

What are we missing? Sample Preparation, Purification, Enrichment and Separation Techniques in Osteoarthritis Research

EO.2.S11-O1 Synovial Joint Fluid Chromatography Outcomes in Osteoarthritis: Do Affinity Sorbents Improve

the Outcome of Multi-Omics Studies?

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19 June 2025, Davos, Switzerland

Conflict of Interest

- Outcomes of the TÜBİTAK  project #223S509 (ongoing)
- EU COST CA21110 – “Building an open European Network on OsteoArthritis research (NetwOArk)” Action (<https://netwoark.eu/>). 
-  Updated information on outcomes. (<https://chondromics.org/>).
- PhD Thesis Outcomes of Serhat ALADAĞ.

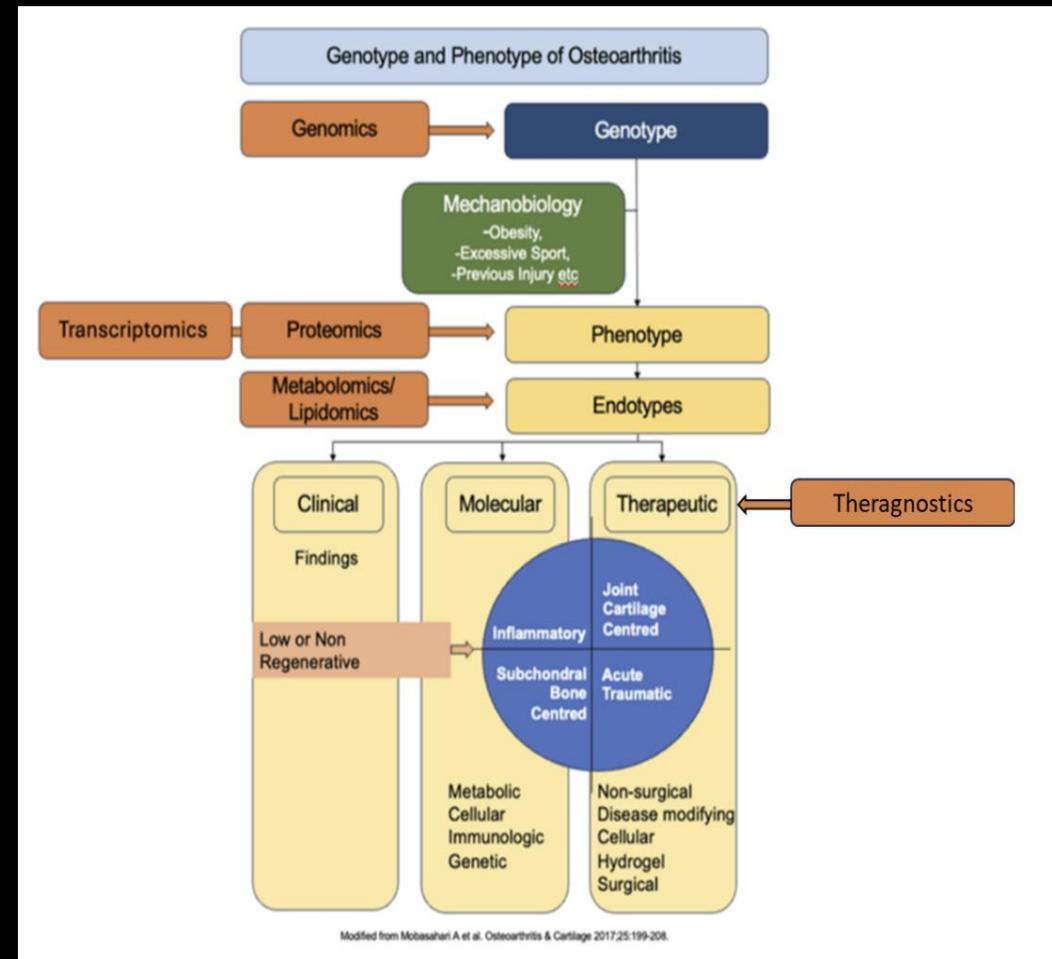
Background and Rationale



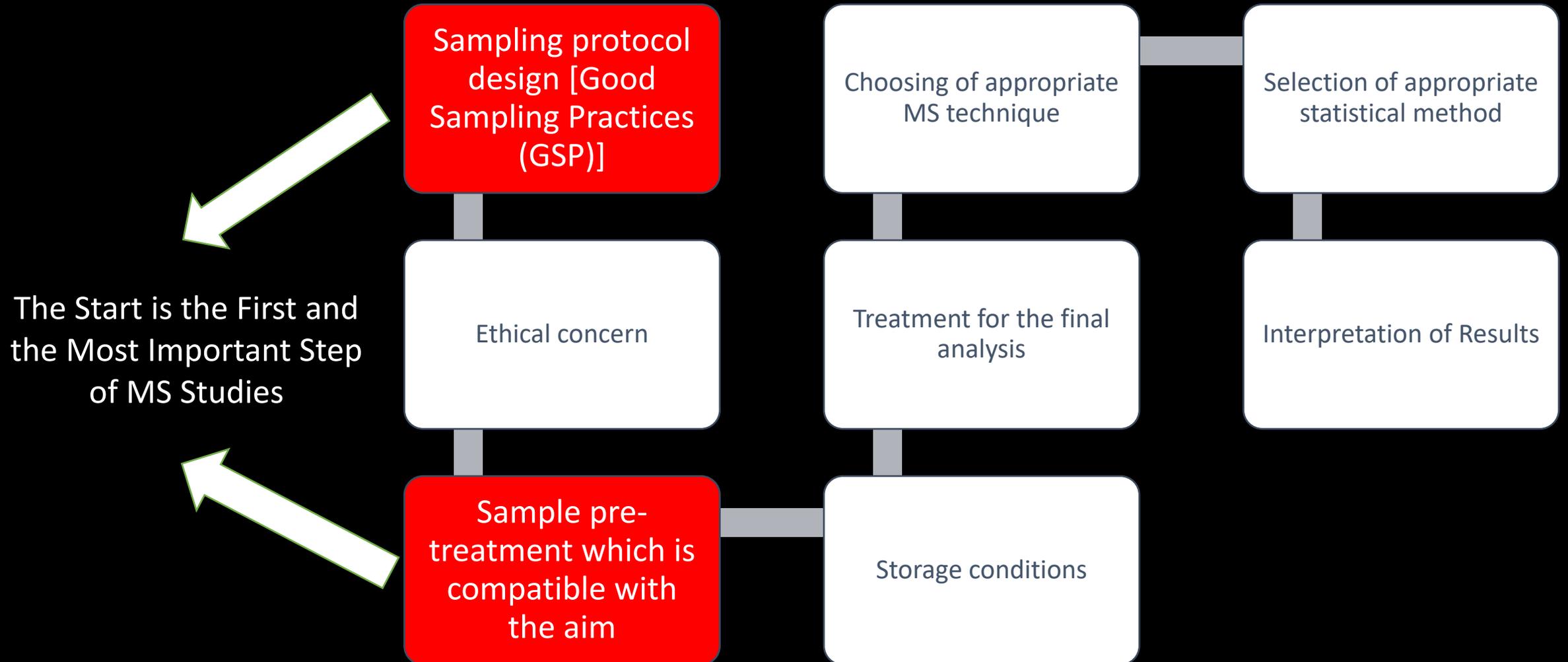
- 1. Proteomics, metabolomics and lipidomics studies of the synovial joint fluid (SJF) may quantify the early diagnosis, progression and response to treatment in knee joint osteoarthritis (KOA).
- 2. Analysing the SJF however can be challenging as it contains albumin.
- 3. Developing affinity particles/sorbents for the analysis of the SJF may enhance the emphasis of inflammatory, degenerative and regenerative metabolites.

Research Questions and Hypothesis

- H1. Sorbents will enhance the SJF chromatography outcomes during KOA multi-omics studies.
- R1. To develop a microextraction sorbent for the specific and selective detection of phospholipids (PLs) and also phosphoproteins. SiO_2 , $\text{SiO}_2@PEI$ and $\text{SiO}_2@PEI@Ti(IV)$.



Methods-Design (Sample Preparation – What are we missing?)



Methods-Design (Sample Preparation – What are we missing?)

