Characterization and Drug Solubility Studies in Rheumatoid Arthritis and Osteoarthritis Synovial Fluid toward the Development of In Vitro Disease State Biorelevant Media

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Purpose
In order to predict drug’s solubility and dissolution behaviour in the joint a biorelevant medium similar to disease state synovial fluid is needed. In this study the physicochemical properties of the in vivo synovial fluid were characterised in detail, and the solubility of a model drug for intra-articular use in the in vivo pathogenic synovial fluid was measured.

Methods
10 samples of Rheumatoid Arthritis (RA) and 15 samples of Osteoarthritis (OA) synovial fluids were collected from volunteers at the Attikon University Hospital (Greece) respectively and were kept at -80°C. The pH values were measured by a pH electrode connected to a S220 SevenCompact pH/Ion pH meter (Mettler-Toledo) at room temperature. The osmolality was measured by freezing point depression using an Advanced Micro-Osmometer Model 3300 (Advanced Instruments Inc.), while surface tension was determined with a Du Nouy ring using a Force Tensiometer – Sigma 700/701 (Dyno Testing) with a measurement conducted every 30 s until a stable surface tension was indicated at room temperature. Viscosity was measured by a High Resolution C-Vor torque balance Rheometer with the cone and plate method at 25°C. Solubility was determined by the shake-flask method with the use of a shaking water bath at 37°C. An excess of Triamcinolone Acetonide (TA) was added in 2 mL of RA and OA samples which were vortexed for 30 s and then incubated into the water bath. After 24 h samples were withdrawn and treated with an Hyaluronidase solution. The samples were filtered through 0.45μm RC filters and then analysed with HPLC. Solubility quantification was performed based on calibration curves constructed with standard solutions of TA spiked in OA and RA samples and treated as the samples.

Results
The pH of the RA samples was 7.56±0.11 that is close to the value of the synovial fluid in physiological conditions. The pH of the OA samples was at 8.04±0.41 making it slightly basic. The osmolality of the RA samples compared to the OA samples was similar and close to isosmotic values (299.5±9.87 and 301.89±9.55 mOsm/kg, respectively). Surface tension was 47.66±2.19 mN/m for RA and 45.44±3.78 mN/m for OA. Although different amount of phospholipids are present in OA and RA synovial fluids, the surface tension which is primarily affected by the presence of phospholipids, seems to be similar in the OA and RA synovial fluids. The viscosity of the in vivo synovial fluids was measured at different shear rates as non-Newtonian properties are present. In high shear rates the fluids tend to have higher viscosities and a higher variation compared to lower shear rates where the viscosity and the variation is smaller. For shear rates from 0.07 to 1000 1/s the viscosity values for RA fluids had an average value of 0.36 to 0.01 Pa s and the OA fluids had an average value of 1.12 to 0.01 Pa s. The solubility studies of TA in the two disease state synovial fluids showed that in RA the drug has a solubility of 54.15±19.1 μg/mL and in OA 29.84±9.46 μg/mL. The solubility of TA in RA synovial samples is higher than its solubility in the OA samples suggesting that the different viscosity of the two pathogenic synovial fluids affect the solubility and probably the dissolution rate of the drug in the joint.

Conclusion
The complete characterisation of the physicochemical properties of the in vivo pathogenic synovial fluids allows the development of biorelevant media for solubility and dissolution studies for drugs used for intraarticular delivery. The expected differences in the drugs’ solubility and dissolution in the joint of OA and RA patients could affect the therapeutic outcomes.