Development of a Novel Immobilized Metal Affinity Chromatography (IMAC) Sorbent for Phosphopeptidomic Analysis of Synovial Fluid

Serhat ALADAĞ[1], İlayda DEMİRDİŞ[2], Burcu GÖKÇAL[3], Ozan KAPLAN[4], Selinay ÖZEL[5], Batuhan Erhan AKTAŞ[6], Mustafa ÇELEBİER[4], Ömür ÇELİKBIÇAK[5], Süleyman Ali TUNCEL[3], Feza KORKUSUZ[6,]

(1)Departments of Bioengineering, (2) Biology, (3) Chemical Engineering, (4) Basic Pharmacy Sciences, (5) Chemistry and (6) Sports Medicine, Hacettepe University, Ankara, Türkiye

Feza KORKUSUZ, MD
Sports Medicine, Hacettepe University

Cells & Extracellular Templates - 2nd International Conference
June 2023, Rome, Italy

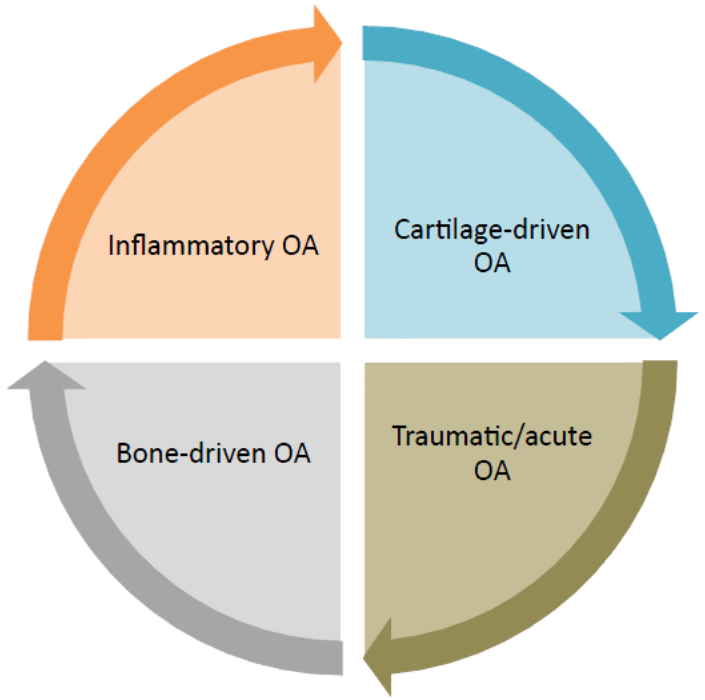
Conflict of Interest

Hacettepe University Medical Faculty

Member of the Turkish Academy of Sciences (TUBA)

MC of the EU:COST-NetwOArK (www.netwoark.eu) Project

Osteoarthritis

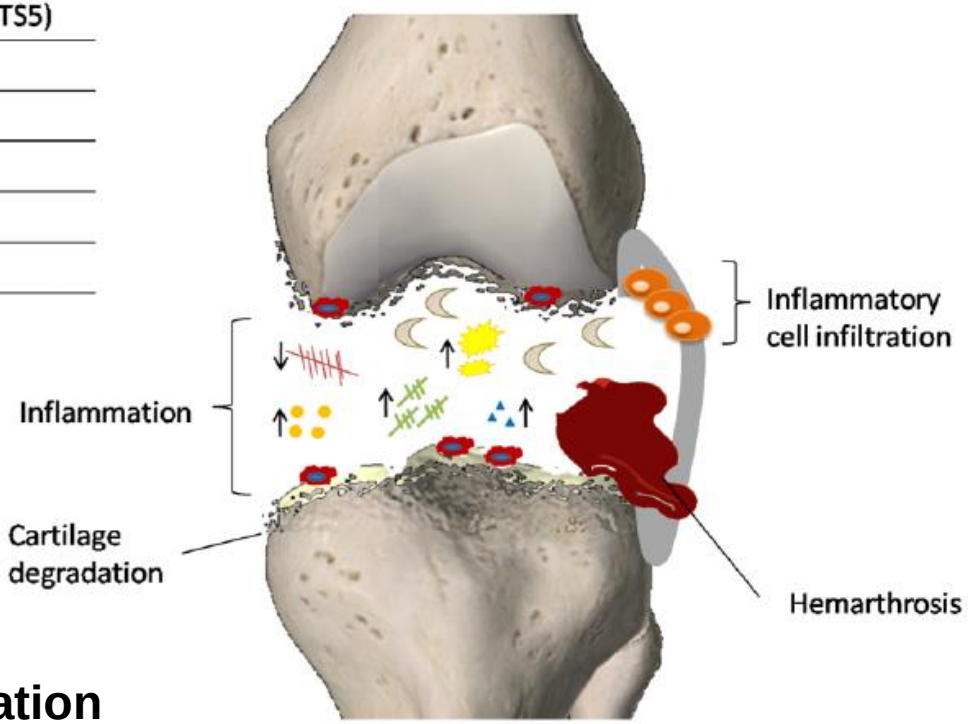


A. Mobasheri et. al., Osteoarthritis and Cartilage 25 (2017) 199-208

disabling condition with morbidity and mortality.