RESEARCH

Open Access

Optimizing Human Synovial Fluid Preparation for Two-Dimensional Gel Electrophoresis

Carl PC Chen¹, Chih-Chin Hsu², Wen-Lin Yeh³, Hsiu-Chu Lin⁴, Sen-Yung Hsieh⁴, Shih-Cheng Lin¹, Tai-Tzung Chen¹, Max JL Chen¹ and Simon FT Tang^{1*}

Journal of
proteome
research

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Article

Optimization of Synovial Fluid Collection and Processing for NMR Metabolomics and LC-MS/MS Proteomics

James R. Anderson, Marie M. Phelan, Luis M. Rubio-Martinez, Matthew M. Fitzgerald, Simon W. Jones, Peter D. Clegg, and Mandy J. Peffers*

Cite This: *J. Proteome Res.* 2020, 19, 2585–2597

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research articles **Journal of proteome** research

Profiling of Endogenous Peptides in Human Synovial Fluid by NanoLC–MS: Method Validation and Peptide Identification

Jurre J. Kamphorst,^{†,‡} Rob van der Heijden,^{*,†,‡} Jeroen DeGroot,[§] Floris P. J. G. Lafeber,^{||} Theo H. Reijmers,[†] Benno van El,[§] Ubbo R. Tjaden,[†] Jan van der Greef,^{†,‡,§} and Thomas Hankemeier^{†,‡}

 molecules



Article

Hexagonal Mesoporous Silica as a Rapid, Efficient and Versatile Tool for MALDI-TOF MS Sample Preparation in Clinical Peptidomics Analysis: A Pilot Study

Rosa Terracciano^{1,*}, Mariaimmacolata Preianò¹, Giuseppina Maggisano¹, Corrado Pelaia² and Rocco Savino¹

Original Article

DOI: 10.7860/JCDR/2018/31333.11790

Role of Synovial Fluid Examination in Diagnosis of Joint Diseases

Pathology Section

PRAVEEN GARG¹, VIBHUTI GOYAL²

Methods-Design (Sample Preparation – What are we missing?)

Group	Category	Gross	Viscosity	Cell count per mm ³	% *PMNs	Other
I	Normal	Yellow, Clear	Normal	<200	<25	-
II	Non-inflammatory	Yellow, Clear	Normal	200-2000	<25	-
III	Inflammatory	Yellow, Turbid	Low	2000-50,000	>50	-
IV	Septic	Yellow, Cloudy, Purulent	Low	>50,000	>90	Gram stain, Positive Cultures
V	Crystal induced	Cloudy, Turbid	Low	200- >50,000	<90	Crystal present
VI	Traumatic	Red, Turbid	Variable	Variable	Variable	†RBCs present

[Table/Fig-1]: Reference ranges to differentiate between the different categories.

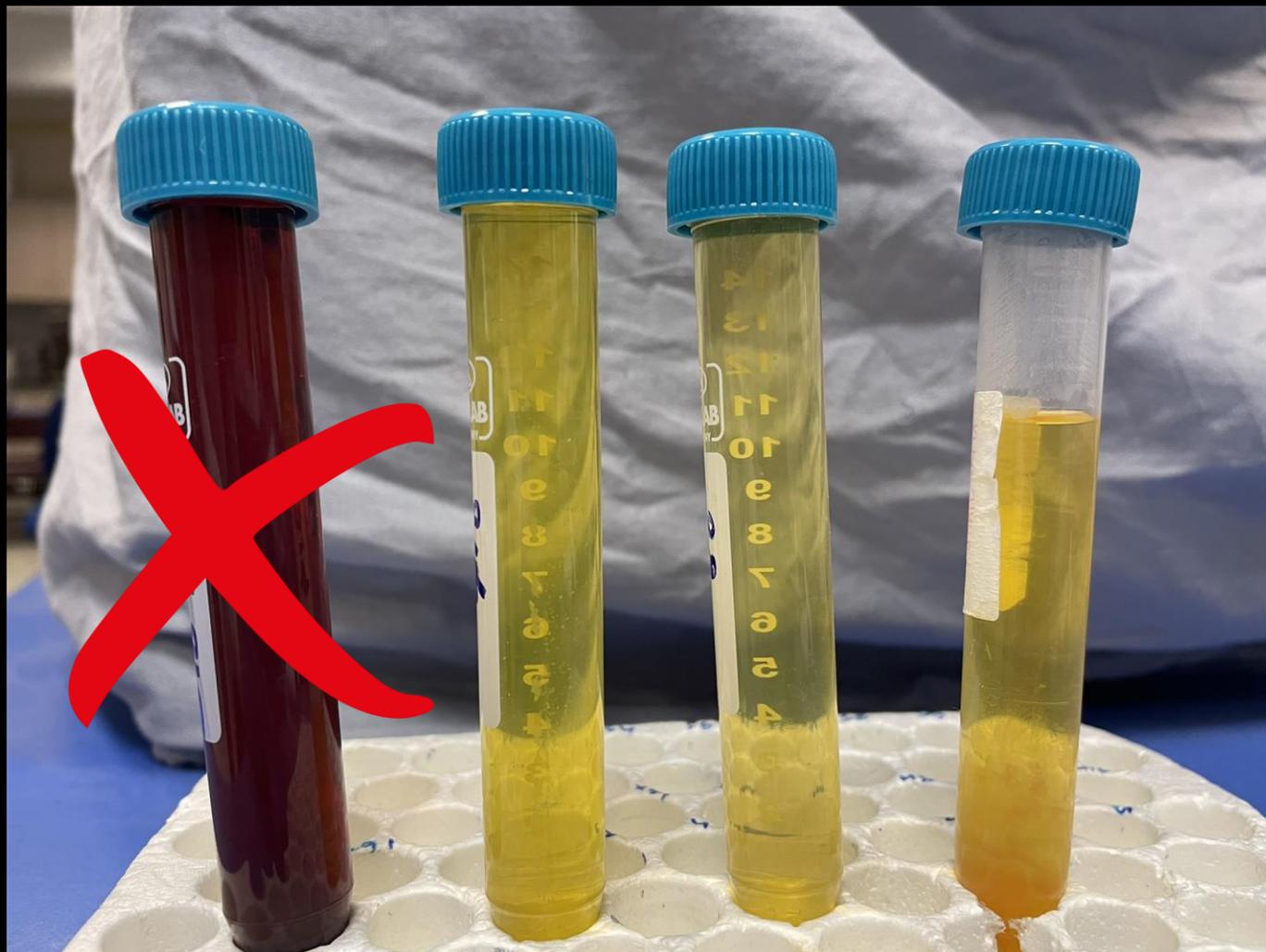
*PMN:Polymorphonuclear neutrophils, †RBC:Red blood cells

Methods-Design (Arthrocentesis-Collection)



19.12.2023, 2023/09-44, SBA23/262

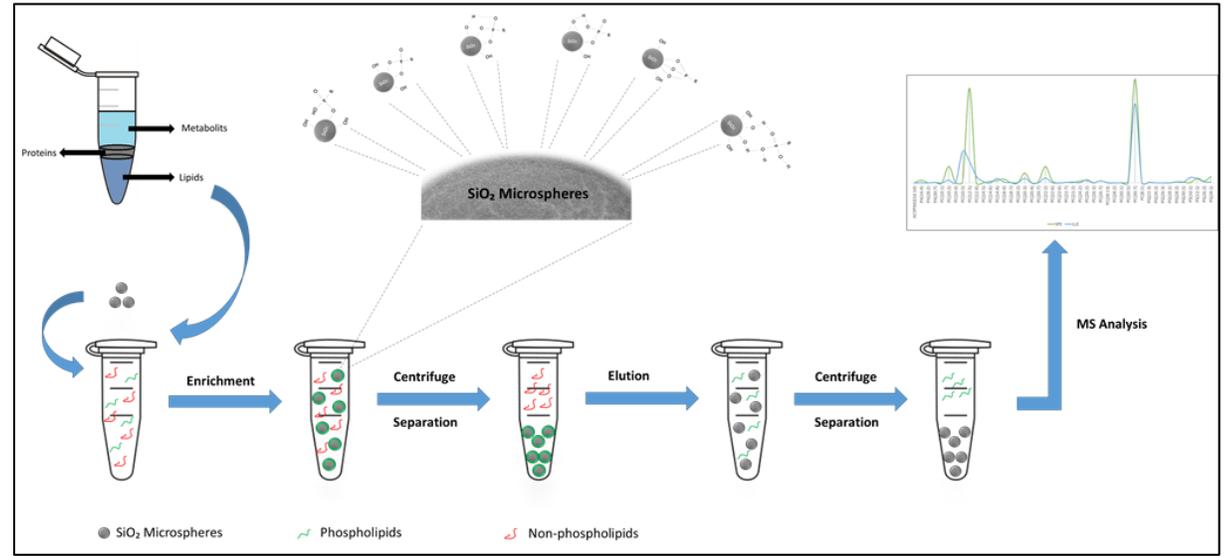
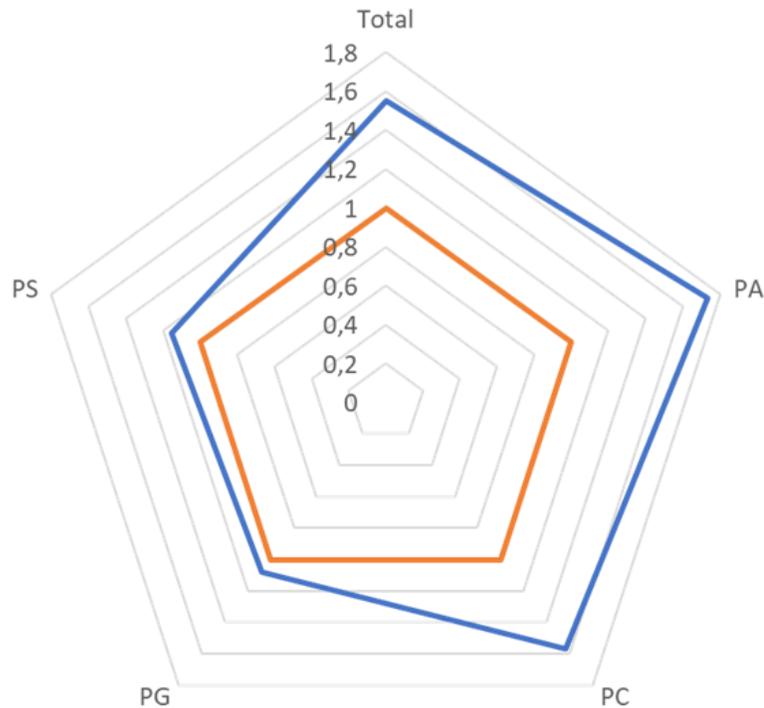
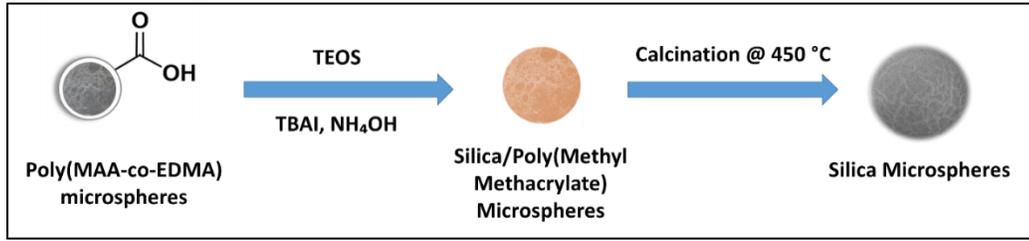
Methods-Design (Arthrocentesis-Collection)



