 0.11 $Log_{10}CFUmL^{-1}$ colonies of *S.aureus* and *P.aeruginosa* respectively. No viable colonies were recovered from positive controls or BPBGS VT/VG samples within the detectable limits, showing an average log reduction of > 5.94 $Log_{10}CFUmL^{-1}$ in *P. aeruginosa* and >4.08 $Log_{10}CFUmL^{-1}$ in *S. aureus* ($p < 0.001$).

Conclusions: Antibiotic loaded BPBGS can successfully eradicate *S.aureus* and *P.aeruginosa* biofilms *in vitro*, indicating a potential role in the prevention and management of infection. Further investigations are required to determine *in vivo* efficacy.

Disclosure: Craig Delury, Sean Aiken, Leanne Cornes and Phillip Laycock are all employees of Biocomposites Ltd.

751

IN VITRO ELUTION AND EFFICACY OF FLUCLOXACILLIN RELEASED FROM CALCIUM SULFATE BEADS

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Aims: Local application of antibiotics can provide high local concentrations whilst minimising risk of systemic toxicity. This *in vitro* study evaluated setting characteristics, elution and bactericidal efficacy of flucloxacillin released from synthetic recrystallised calcium sulphate (RCS)*.

Methods: A high purity synthetic RCS was mixed with 1000mg/10cc flucloxacillin. Both powders were combined, then the mixing solution provided was added and mixed for 30-45 seconds and moulded into 4.8mm and 6mm hemispherical beads.

Six-millimetre beads were placed onto tryptone soy agar plates seeded with *Staphylococcus aureus* then incubated for 24 hours at 33+/- 2°c. On removal from the incubator, plates were examined for the absence of growth, indicated by a zone of inhibition (ZOI) around the test material. Zones were measured and photographed.

Three grammes of 4.8mm beads were added to vials containing 4ml phosphate buffered saline (PBS) and stored in an incubator at 37+/-2°c . Two millilitres were extracted at regular intervals for analysis and the volume replenished with fresh PBS. All samples were analysed in triplicate by Liquid Chromatography-Mass Spectrometry (LC-MS).

Results: Beads containing flucloxacillin were successfully mixed and set in three to five minutes. LC-MS analysis showed initial elution of flucloxacillin at approximately 6200µg/mL and retained elution of 2500µg/mL for 10 days. Rate of elution steadily decreased to below 300µg/mL after 14 days. Beads also produced ZOI with diameters ranging between 48-49mm against *S.aureus*.

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Conclusions: ZOI analysis demonstrated that flucloxacillin released from RCS maintained efficacy against *S.aureus in vitro*. Additionally, analysis of the elution concentrations demonstrated high levels of flucloxacillin, above MIC for *S.aureus,* was released out to the 14 days investigated. *In vitro* doesn't correlate to *in vivo* and conditions at the implant site may affect elution and absorption rates.

References: *Stimulan Rapid Cure, Biocomposites Ltd.

Disclosure: All authors are employees of Biocomposites Ltd.

783

GLUTAMATE RECEPTOR EXPRESSION IN THE TIBIAL SUBCHONDRAL BONE CHANGES AFTER MEDIAL OPENING WEDGE HIGH TIBIAL OSTEOTOMY

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Osteoarthritis of the knee in younger, active patients remains a significant clinical problem. For those with isolated medial compartment disease, valgus high tibial osteotomies (HTO) aim to slow, halt or even reverse the progression of osteoarthritis. However the mechanism underlying this remains unclear.

Glutamate signalling is mechanically regulated and drives inflammation, pain and joint degradation in osteoarthritis in animal models. We hypothesised that realignment of the weightbearing axis of the knee would regulate glutamate receptor expression after HTO surgery.

Patients, recruited as part of the Arthritis Research UK Biomechanics and Bioengineering Centre HTO study, underwent medial opening wedge HTO. Subchondral bone samples were taken from four quadrants of the knee both at the time of surgery (pre-HTO) and at plate removal approximately 12 months later (post-HTO). Glutamate receptors, NR2D and GRIK4, mRNAs were measured using quantitative reverse transcription polymerase chain reaction (RT-qPCR). The expression within each compartment as a proportion of whole joint expression was compared longitudinally between pre and post samples and a one sample t-test performed.