Joint Physiology

●	Cytokines (IL-1, IL-6, IL-8, TNF)
▲	Metalloproteinase (MMP-1, -3, -8, -9, -13; ADAM-TS5)
⟋	Proteoglycan
⬭	Chondrocyte death
ROS	Reactive oxidant species ($O_2^{\bullet-}$)
⟋	Lubricin
☾	Cartilage fragments
⬭	Inflammatory cells (macrophages, lymphocytes)



Inflammation-Degeneration-Regeneration

Punzi L et. al., **Post-traumatic arthritis: overview on pathogenic mechanisms and role of inflammation**, RMD, 2016

KNEE OSTEOARTHRITIS TREATMENT ALGORITHM*

(1) Non-Surgical — **Modifying the joint axis, BMI management, Exercise, Corticosteroids, NSAIDs**

(2) Disease Modifying — **GAG & CS supplements**
Intra-articular hyaluronan injections: (ESCEO)

ESCEO: The European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases

(3) Cellular† — **PRP, PRGF, SVF, Stem cells, Extracellular vesicles**

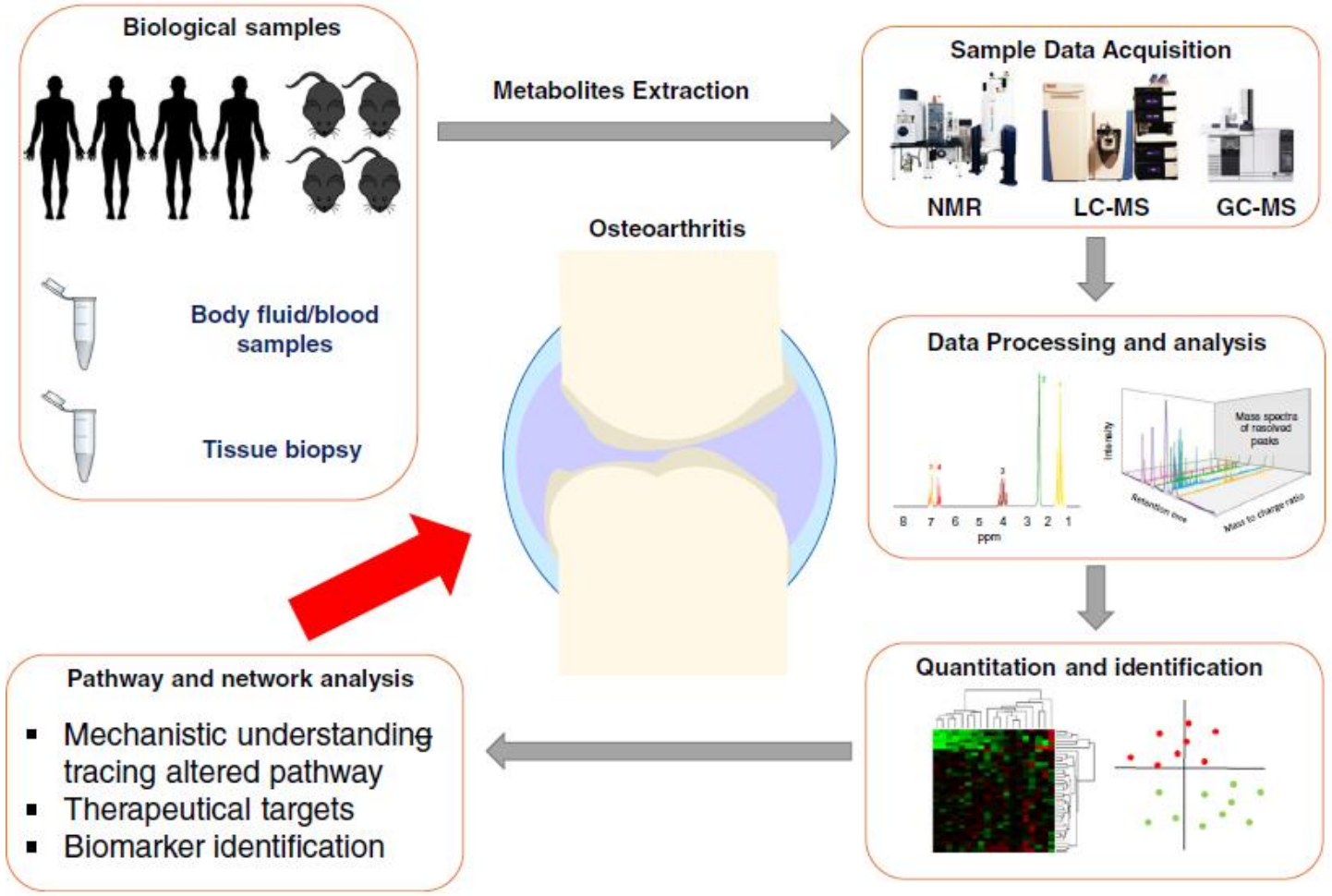
†Experimental (limited # of patients and follow up). (Modalities written in red are experimental.)

