

Evaluating Synovial Fluid by **Omic Technology:** Where are we now?

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Disclosures

- Research Funds:



- Scientific and Technological Research Council of Türkiye (TÜBİTAK), Technology and Innovation Grant Programs Directorate (TEYDEB) grant number 3210893.
- Scientific and Technological Research Council of Türkiye (TÜBİTAK)(Grant # 122S675) entitled "*Metabolomic Analysis of Leucocyte Rich (L-PRP) and Poor (P-PRP) (PRP) in Normal and Osteoarthritic Chondrocyte Differentiation*".



- Scientific and Technological Research Council of Türkiye (TÜBİTAK)(Grant # 223S5094) entitled "*Development of a new Immobilized Metal Affinity Chromatography (IMAC) Sorbent for Phosphopeptide Enrichment for Monitoring the Diagnosis, Course, and Treatment of Osteoarthritis and Implementing the **Omic Mapping of Synovial Fluid** in Proteomic, Metabolomic and Lipidomic Studies*".



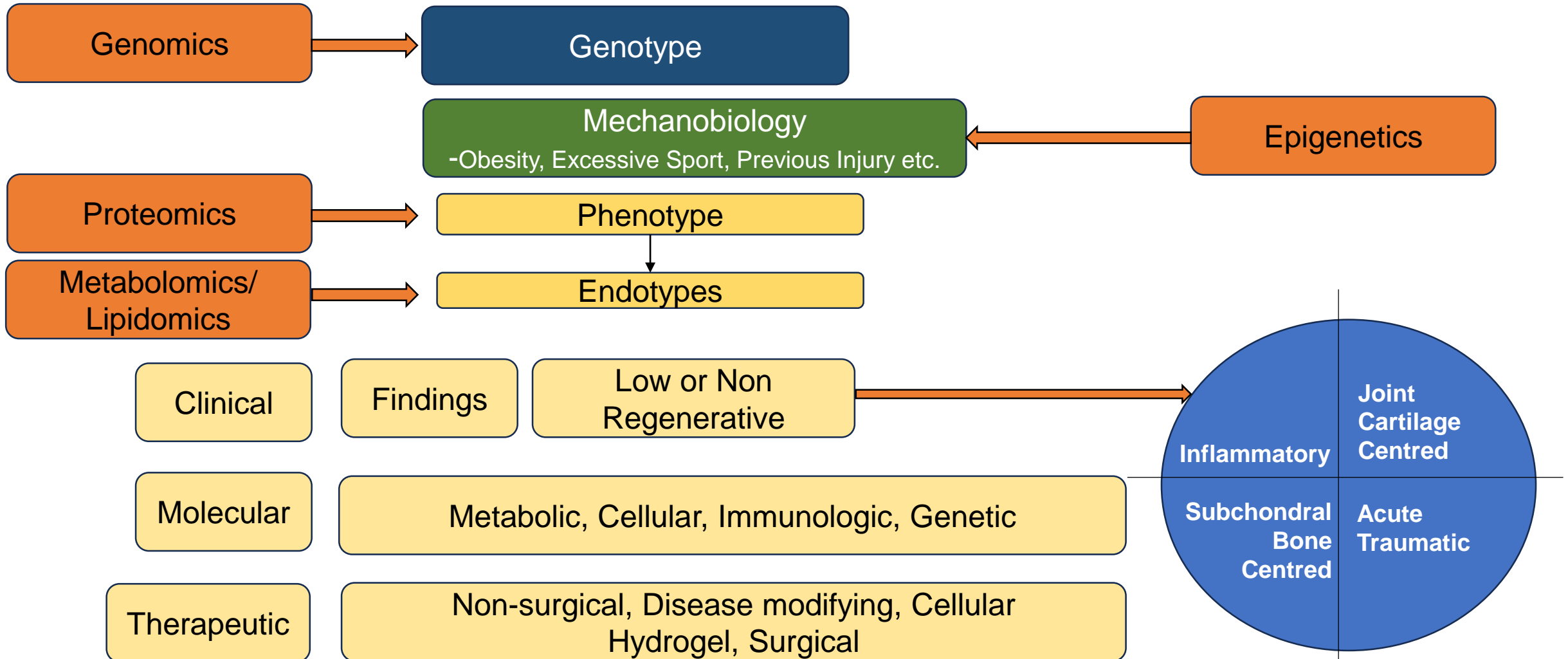
- Hacettepe University (Grant # THD-2020-18692),
- The Turkish Orthopedic and Traumatology Society (TOTBID).

- Non-Interventional Clinical Research Ethical Board of Hacettepe University approved studies.



- Feza Korkusuz MD is an active member of the Turkish Academy of Sciences (TÜBA)

Background: "OA Geno- and Phenotypes"



Genome

Transcriptome

Proteome

Gen



mRNA



Protein



Modified Protein



2×10^4

$> 10^6$

$> 10^6$

$> 10^7$

Transcription

Translation

Posttranslational modifications

Biochemical Reactions

Phenotype



Metabolome

Metabolites

$> 100,000$



Genomics



Proteomics



Metabolomics

Articular Cartilage Treatment Algorithm*

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

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

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

(3) **Non-Degradable Polymeric Hydrogel Spacers**

(4) Cellular[†]

PRP, PRGF, SVF, Stem cells and Extracellular vesicles

[†]Experimental (limited # of patients and follow up).