Methods-Design (Arthrocentesis-Collection)



Methods (Microsphere Synthesis)

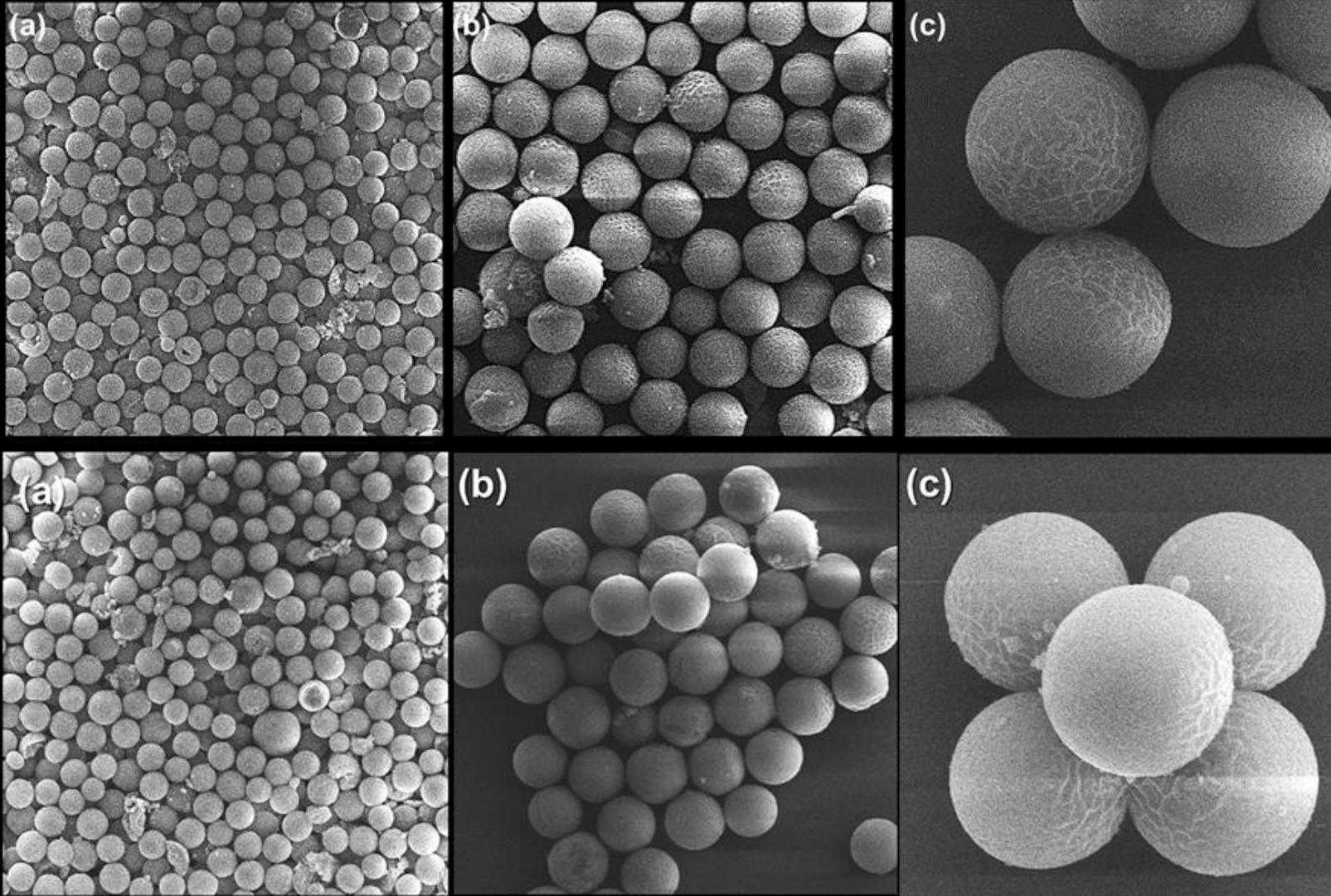


Article

A Facile and Efficient Protocol for Phospholipid Enrichment in Synovial Joint Fluid: Monodisperse-Mesoporous SiO₂ Microspheres as a New Metal Oxide Affinity Sorbent

Serhat Aladağ¹ , İlayda Demirdiş² , Burcu Gökçal Kapucu³, Emine Koç⁴, Ozan Kaplan⁴, Batuhan Erhan Aktaş⁵, Mustafa Çelebier^{1,4}, Ali Tuncel^{1,3} and Feza Korkusuz^{1,5,*}

Methods (Microsphere Characterization-SEM)



SiO_2

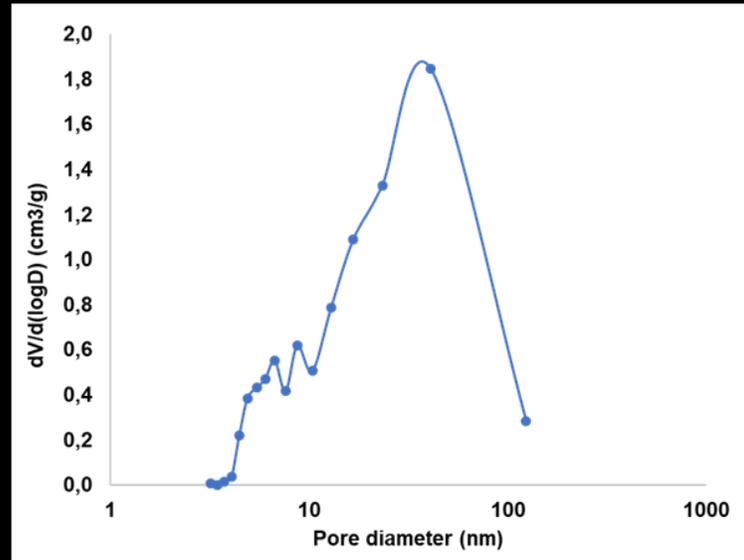
and

$\text{SiO}_2@PEI@Ti(IV)$

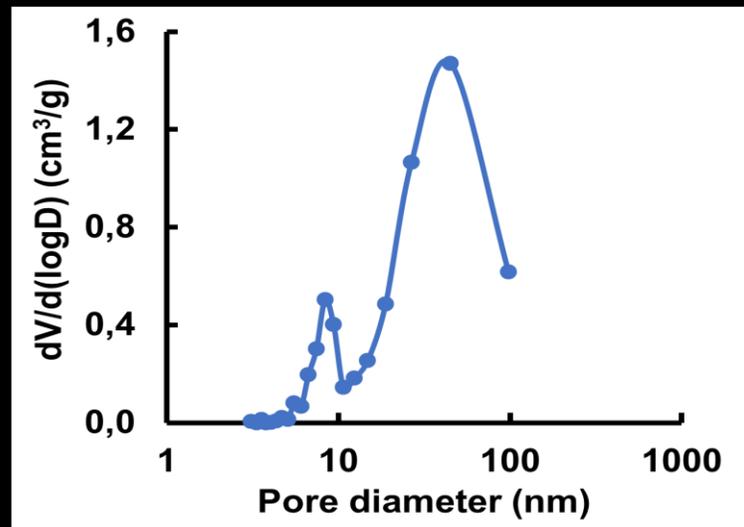
SEM photographs of
microspheres at
different scales

(a: 20 μm , b: 10 μm , c:
2 μm).