(4) Non-Degradable Polymeric Hydrogel Spacers

(5) Surgical — **Micro- or Nano-fx., Mosaicplasty, Allografts, MACI, High tibial osteotomy**
Partial or total joint replacement surgery.

*Modified from the OARSI Guidelines.

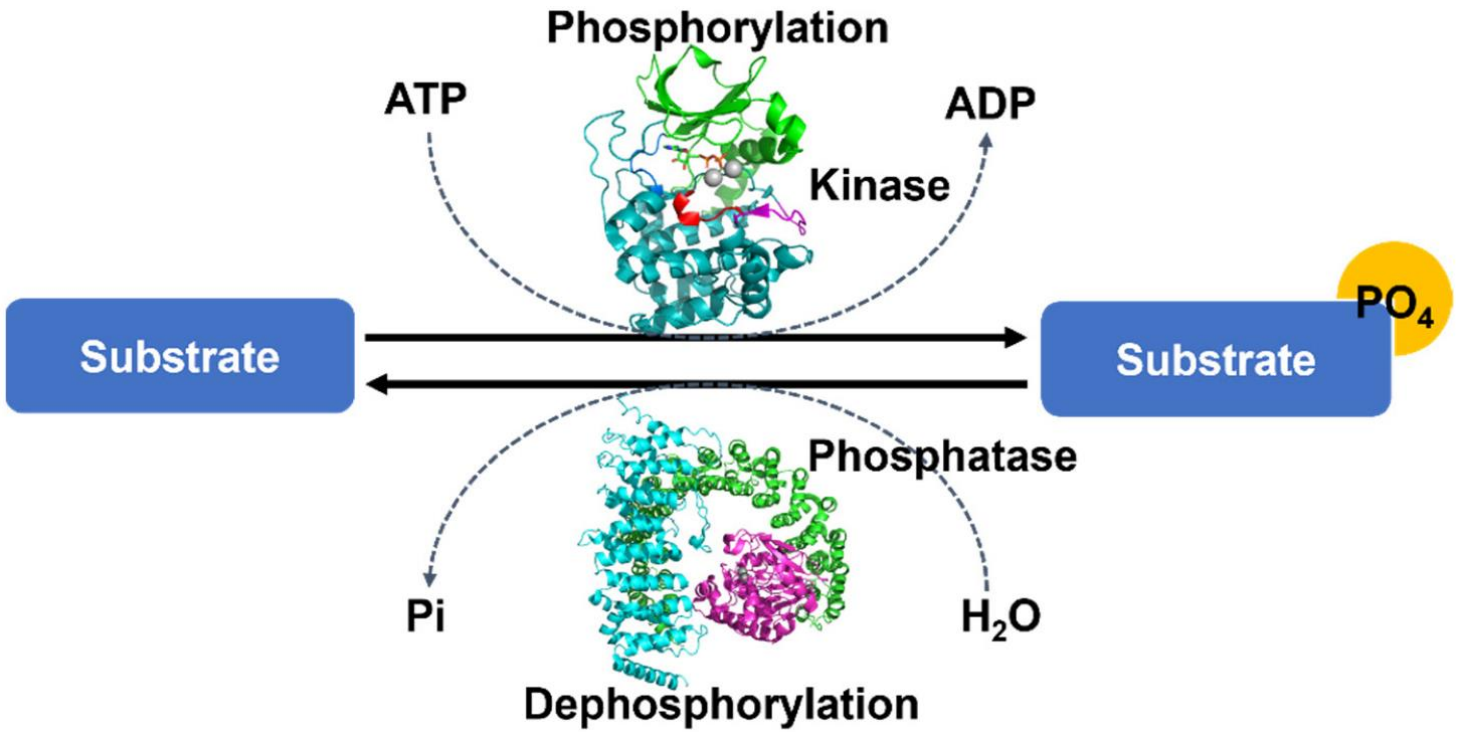
Omics Workflow



Salah Ali A. Showiheen et. al., **Application of Metabolomics to Osteoarthritis: from Basic Science to the Clinical Approach**, Curr Rheumatol Rep, 2019