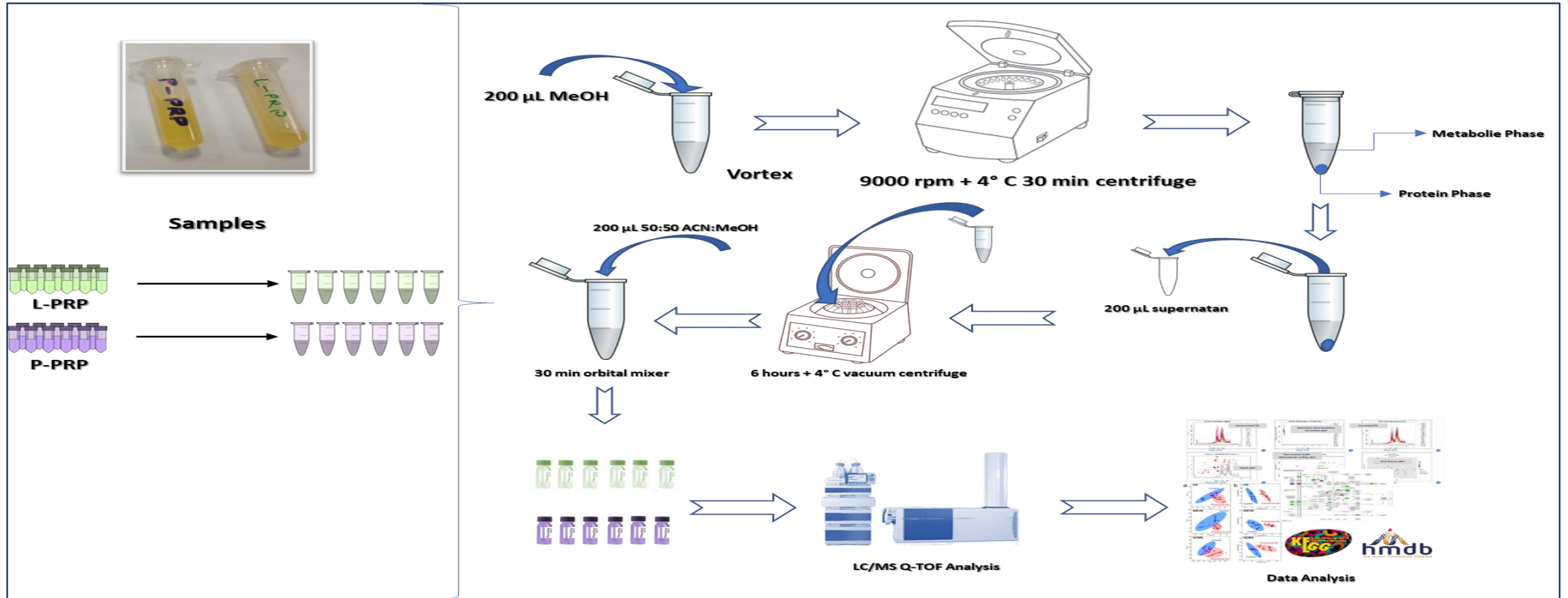
(Modalities written in red are experimental.)