Methods (Microsphere Characterization-BET)

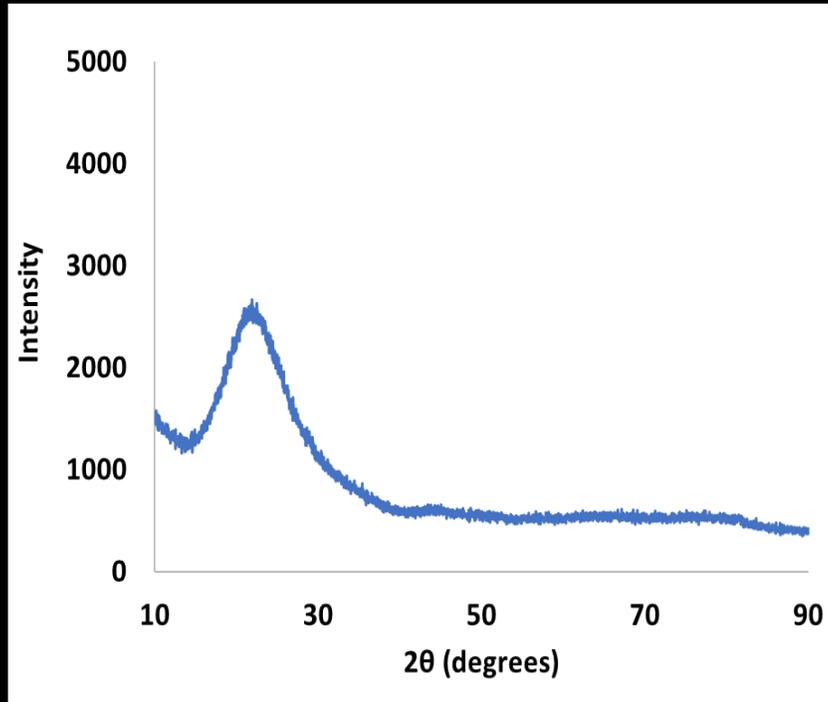


	Specific surface area (m ² /g)	Pore volume (cm ³ /g)	Pore diameter (nm)	Mode pore size (nm)
SiO ₂	161,5	1,1	6,7	41,1

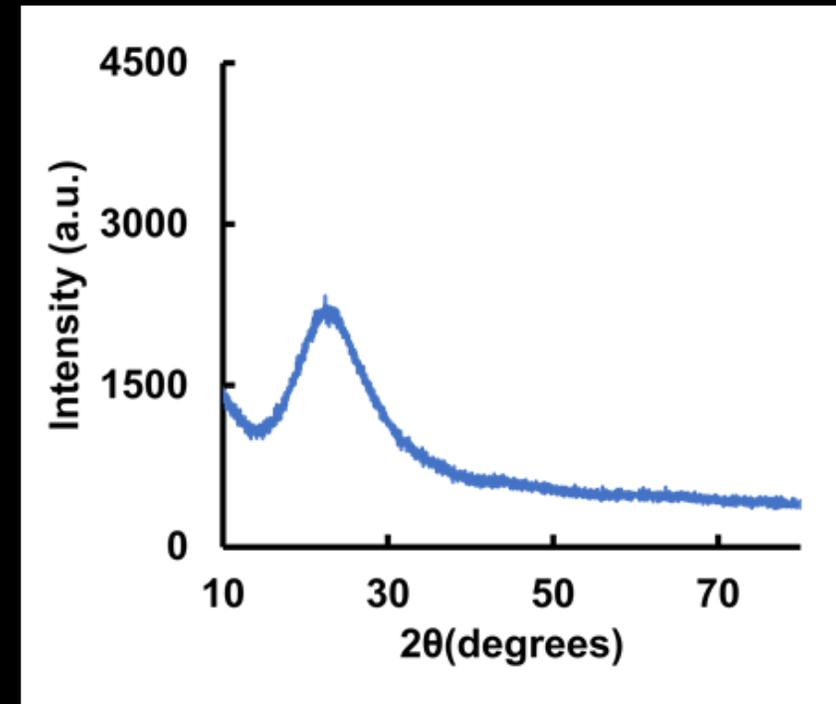


	Specific surface area (m ² /g)	Pore volume (cm ³ /g)	Pore diameter (nm)	Mode pore size (nm)
SiO ₂ @PEI@Ti(IV)	132,2	1,0	8,4	44,8

Methods (Microsphere Characterization-BET)

