WP3 Metabolomic, Lipidomic and Proteomic Analyzes

Technical Flowchart

WP3.1. Examination of synovial joint fluid at the metabolome level

Metabolite, lipid and protein fraction obtainment (liquid-liquid extraction method)

- 100 µL of samples will be extracted using 300 µL chloroform, 300 µL methanol and 100 µL Milli-Q water.
- 60 s vortexing- 10 m centrifugation at 10,000 rpm.

Metabolite solution in the methanol phase and
Lipid solution in the chloroform phase will be collected.

WP3.2. Examination of synovial joint fluid at the lipidome level

Evaporated lipid phase will be dissolved in 100 µL isopropyl alcohol.

Treated particles and phosphate-containing lipids will be enriched.
Analysis by reverse phase chromatography.
(Water and acetonitrile:isopropyl alcohol (ACN:IPA) 70:30 (v/v)).
(1% ammonium acetate - 0.1% acetic acid)

Scanning range: 100-1700 m/z
Column temperature: 60°C
Drying gas temperature: 350°C
Capillary voltage: 3.500 V.

WP3.3. Examination of synovial joint fluid at the proteome level

Phosphoproteomic studies:

- Protein pellet will be dissolved in 20 mM NH_4HCO_3 buffer.
- Proteins will be treated with 10 mM DDT and 10 mM iodoacetamide and separated into peptides with trypsin enzyme.
- Peptides obtained after 16 hours of incubation at room temperature will be treated with cartridges prepared in IP2 and phosphopeptide enrichment will be carried out.

Proteomic studies:

- LC-QTOF mass spectrometry using the PreOmics kit (PreOmics GmbH).
- Reverse phase nanoLC (nano liquid chromatography) analyses.
- Ions obtained using nano electrospray ionizer will be sent to the mass spectrometer.

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