(5) Surgical

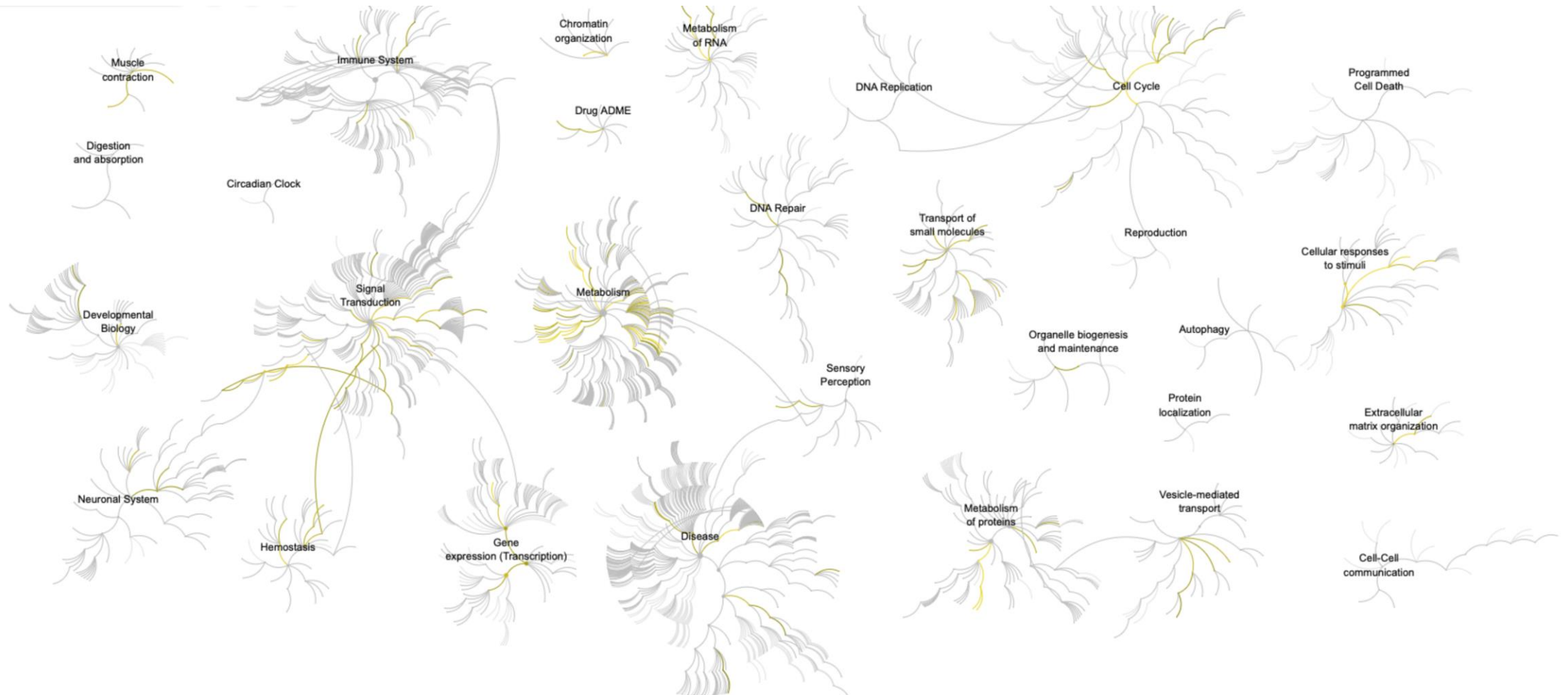
Micro- or Nano-fx., Mosaicplasty, Allografts, MACI, High tibial osteotomy,
Subchondral bioplasty, Partial or total joint replacement surgery.

*Modified from the OARSI Guidelines.

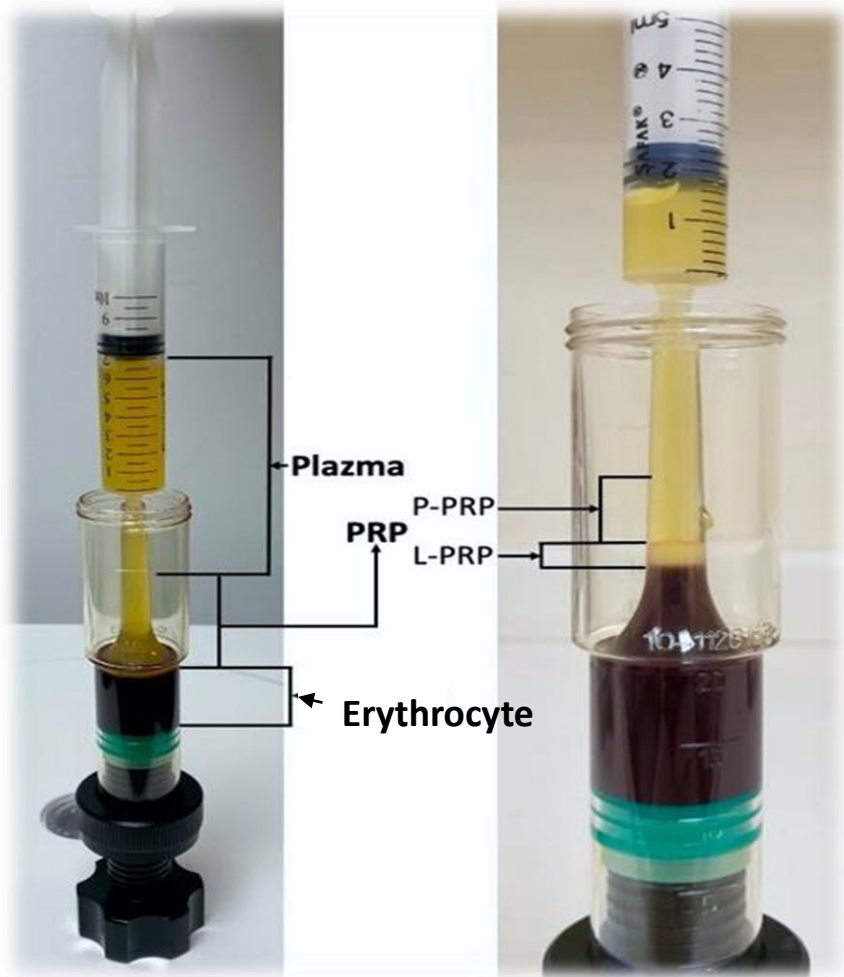
Omic Technology (LC-MS Q-TOF/MALDI-TOF)



Omics Technology (LC-MS Q-TOF/MALDI-TOF)



