post-tests, changes were observed only at exercise groups. In running group, significant increase (P = 0.049) occurred immediately after the walking exercise. Also in swimming group COMP serum concentration increased significantly (P = 0.084) immediately after the walking exercise. Significant decrease (P = 0.051) of serum COMP concentration were observed at direct impact running group at 30-minutes recovery after walking exercise.
Conclusions: In this age group, moderate walking activity can have little influence on the increase serum COMP concentrations. However, significant decrease of serum COMP concentration after walking exercise observed only in running group. This change, after 12-weeks regular weight-bearing impact exercise, will be explained with the functional adaptation of articular cartilage to specific environmental requirements.

112 NEW APPROACHES IN THE DETECTION OF CALCIUM PHOSPHATE CRYSTALS IN SYNOVIAL FLUID

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Purpose: Calcium phosphate crystals such as basic calcium phosphate (BCP) and calcium pyrophosphate dihydrate (CPPD) are known to occur in intra-articular synovial fluid (SF) and may be associated with a number of destructive arthropathies. For instance, BCP crystals are found in up to 70% of osteoarthritic joints and current data suggests that BCP crystals represent a potential therapeutic target. Previous studies have used enzymatic and chemical methods to reduce organic contamination in the biological sample in an attempt to facilitate crystal detection. However, by doing so, valuable information from other biomarkers in SF sample will be lost. Bisphosphonates are commonly used as pharmaceutical drugs for the treatment of osteoporosis and are known to bind very specifically and strongly to the surface of calcium phosphate. We have exploited this chemical specificity to create an extraction platform which can isolate calcium phosphate crystals from SF samples in a simple and selective manner.
Methods: Spiked samples were prepared by suspending synthetic calcium phosphate crystals (HA/CPPD) in artificial SF (3% hyaluronan in human serum) at concentrations normally found in vivo. SF samples were aspirated from the affected joints (mainly knees and shoulders) of patients with OA and/or other arthropathies. Commercially available superparamagnetic beads (Dynal) were derivatised with bisphosphonates (neridronate) using carbodiimide coupling chemistry. Bisphosphonate-modified superparamagnetic beads (BPSM beads) were mixed and incubated with the SF samples for one hour. Removal of unwanted biological material and washing was achieved by using a small permanent magnet to pull the beads to the side of the vessel. Scanning electron microscopy (SEM) and energy dispersive x-ray (EDX) spectroscopy (Hitachi SN-3000) was used to confirm the presence/absence of crystals captured by the beads. Calcium content was measured using an o-cresolpthalein complexone assay on a Biotek Powerwave XS 96-well reader. Raman spectra were collected on a Renishaw InVia Raman microscope (514/785 nm laser excitation).
Results: SEM/EDX measurements of spiked SF showed that HA/CPPD crystals were successfully isolated by the BPSM beads. Typically, high levels of calcium measured using colorimetry were consistent with the presence of crystals in the sample as found in SEM. Raman spectral features indicated that there are certain marker bands that are specific to the type of crystal species present, such as the specific signature modes at 960 cm^{-1} (BCP) and 1043 cm^{-1} (CPPD). Hence, Raman microspectroscopy provides a means of detecting the type of crystal present following the initial separation step.
Conclusions: In this approach, the surface of superparamagnetic polymer beads was modified with commercially available bisphosphonates, essentially providing the beads with the capability to capture calcium phosphate crystals from SF samples. Once isolated, the crystals were successfully detected and characterised using colorimetry, scanning electron microscopy and Raman microspectroscopy. The main challenge is to correlate the high levels of calcium with the presence of crystals and the proposed diagnosis. This aspect will require the analysis of a larger pool of samples and may provide a tool for improved diagnosis and characterisation of OA and other crystal related pathologies.

113 COLORIMETRY AS A POTENTIAL DIAGNOSTIC TOOL IN OSTEOARTHRITIS

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Purpose: Synovial fluid examination is a valuable but under-utilised diagnostic tool for arthritis. The link between basic calcium phosphate (BCP) crystals in synovial fluid (SF) and osteoarthritis (OA) is well described. BCP crystals are unique OA and may serve as a biomarker and aid to more accurate diagnosis of the disease. The detection and quantification of BCP crystals in OA is particularly difficult due to their submicron dimensions and the challenges of finding them within the complex and viscous synovial fluid matrix. A further complication resides in the ability to distinguish them from the other calcium-containing crystals that can co-exist in synovial fluid such as calcium pyrophosphate dihydrate (CPPD), commonly associated with pseudogout. The aim of our work is to develop semi-quantitative, colorimetric assays for detecting calcium phosphate crystals in SF.
Methods: Model SF samples were prepared by suspending synthetic hydroxyapatite (HA) and CPPD crystals in simulated SF (3% hyaluronan in human serum) at concentrations normally found *in vivo*. Patient SF samples were aspirated from the affected joints (mainly knees and shoulders) of 20 people with OA and/or other arthropathies. For crystal extraction, SF samples (100 μl) were first treated with sodium hypochlorite and ultrasonicated to break down the organic matrix. Samples were then centrifuged to pellet the crystals. This was followed by a number of washing steps with water. The pellet was dissolved in 1N nitric acid to dissociate the crystals into their constituent ions. Calcium concentration was measured using an o-cresolpthalein complexone assay (1−10 mg/l range). Phosphate concentration was measured using a modified molybdate blue method (1−50 mg/l) on a Biotek Powerwave XS 96-well reader and also determined using a vanadate-molybdate reagent with 0.002 M solution of malachite green (MG) as a chromophore. Values obtained for the samples were normalised against analytical grade calcium and phosphate standards. Scanning electron microscopy (SEM) and energy dispersive x-ray (EDX) spectroscopy (Hitachi SN-3000) were used to confirm the presence/absence of crystals isolated within the pellet.
Results: Sodium hypochlorite solution was found to be an effective deproteinising agent that helps to degrade the organic material but does not break down HA and CPPD crystals due to their stability in basic solution. SEM of spiked SF showed that HA/CPPD crystals were successfully isolated from the organic matrix with no signs of degradation. Calcium levels found in patient SF samples ranged from 40–200 (mg/l). The presence of BCP or CPPD could be diagnosed using the Ca:P ratio. The ratio closer to 1:1 indicated excess of CPPD and closer to 1:1 – excess of HA crystals. Typically, high levels of calcium and phosphate levels were consistent with the presence of crystals in the sample as found in SEM.
Conclusions: We present a simple, semi-quantitative method for detecting calcium phosphate crystals in SF. The main challenge is to correlate the high levels of calcium/phosphate with the presence of crystals and the proposed diagnosis. This aspect will require the analysis of a larger pool of samples and may provide a tool for improved diagnosis and characterisation of OA and other crystal related pathologies. The MG method is suitable for naked-eye categorising of SF samples for high/medium/low phosphates content.