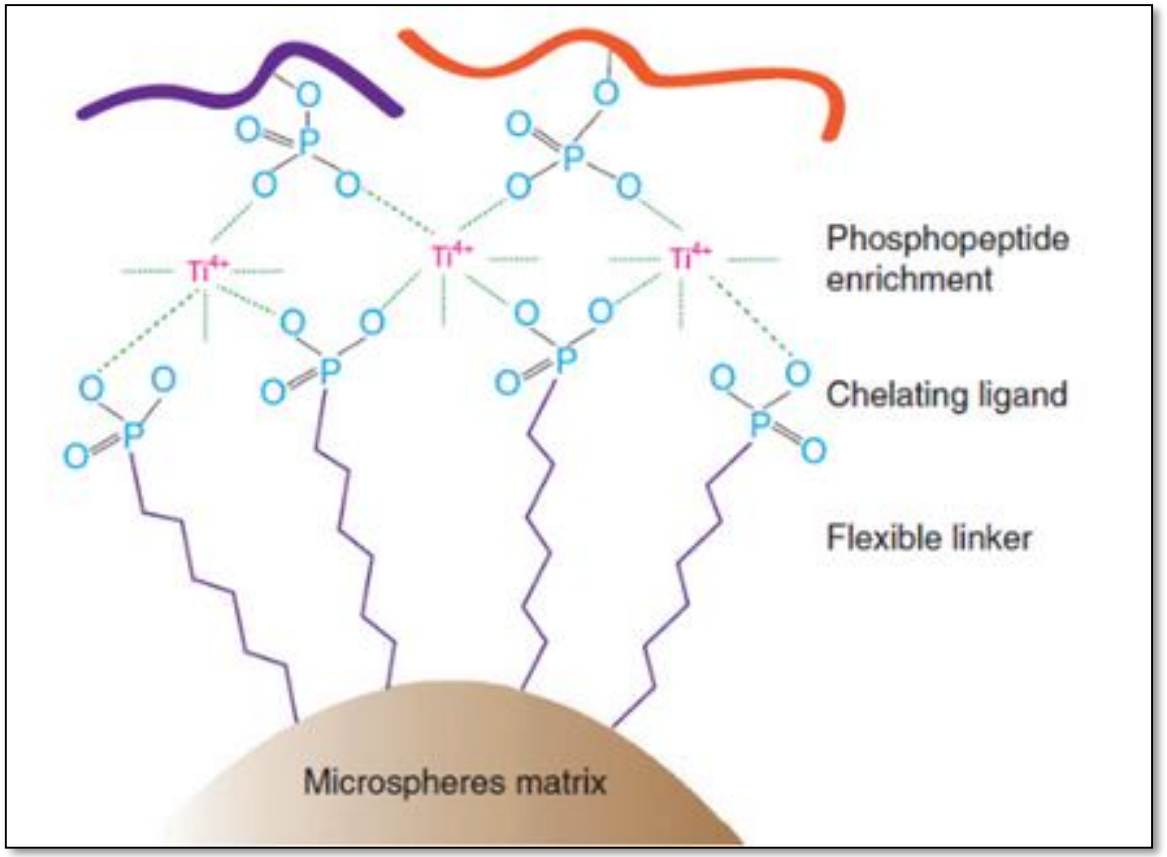
Phosphorylation

Phosphorylation, which is one of the changes that occur at the molecular level; regulates proliferation, signal transduction and apoptosis at the cellular level. **The present study aims to develop a new affinity sorbent for the determination of phosphopeptides for diagnosis, treatment and monitoring of arthropathic diseases and their determination by mass spectrometry**.