Platelet Rich Plasma



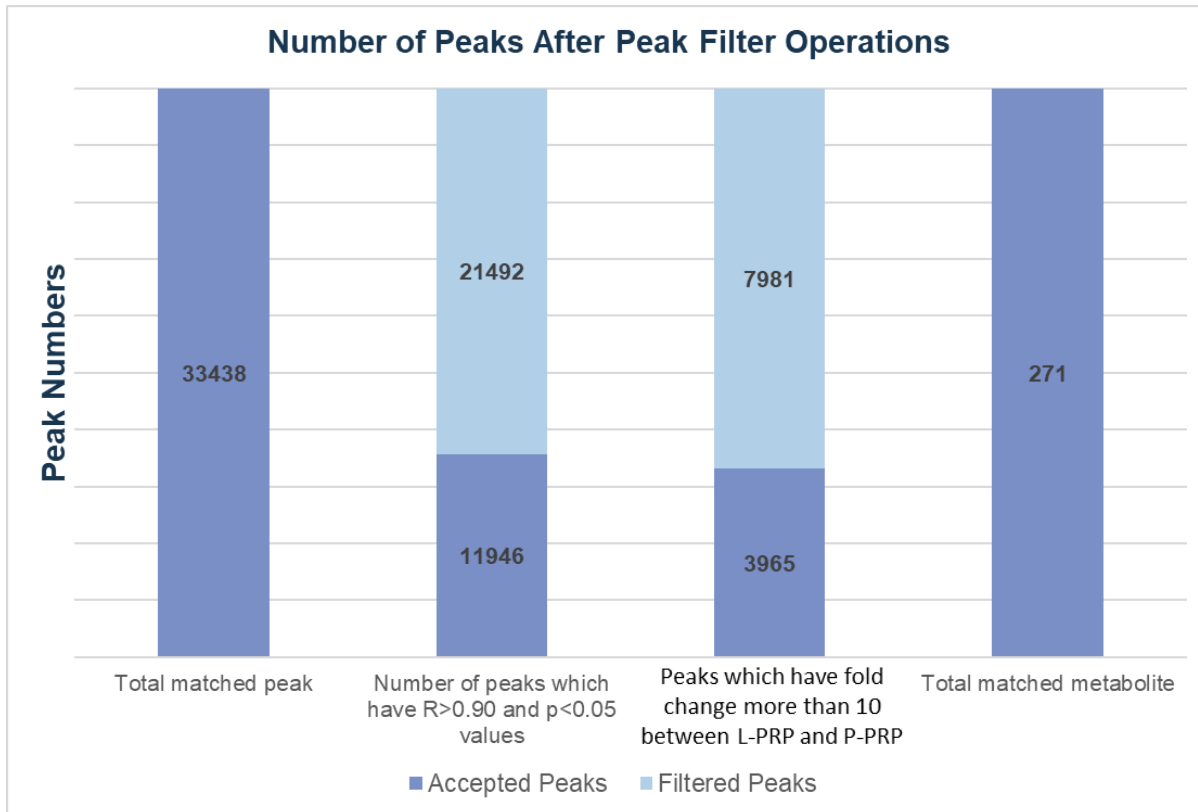
A **cross-sectional** clinical study in **six recreational male athletes** between the ages of **18 and 35**.

3 mL P-PRP and 3 mL L-PRP was prepared from 60 mL of venous blood after treating with 9 mL of sodium citrate and centrifugation at 2.700 rpm for 10 min.

Half of the prepared PRP's were frozen at **-20 degree-centigrade** for a week.

After thawing to room temperature, fresh and frozen samples were analyzed at the **Q-TOF LC/MS** device.