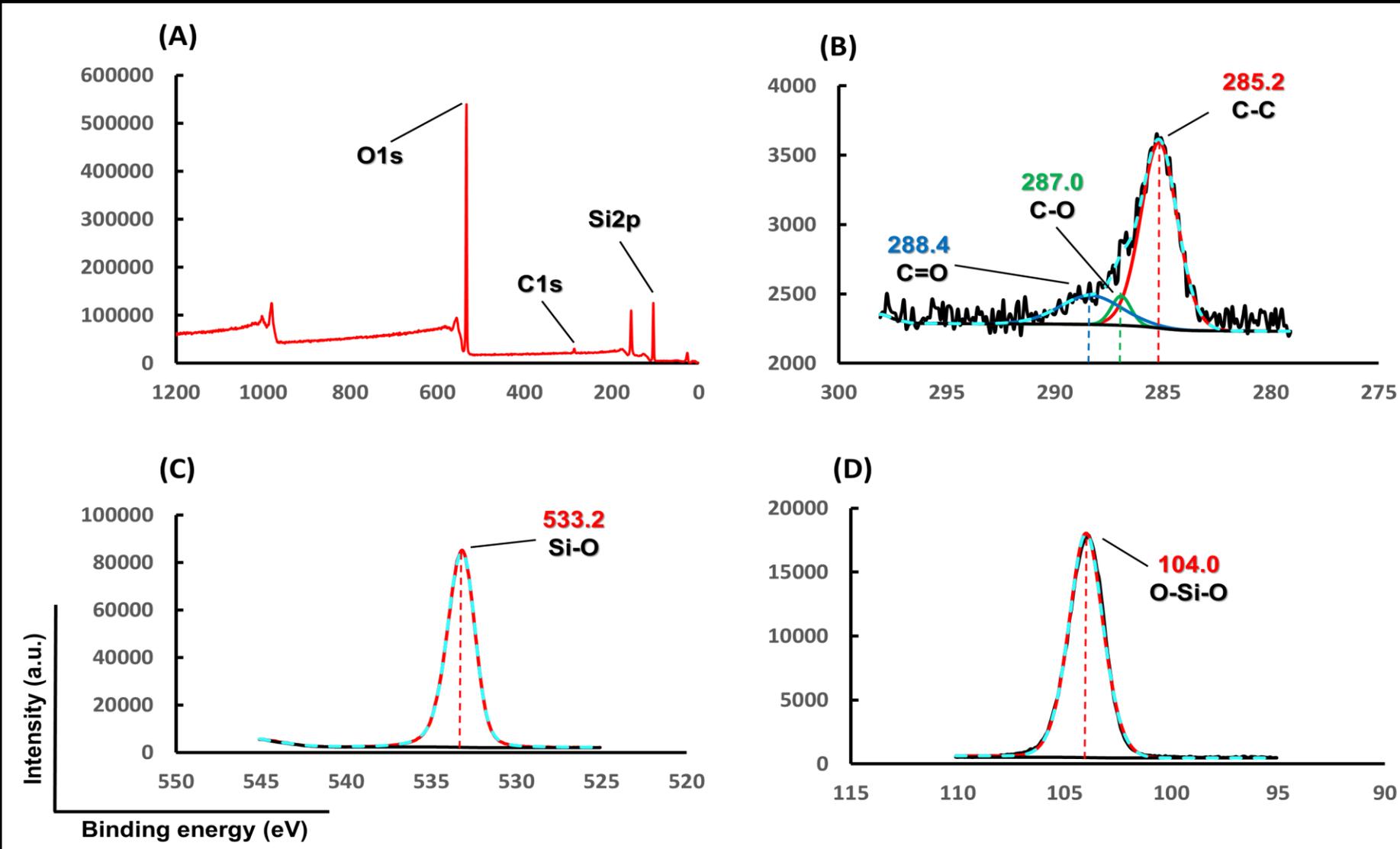


SiO₂

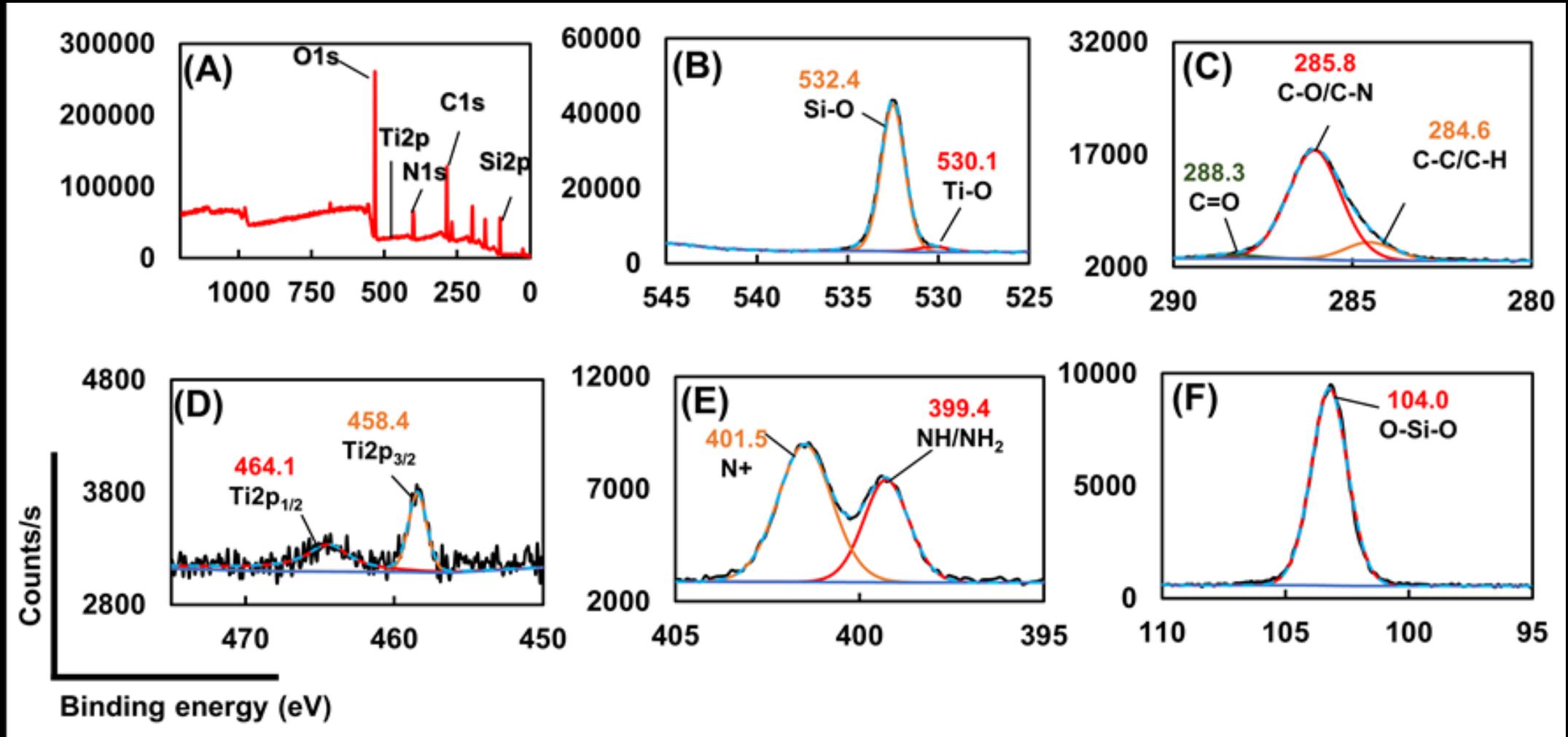


SiO₂@PEI@Ti(IV)

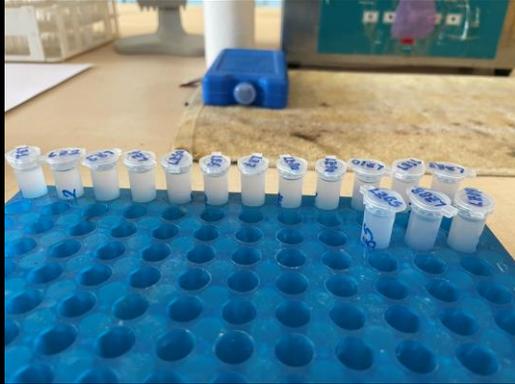
Methods (Microsphere Characterization-SiO₂-XPS)



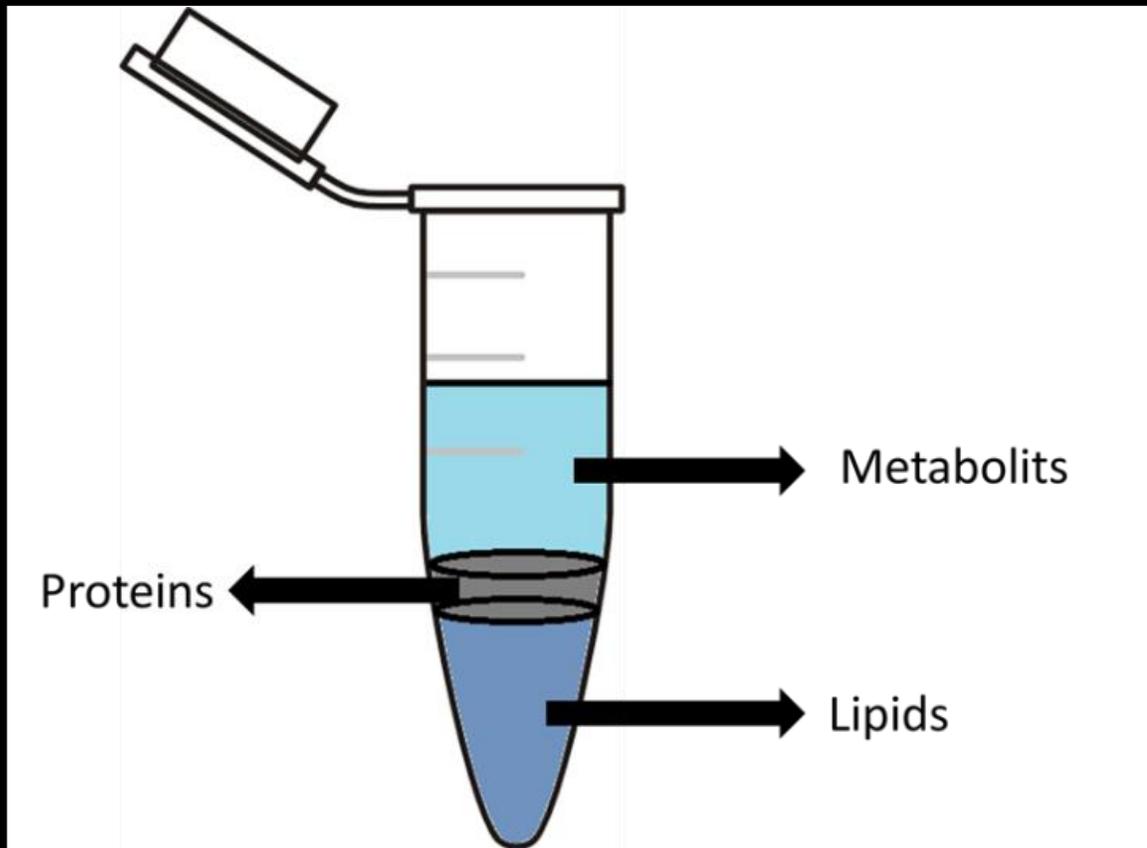
Methods (Microsphere Characterization-SiO₂@PEI@Ti(IV)-XPS)



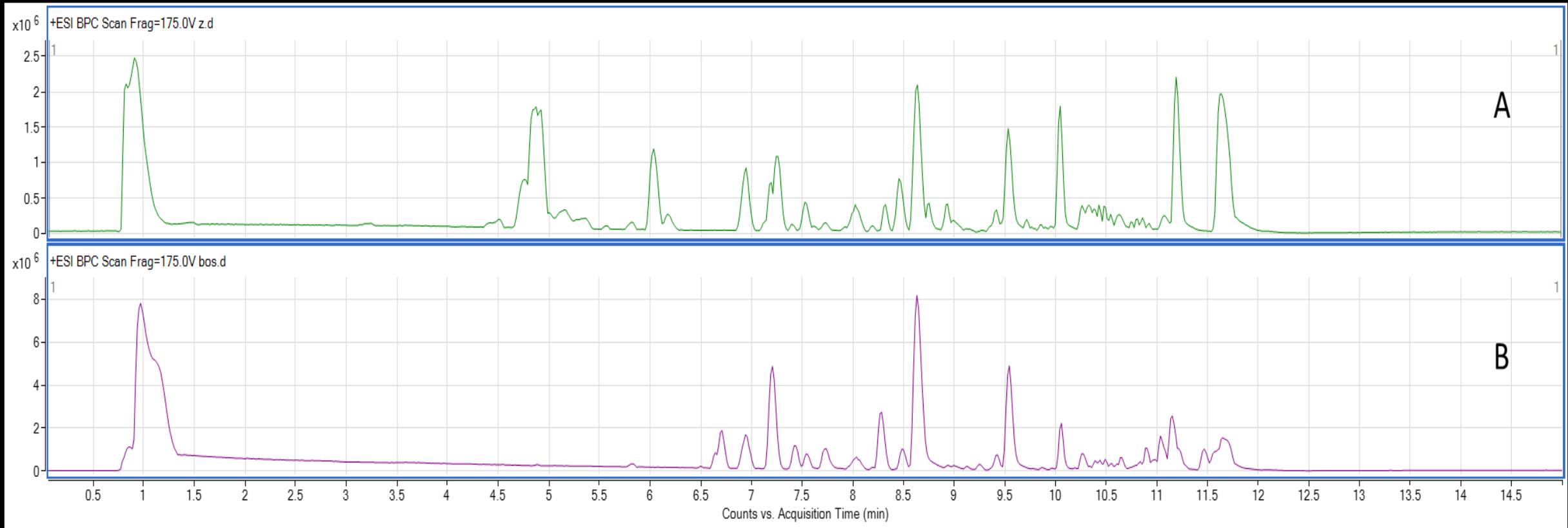
Methods (Lipid Extraction)



Methods (Lipid Extraction)