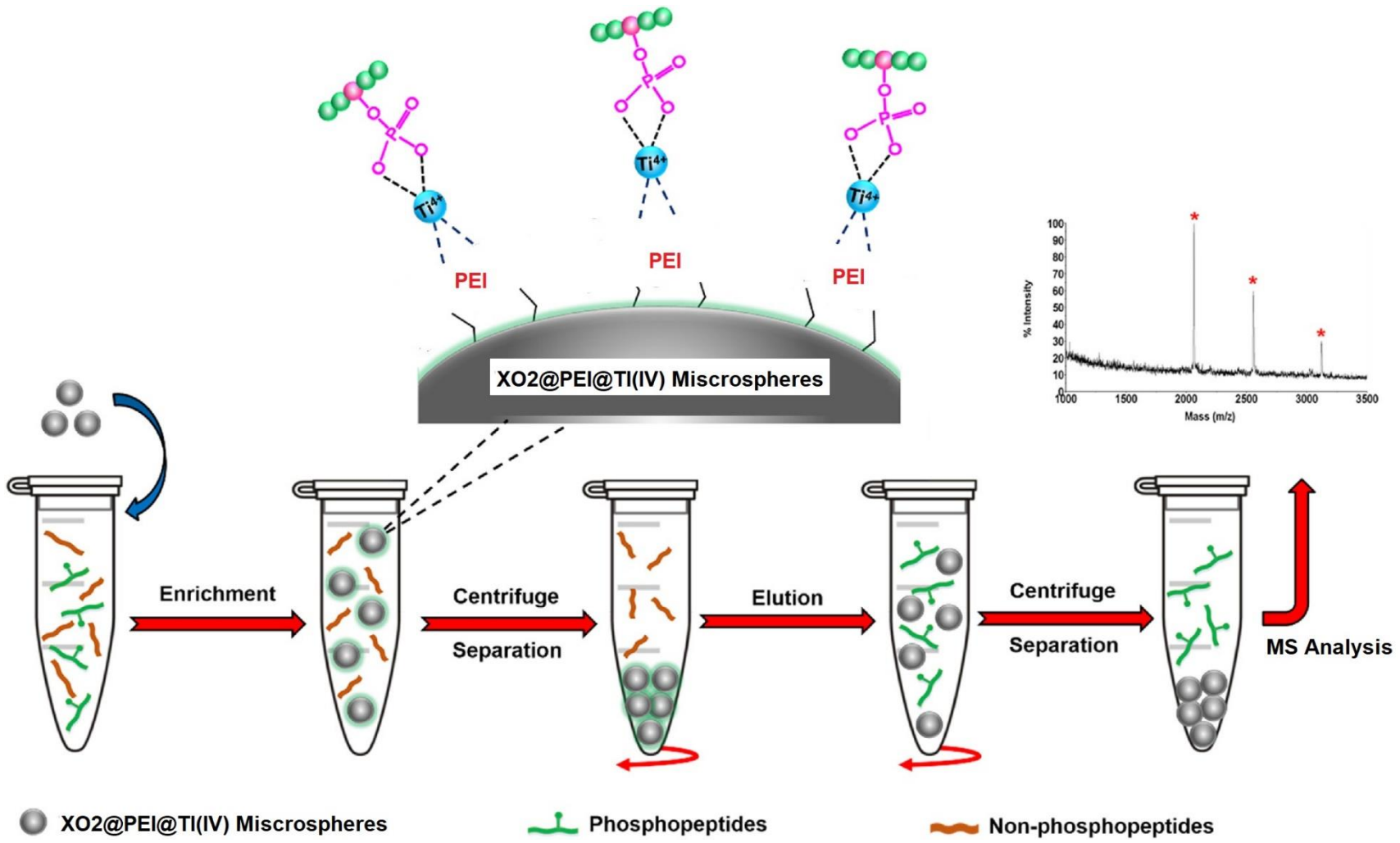
Phosphopeptide Enrichment Workflow

- Phosphoproteins/phosphopeptides can be attached to the surface by using the specific interaction between the phosphate groups in the phosphoproteins and the immobilized Ti(IV) cations of the sorbent material. The phosphoprotein or phosphopeptide enrichment method with Ti(IV) – IMAC (Ti(IV)-immobilized metal affinity chromatography) sorbent generally consists of three steps.
- In the first step, the sample is loaded. For this, phosphoprotein solution is prepared and the prepared solution is interacted with Ti(IV) - IMAC material until the adsorption equilibrium is reached.
- In the second step, non-specific peptides and proteins are removed. The sorbent is separated from the solution by centrifugation and the aqueous phase containing the non-adsorbed proteins is separated from the Ti (IV) – IMAC sorbent.



- In the last step, the elution of the captured phosphoproteins is made. Phosphoprotein/phosphopeptide - loaded Ti(IV) - IMAC sorbent is transferred to the elution medium and the phosphoproteins are separated from the surface.