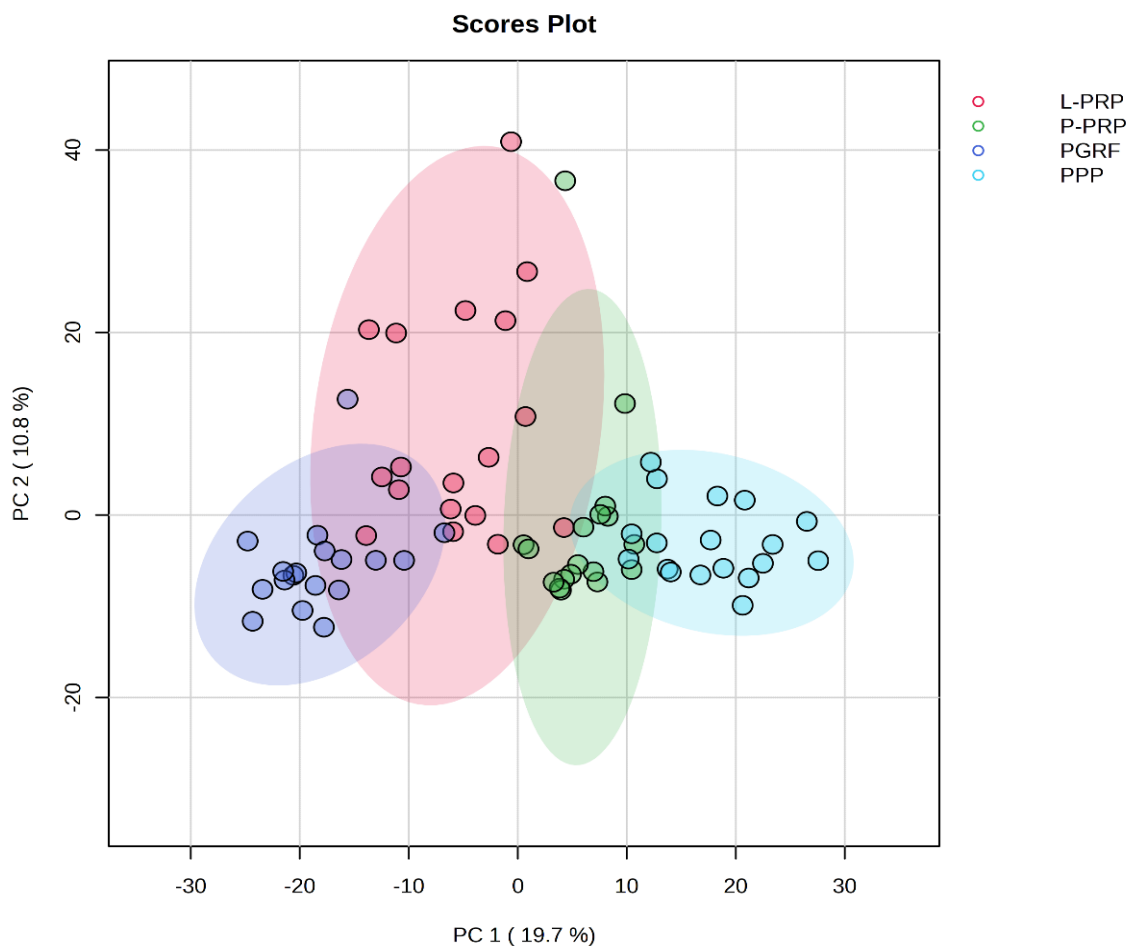
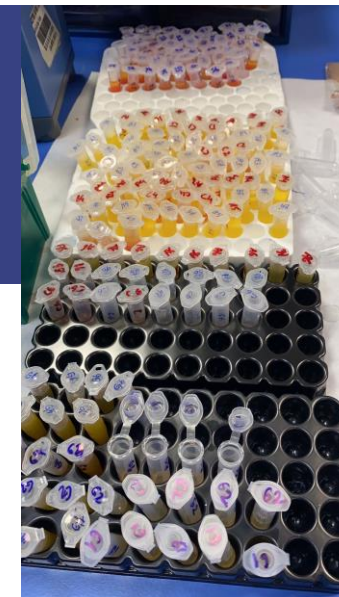
Findings



A total of **33.438 peaks** were found from the injected samples.

Statistically significant ($p < 0.05$) peaks were uploaded to the MetaboAnalyst 5.0 platform. Exogenous out of metabolites were eliminated and metabolites found significant for our study were subjected to pathway analysis.