NR2D and GRIK4 were significantly reduced within the posteromedial compartment. Median NR2D expression (n = 18) was reduced by 17.4% (p=0.03), whereas median GRIK4 expression (n = 17) was reduced by 15.5% (p = 0.04) in posteriomedial subchondral bone post-HTO.

These results suggest that expression of NR2D and GRIK4 receptors may be mechanically regulated in human subchondral bone. This supports the hypothesis that altering knee biomechanics via HTO causes changes in subchondral bone biology which are related to the progression of knee osteoarthritis.

Disclosure: Nothing to disclose.

831

CLINICAL EFFECTIVENESS OF ASPIRIN AS MULTIMODAL THROMBOPROPHYLAXIS IN PRIMARY TOTAL HIP AND KNEE ARTHROPLASTY- A REVIEW OF 6078 CASES

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Background: Venous thromboembolism (VTE) is a serious complication after total hip and knee arthroplasty. There is still no consensus regarding the best mode of thromboprophylaxis after lower limb arthroplasty. The aim of this study was to ascertain the efficacy, safety profile and rate of adverse thromboembolic events of aspirin as extended out of hospital pharmacological anticoagulation for elective primary total hip and knee arthroplasty patients and whether these rates were comparable with published data for low molecular weight heparin (LMWH).

Methods: Data was extracted from a prospective hospital acquired thromboembolism (HAT) database. The period of study was from 1st January 2013 to 31st December 2016 and a total of 6078 patients were treated with aspirin as extended thromboprophylaxis after primary total hip and knee arthroplasty.

Results: The primary outcome measure of deep vein thrombosis and pulmonary embolism within 90 days postoperatively was 1.11%. The secondary outcome rates of wound infection, bleeding complications, readmission rate and mortality were comparable to published results after LMWH use.

Conclusions: The results of this study clearly show that Aspirin, as part of a multimodal thromboprophylactic regime, is an effective and safe regime in preventing VTE with respect to risk of DVT or PE when compared to LMWH. It is a cheaper alternative to LMWH and has associated potential cost savings.

Disclosure: Nothing to disclose.

848

THE ELECTROSTATIC CONTRIBUTION OF PROTEOGLYCANS TO MECHANICAL STIFFNESS OF THE HUMAN MENISCUS

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Background: Load transmission is a principal role of the meniscus. In articular cartilage, proteoglycans contribute to mechanical stiffness via negatively charged moieties which generate Donnan osmotic pressures. Such a role for proteoglycans in meniscal tissue has not been established.

Method: Discs of human meniscal tissue two millimetres thick and of five millimetres diameter were placed within a custom confined compression chamber and bathed in 0.14M PBS (mimics cellular environment), deionised water (negates effect of mobile ions) or 3M PBS (negates all ionic effects). The apparatus was mounted within a materials testing machine and a 0.3N preload applied. Once equilibrium was attained, a 10% ramp compressive strain was followed by a 7200 second hold phase. Resultant stress relaxation curves were fitted to a nonlinear poroviscoelastic model with strain dependent permeability using finite element modelling. All samples were assayed for proteoglycan content. Comparison of resultant parameters was undertaken using multivariate ANOVA with Bonferroni adjustment for multiple comparisons.

Results: Thirty-six samples were tested. A significant difference ($p < 0.05$) was observed in the value of the Young's modulus (E) between samples tested in deionised water compared to 0.14M/3M PBS, with the meniscus found to be stiffest in deionised water (E = 1.15 MPa) and least stiff in 3M PBS (E = 0.43 MPa). No differences were observed in the zero strain permeability or the exponential strain dependent/stiffening coefficients. Proteoglycan content was not found to differ with solution, but was found to be significantly increased in the middle meniscal region of both menisci.

Conclusions: Proteoglycans make a significant electrostatic contribution to mechanical stiffness of the meniscus, increasing it by 58% in the physiological condition, and are hence integral to its function.

It is therefore critical that meniscal regeneration strategies such as scaffolds or allografts attempt to preserve, or compensate for, the function of proteoglycans to ensure normal meniscal function.

Disclosure: Nothing to disclose.