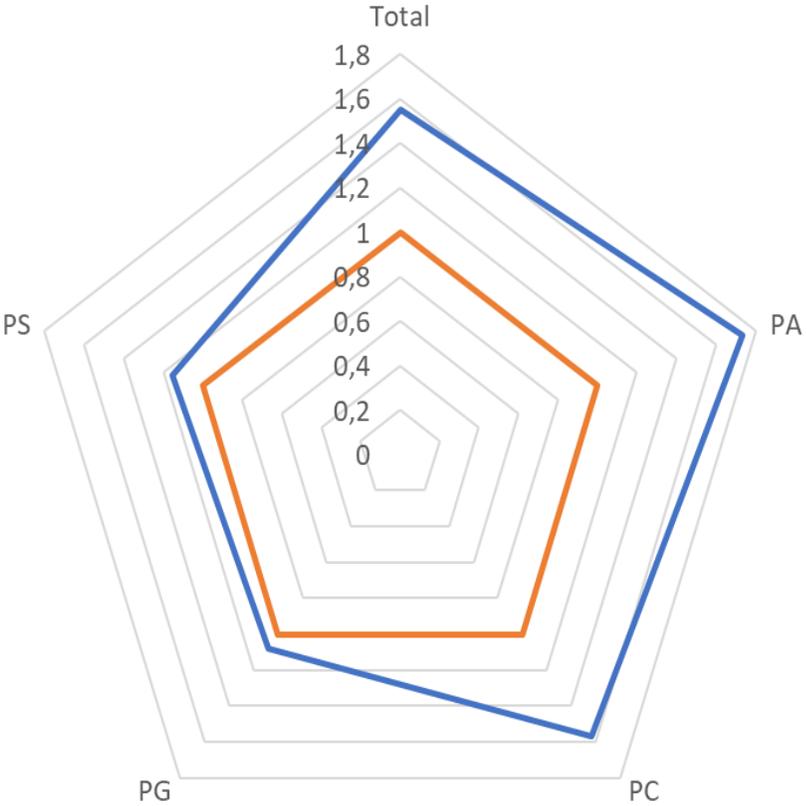
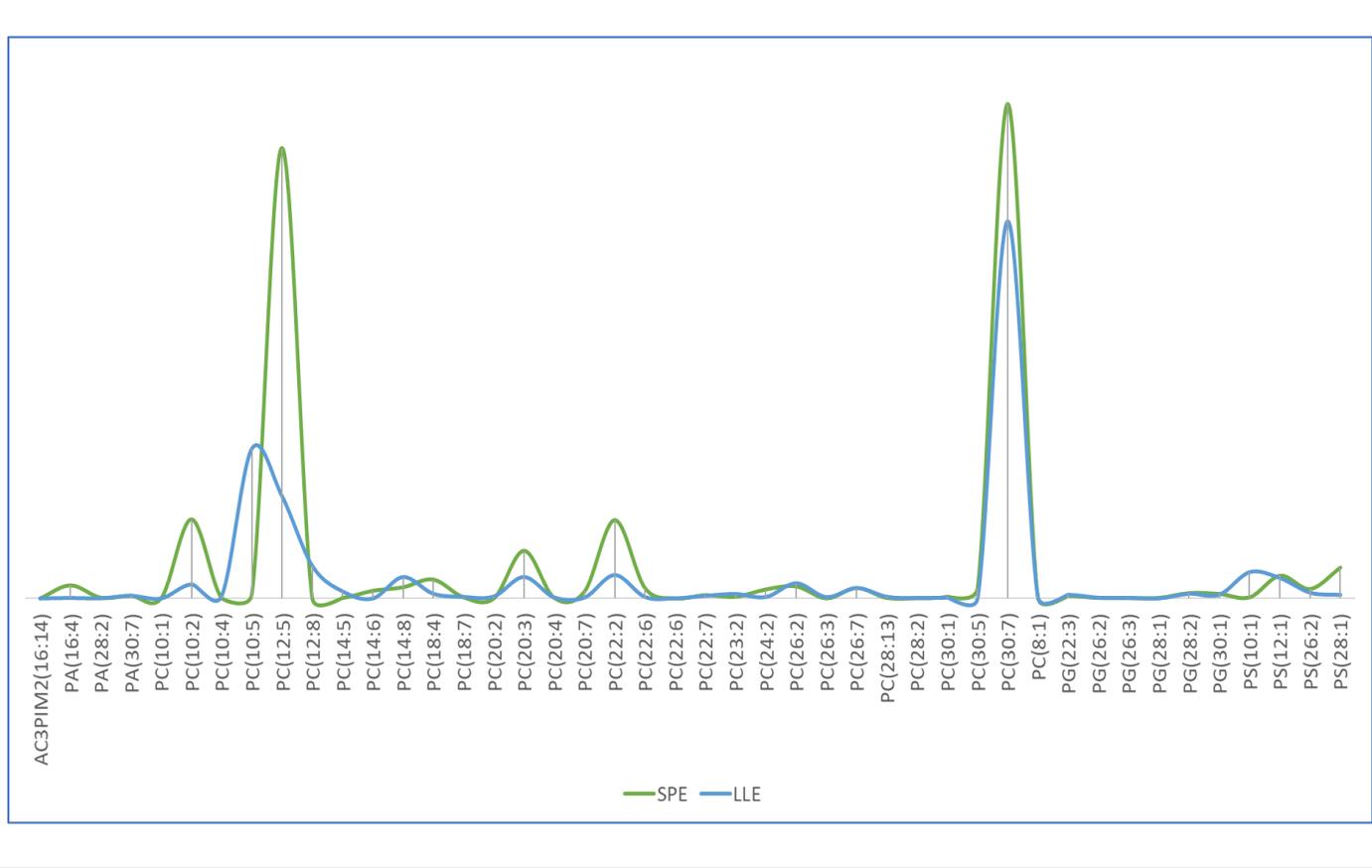
Results (Lipidomics Findings)