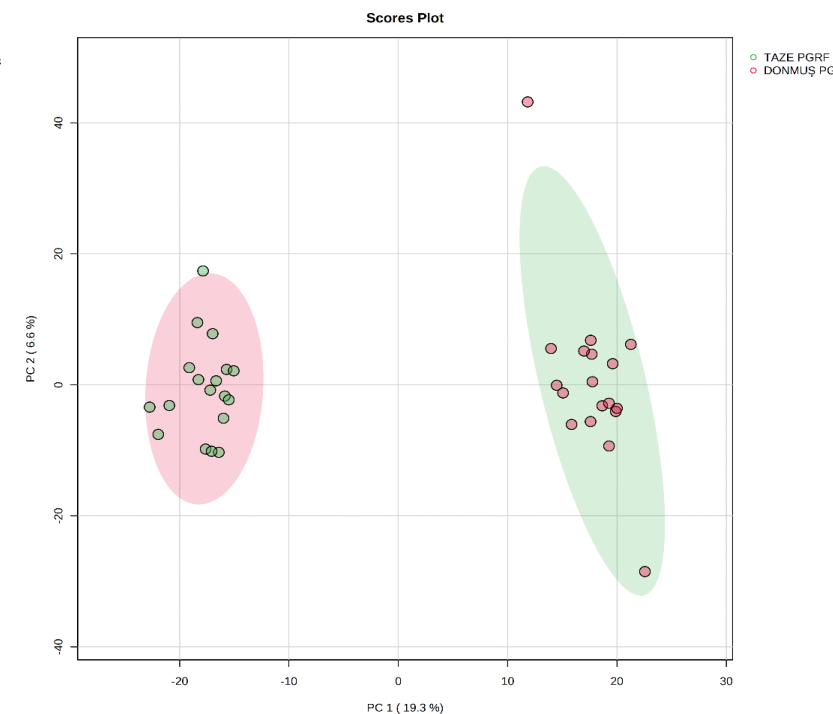
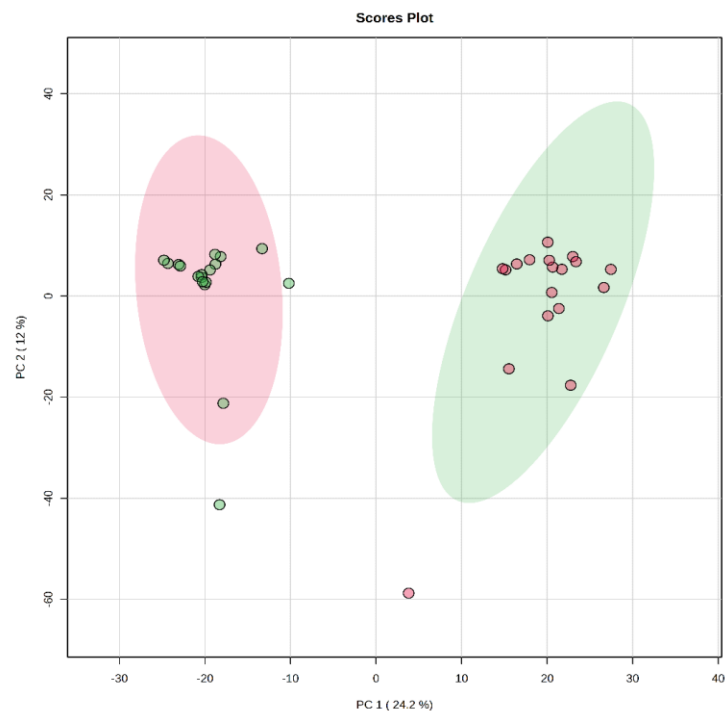
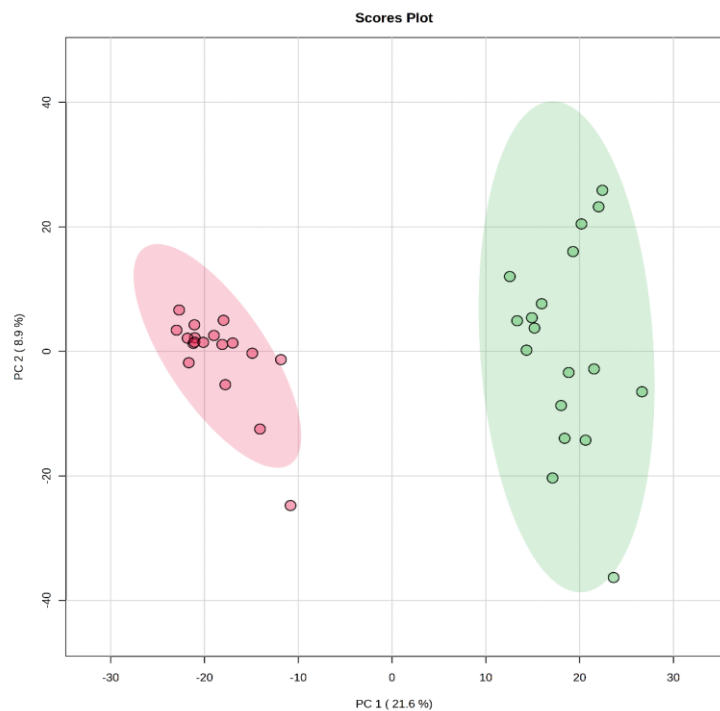
Findings



Principal component analysis (PCA) revealed that L-PRP, P-PRP, PGRF and PPP relatively differ from each other at the metabolome level.

Not all the PRPs are the same!

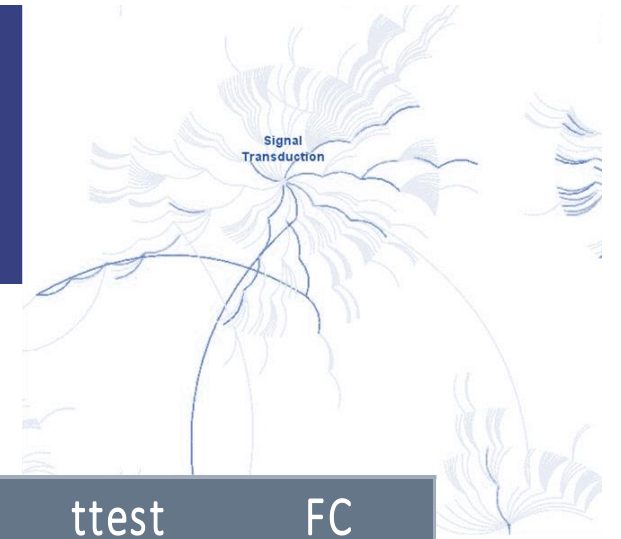
Findings



Freezing and thawing PRPs changes their metabolomic profiles.

Findings

Signaling pathways are effected as the most.