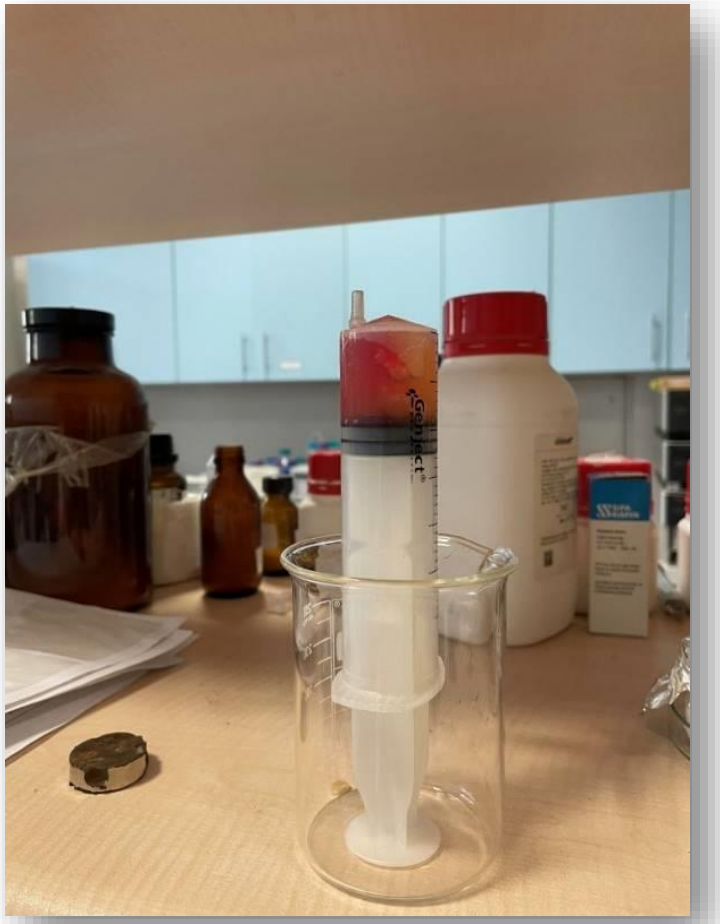
Phosphopeptidomics via Affinity Sorbent

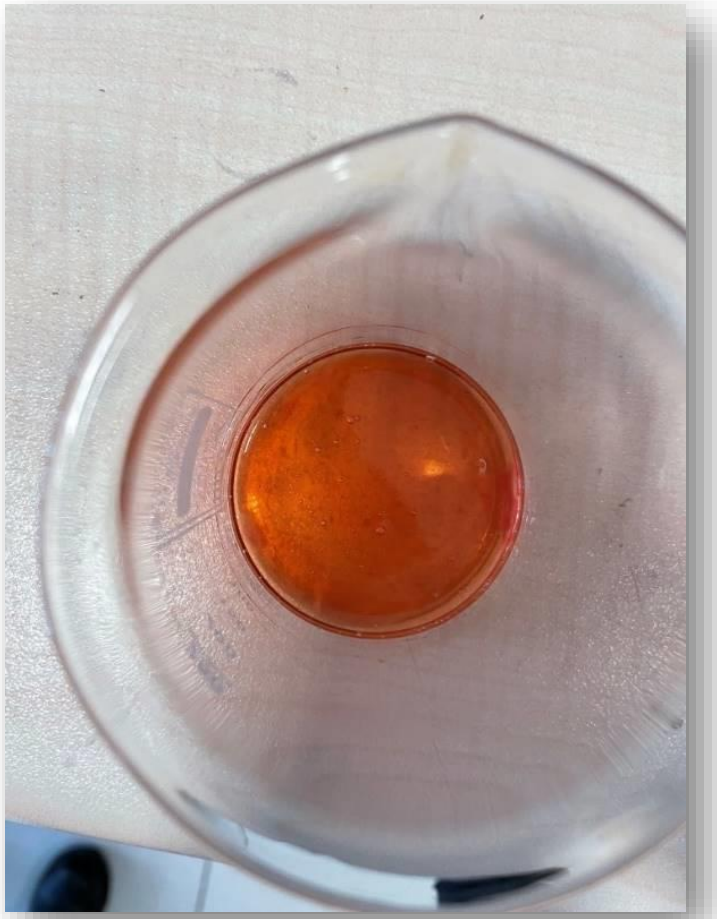


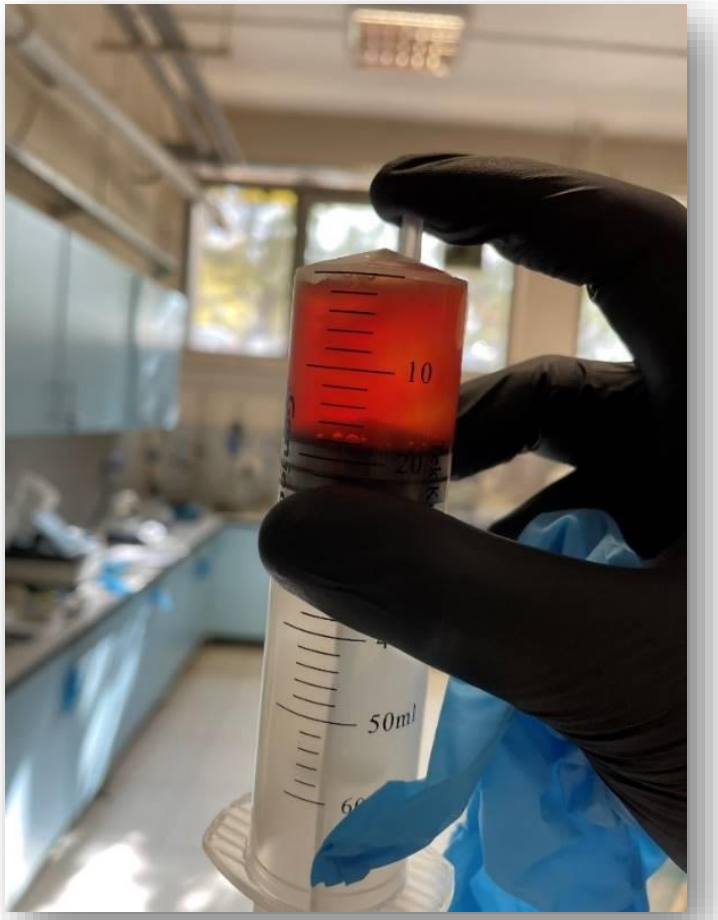
Preperation of Synovial Fluid

Bilateral Grade 4, Knee OA, Male, 57

Preperation of Synovial Fluid

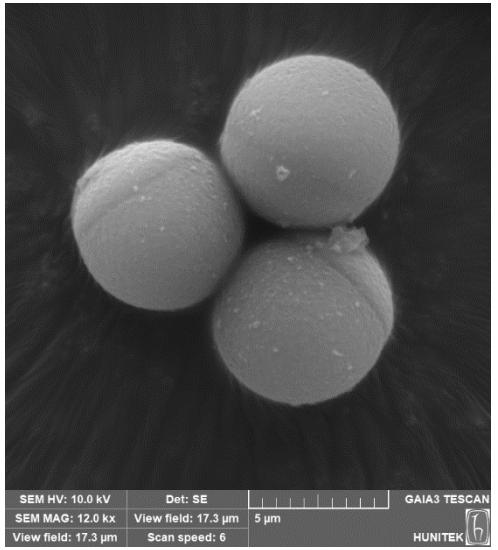


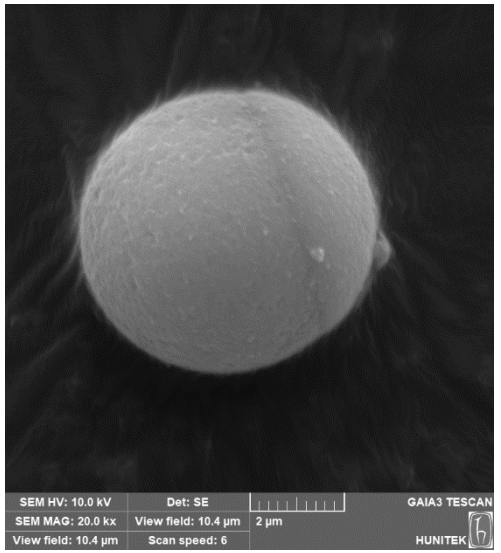




Synthesis of Affinity Sorbent

- Monodisperse, porous and high surface area SiO_2 microspheres were obtained using polyacrylate microspheres were as mold material. Polyethyleneimine (PEI) was used as the interface layer to immobilize Ti(IV) ions on SiO2 microspheres.
- For the synthesis of Ti(IV) attached-polyethyleneimine-silica [SiO_2@PEI@Ti(IV)] microspheres as the Ti(IV)-IMAC sorbent, Ti(IV) cations were linked to bound PEI chain on the microspheres. In order to determine the possible phosphopeptide isolation performance of the affinity sorbent, some known phosphoproteins α-casein and β-casein were used as the reference.
- Tryptic digestion and phosphopeptide enrichment via IMAC were then performed.
- The eluent obtained by IMAC enrichment of the sample prepared by tryptic digestion of phosphoprotein was subjected to mass spectrometry analysis.
- The IMAC enrichment was also applied for the synovial fluid and the eluent was also analyzed by mass spectrometry.