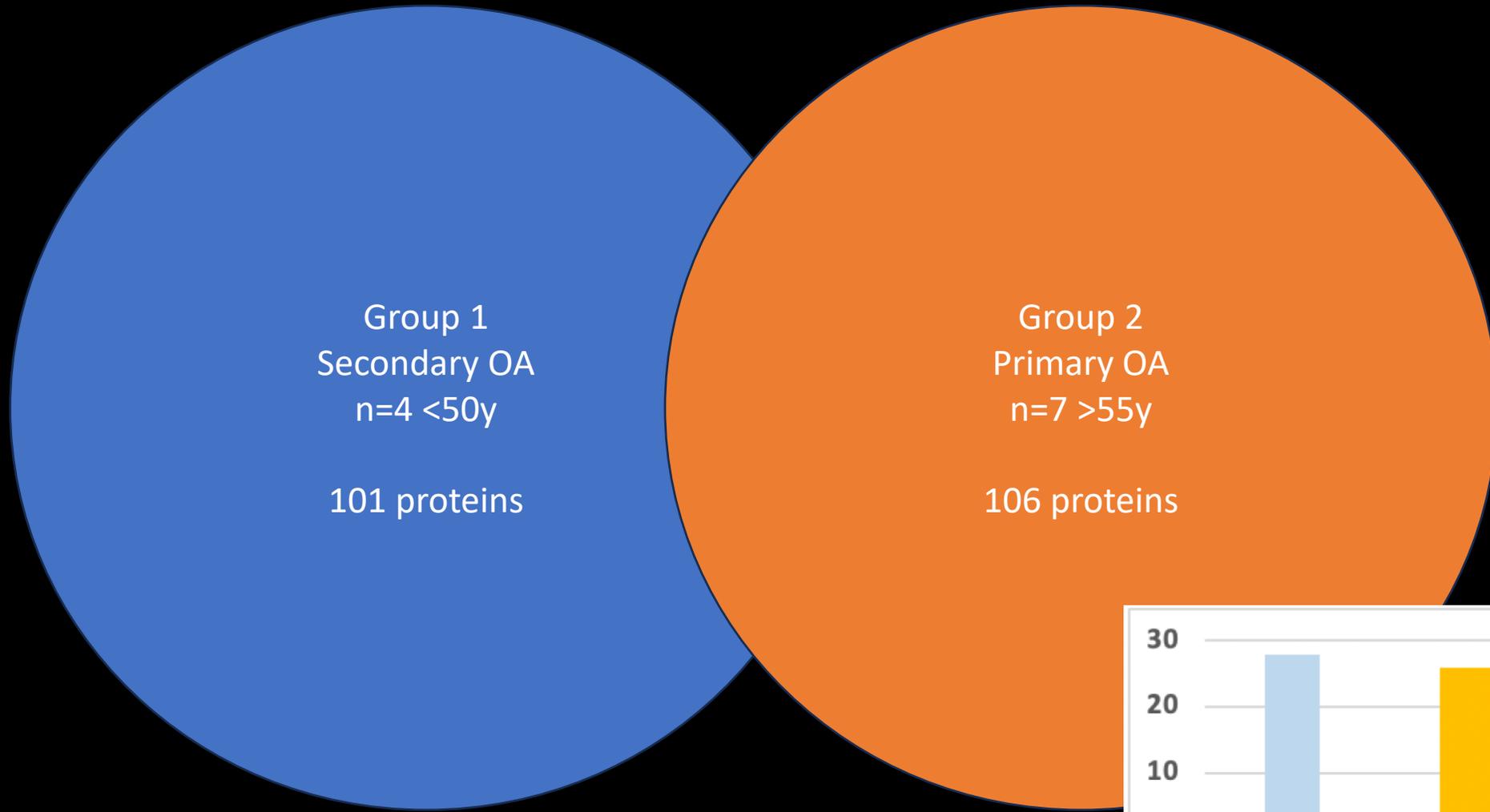
A: LLE + SPE

B: LLE

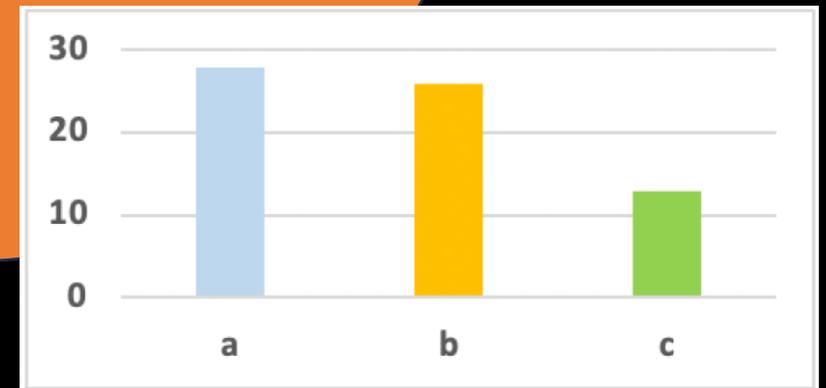
Results (Lipidomics Findings)



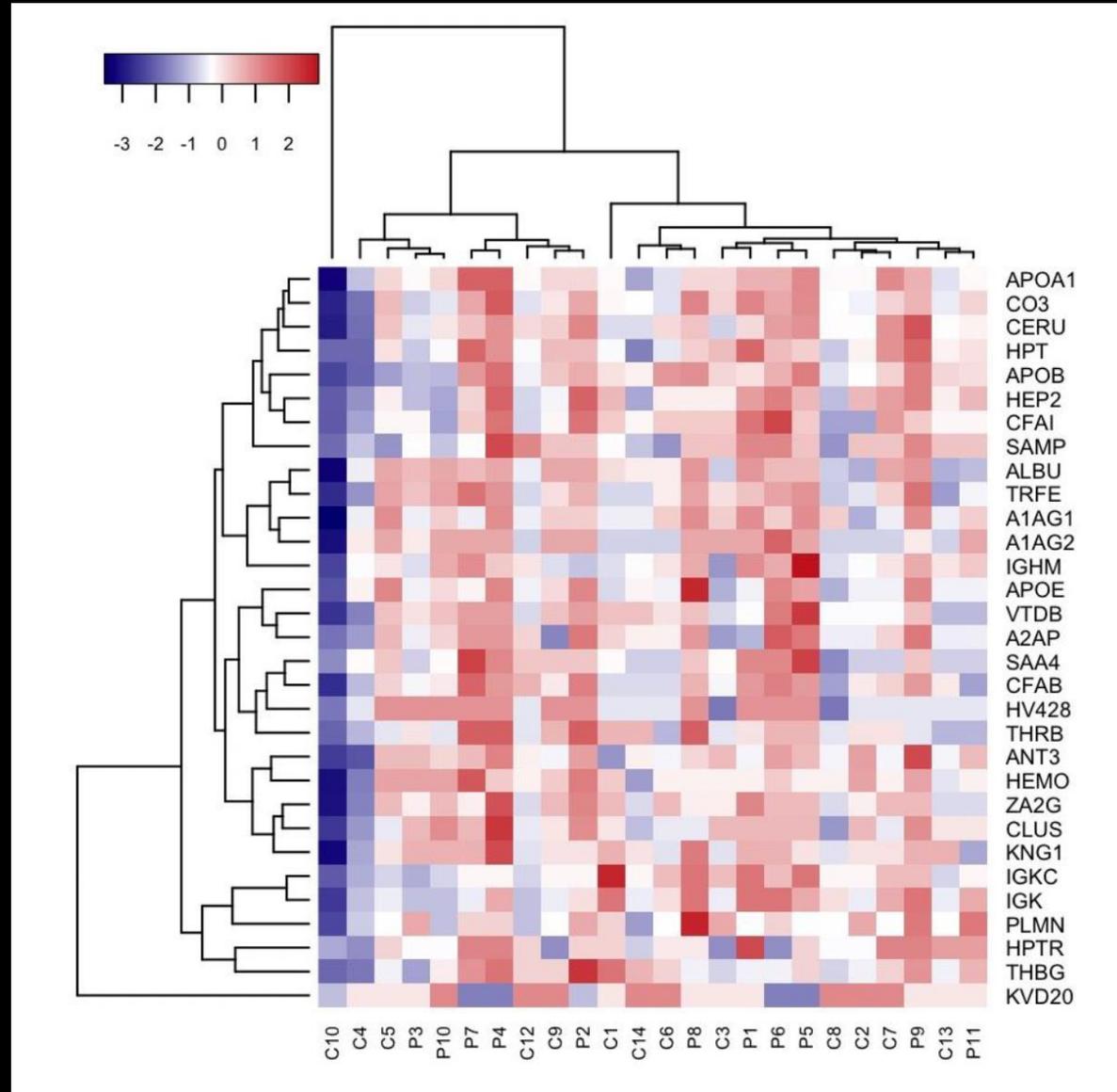
Results (Preliminary Proteomics Findings)