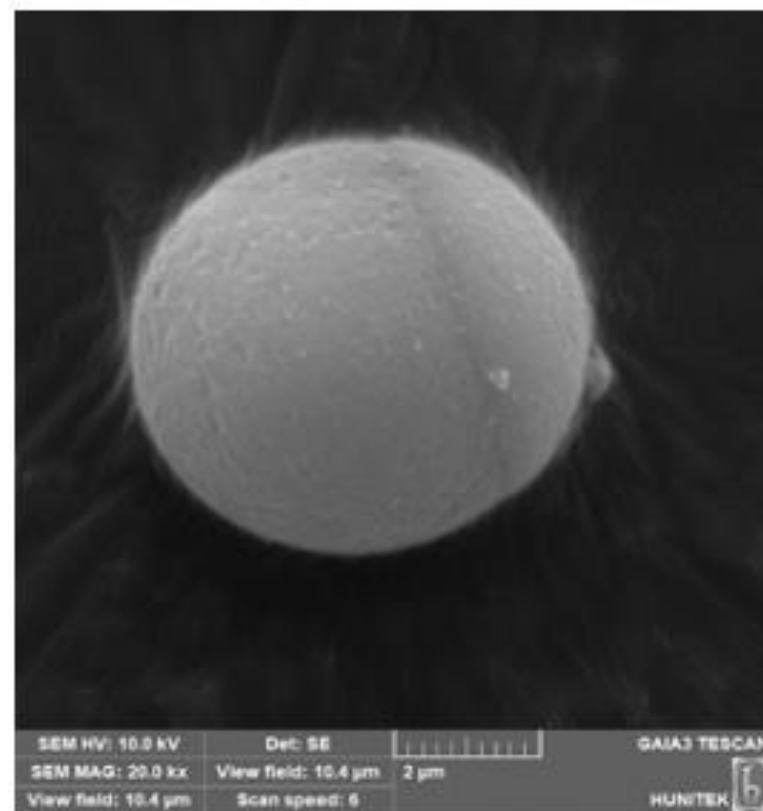
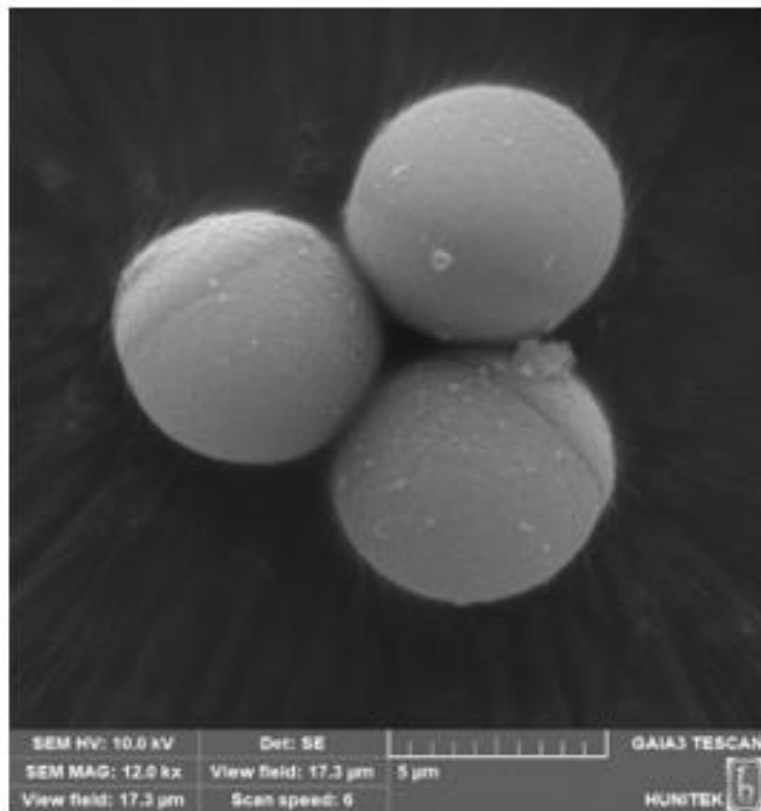


Metabolites	M/Z	Retention time (min)	ttest	FC
Sphinganine	320.32	22.7	7E-07	-1.09
Calcidiol	329.32	22.3	1E-06	1.17
2-Arachidonylglycerol	401.27	19.4	2E-06	1.06
dCTP	449.99	14.2	6E-03	2.27
LysoPC(18:1(9Z))	491.35	15.8	1E-04	3.91
PC(16:0/16:0)	892.65	21.1	4E-08	-4.93
Phosphocreatine	234.02	0.2	6E-03	1.37
Homovanillic acid	241.02	17.6	1E-04	-1.01
1-Phosphatidyl-1D-myo-inositol 3-phosphate	236.02	0.2	2E-03	1.10
4-Imidazolone-5-propionic acid	241.02	17.6	7E-05	1.10