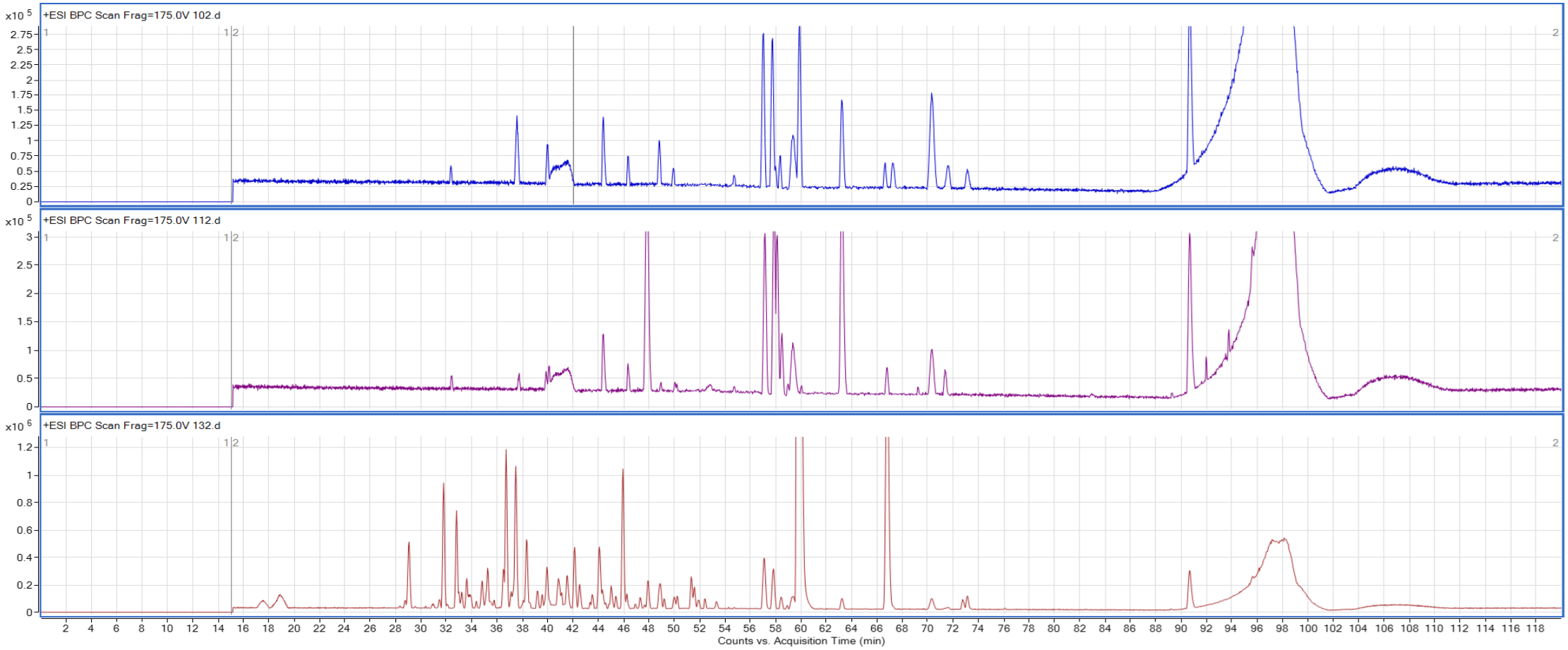




Synovial Fluid Tryptic Digestion and Sample Preparation for MS Analysis

Mass Spectrometry Results

- As a result of the studies performed with LC-MS/MS, the phosphopeptide isolation performance of the affinity sorbent was demonstrated chromatographically.



Key Messages

What is already known about this subject?

- Post-traumatic arthritis is a condition triggered by an acute joint trauma that can lead to osteoarthritis or chronic inflammatory arthropathies.
- No feasible markers and specific treatments for preventing the evolution of post-traumatic arthritis in chronic disease are available yet.

What does this study add?

- To our knowledge, affinity sorbent was synthesized for the first time in synovial fluid for phosphopeptidomic analysis.

How might this impact on clinical practice?

- With this study, it has been demonstrated that alternative biomarker research methods can be developed for the diagnosis and prognosis of osteoarthritis for clinicians.

7-9 June 2023

CELLS AND EXTRACELLULAR TEMPLATES

2nd International Conference

TEMPLATES FOR DAMAGED AND LOST TISSUES

Feza KORKUSUZ, MD

Sports Medicine, Hacettepe University

feza.korkusuz@Hacettepe.edu.tr