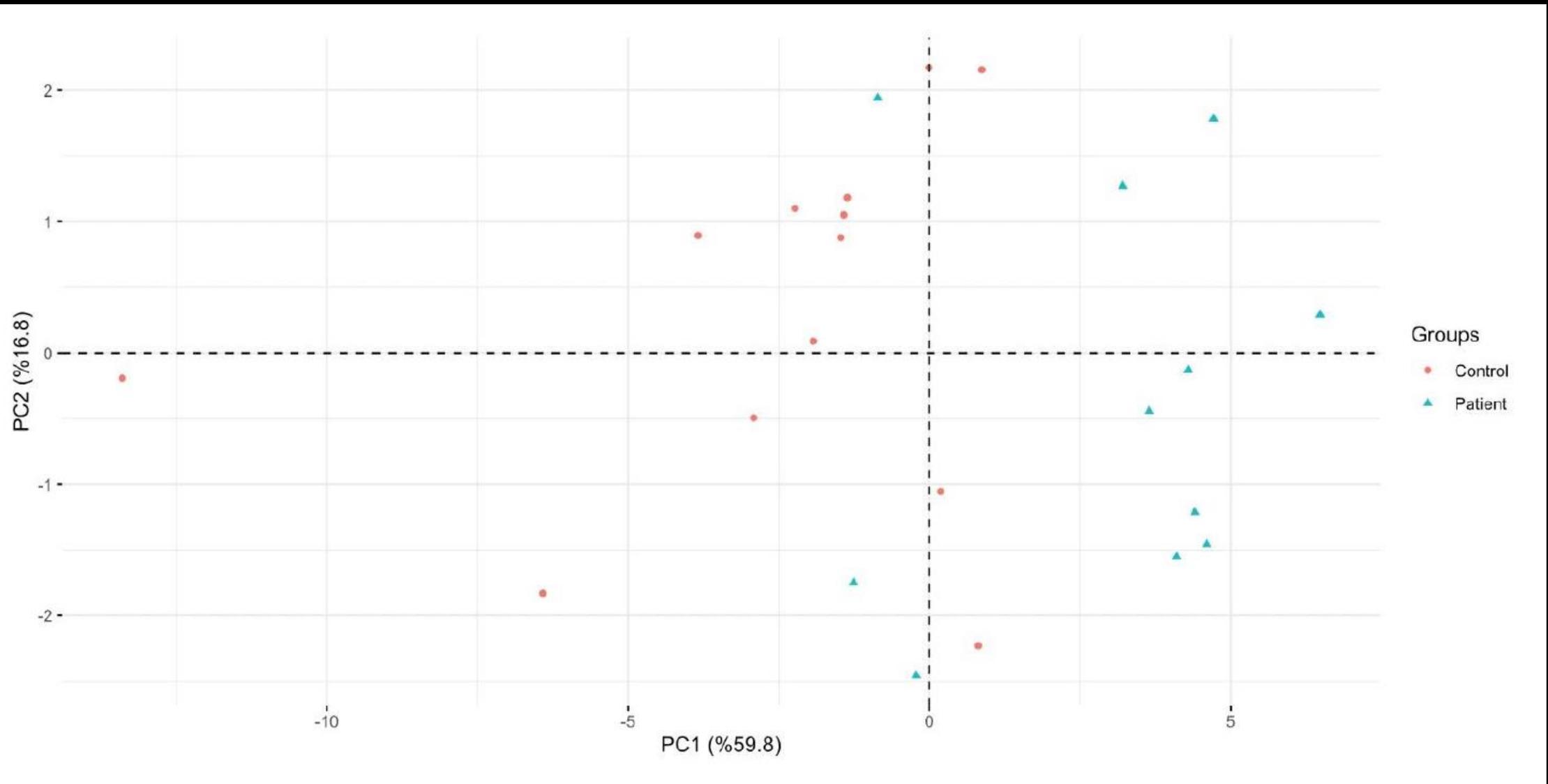
MASCOT Library Comparison



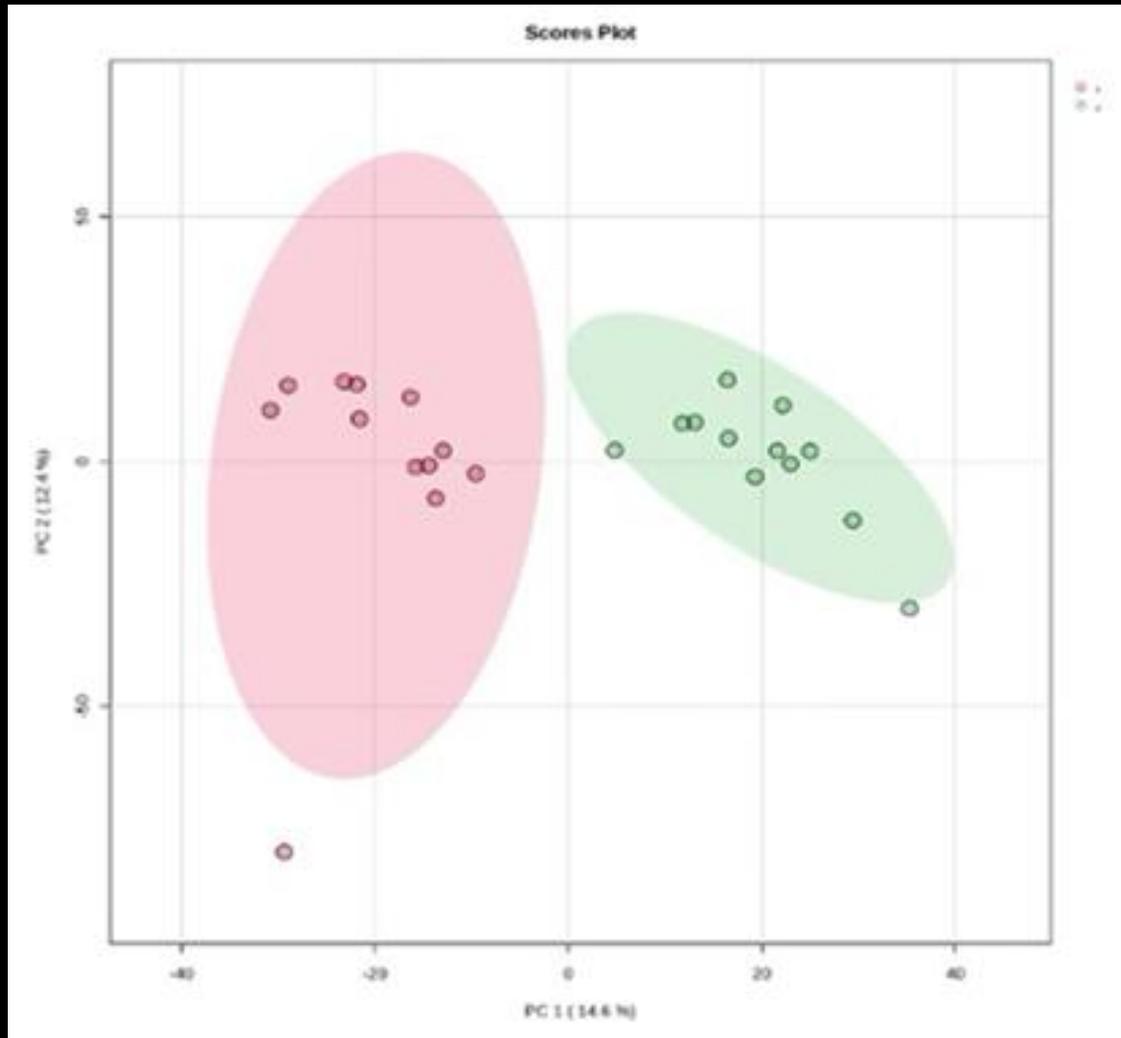
Results (Preliminary Proteomics Findings)



Results (Preliminary Proteomics Findings)



Results (Preliminary Metabolomic Findings-PCA)



Primary (pink) and trauma-induced (green) OA patients PCA graph illustrates different metabolome profiles of SJF samples analyzed using another extraction method.

Results (Preliminary Multi-Omics Findings)

Multi Omics in Personalized Medicine Workshop with International Collaboration

SAVE THE DATE 17 March 2025 Monday

Registration is **free of charge** and **participants**. First come will be served.

For registration we are waiting for your **name, surname, email address, and phone number** to **chondromics.org**. You will be informed with more details.

Register Now!

Registration is **free of charge** and it is limited to 40 participants. If you come will be served first.

For registration we are waiting your e-mail that should include your name, surname, phone number, and e-mail address to **chondromics@gmail.com**. After that you will be informed with confirmation status.

Workshop Details:
The workshop is organized in the context of the COST CA21110 Building an open European Network on Osteoarthritis Research (NetwOArk) (networkark.eu) Action and the Mobility (mobility.net4r) Project Proposal.

Logos: NetwOArk, MObility, chondROMICS

Footer: NetwOArk The European Network on Osteoarthritis, MObility, chondROMICS

<https://chondromics.org/>



Conclusion and Take Home Message

What is already known about this subject?

- Diagnosis, treatment and monitoring of OA depend on various factors such as genetics, epigenetics and patient phenotypes and endotypes. Diagnosis is currently based on patient history, physical examination and medical imaging.

What does this study add?

- Quantitative assessment of SJF may help determine treatment options, especially in young OA patients.

How might this impact on clinical practice?

- There is a need to develop reliable and economical biomarker-based diagnostic kits that can provide rapid results at the bedside for early diagnosis of the disease, if possible before symptoms occur, and for monitoring the success of treatments.

Separations / SCI Expanded / September 2024



Article

A Facile and Efficient Protocol for Phospholipid Enrichment in Synovial Joint Fluid: Monodisperse-Mesoporous SiO₂ Microspheres as a New Metal Oxide Affinity Sorbent

Serhat Aladağ¹, İlayda Demirdiş², Burcu Gökçal Kapucu³, Emine Koç⁴, Ozan Kaplan⁴, Batuhan Erhan Aktaş⁵, Mustafa Çelebier^{1,4}, Ali Tuncel^{1,3} and Feza Korkusuz^{1,5,*}

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- * Correspondence: feza.korkusuz@hacettepe.edu.tr

Abstract: Phospholipids (PLs), essential components of cell membranes, play significant roles in maintaining the structural integrity and functionality of joint tissues. One of the main components of synovial joint fluid (SJF) is PLs. Structures such as PLs that are found in low amounts in biological fluids may need to be selectively enriched to be analyzed. Monodisperse-mesoporous SiO₂ microspheres were synthesized by a multi-step hydrolysis condensation method for the selective enrichment and separation of PLs in the SJF. The microspheres were characterized by SEM, XPS, XRD, and BET analyses. SiO₂ microspheres had a 161.5 m²/g surface area, 1.1 cm³/g pore volume, and 6.7 nm pore diameter, which were efficient in the enrichment of PLs in the SJF. The extracted PLs with sorbents were analyzed using Q-TOF LC/MS in a gradient elution mode with a C18 column [2.1 × 100 mm, 2.5 μM, Xbridge Waters (Milford, MA, USA)]. An untargeted lipidomic approach was performed, and the phospholipid enrichment was successfully carried out using the proposed



Citation: Aladağ, S.; Demirdiş, İ.; Gökçal Kapucu, B.; Koç, E.; Kaplan, O.; Aktaş, B.E.; Çelebier, M.; Tuncel, A.; Korkusuz, F. A Facile and Efficient

Aladağ, S.; Demirdiş, İ.; Gökçal Kapucu, B.; Koç, E.; Kaplan, O.; Aktaş, B.E.; Çelebier, M.; Tuncel, A.; Korkusuz, F. A Facile and Efficient Protocol for Phospholipid Enrichment in Synovial Joint Fluid: Monodisperse-Mesoporous SiO₂ Microspheres as a New Metal Oxide Affinity Sorbent. *Separations* **2024**, *11*, 262. <https://doi.org/10.3390/separations11090262>

Project Information Platform (<https://chondromics.org/>)

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EU-netwOArk

Omics Analysis for More Clues

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The ChondrOMICs Project

Home



The Scientific and Technological Research Council of Türkiye (TÜBİTAK) granted the project in relevance to the EU COST CA21110 – “Building an open European Network on OsteoArthritis research (NetwOArk)” Action.



Funded by
the European Union

Team (<https://chondromics.org/>)



Chem. Eng. Bioeng. Biology



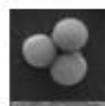
Sports Medicine



Basic Pharmacy Sciences Chemistry



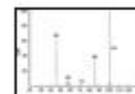
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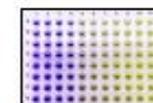
Synthesis & Characterisation



Clinical Side



MS & Data Mining



ELISA



Biostatistics

If you want to go fast, go alone.
If you want to go far, go together.
African proverb.



Thank you...