Synovial Fluid



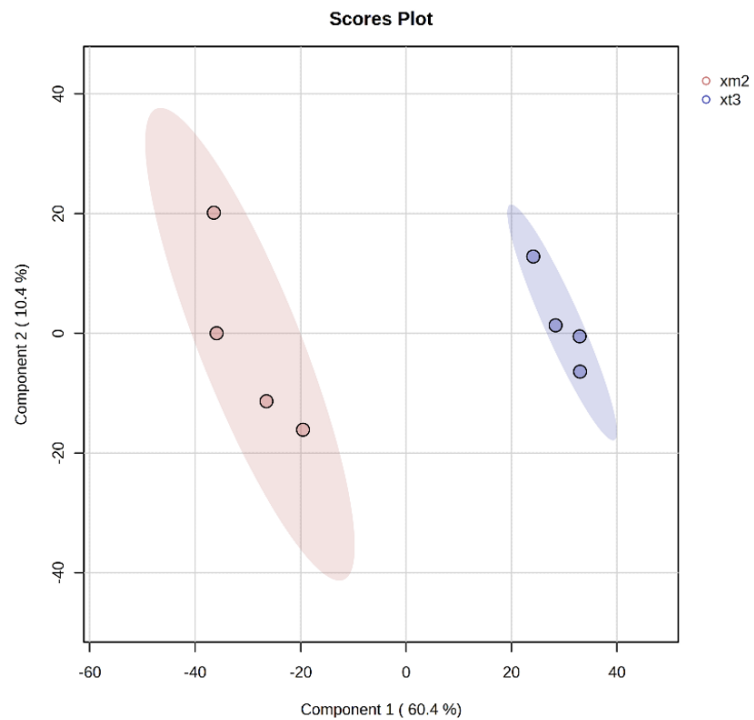
IMAC Sorbents



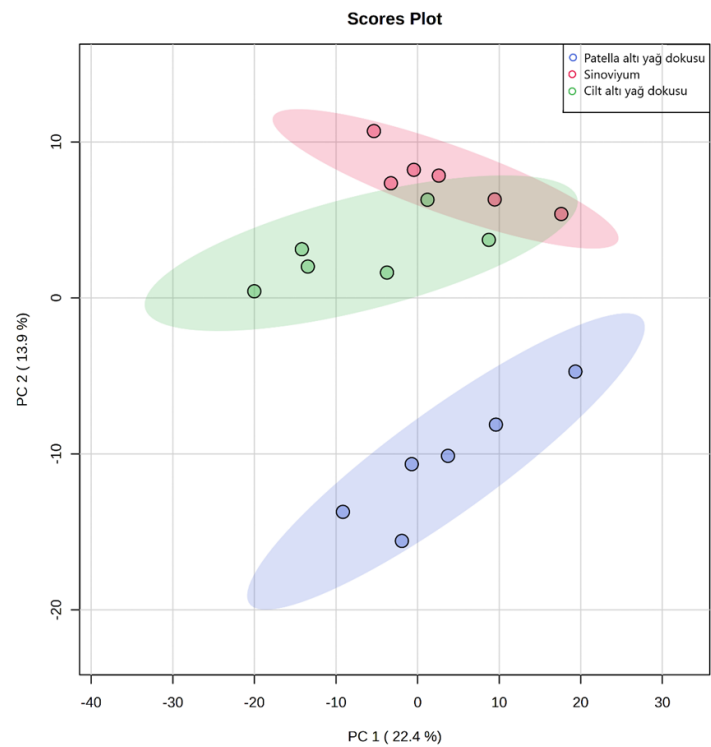
Synovial Fluid

	modification	peptid seq	protein id	score
11+12	Phospho (STY),Phospho (ST)	_AKIVS(Phospho (STY))DPCTT(Phospho (ST))QLGGIEFSK_	REV_Q9NSC7	11,79258
11	Phospho (STY)	_DLELY(Phospho (STY))KK_	REV_Q13107	7,671245
11	Phospho (STY)	_DQIEEPKLQLT(Phospho (STY))KEAR_	REV_P33908	13,90591
UCUNDE ORTAK	Phospho (STY),Phospho (ST)	_EKKS(Phospho (STY))PFWNPDGS(Phospho (ST))FLGDR_	REV_P37802	12,55442
UCUNDE ORTAK	Unmodified	_ERADVNEPMGTVEFVK_	REV_Q5U5Q3;REV_Q6ZN04	9,530286
UCUNDE ORTAK	Unmodified	_ETYWVTSILSSIGASMFVCGAFSAHKSTR_	REV_P56880	16,01398
11+12	Phospho (STY),2 Phospho (ST)	_FEPRIS(Phospho (STY))RPDS(Phospho (ST))S(Phospho (ST))DYIVLVPAGQK_	REV_A0A0A0MS00	10,32942
11+12	Phospho (STY),Phospho (ST)	_GDPLY(Phospho (ST))ERTPELPY(Phospho (STY))WQDR_	REV_Q70J99	9,046585
11+12	Phospho (STY)	_GGHT(Phospho (STY))VVNVSKGWIIYWSTK_	REV_O95398	8,819157
11	Phospho (STY)	_IS(Phospho (STY))SNKSPIFLSLNTKVSVEHK_	REV_Q8NFU7	7,533208
11+12	Phospho (STY),Phospho (ST)	_IT(Phospho (STY))PAT(Phospho (ST))KRCPPPIR_	REV_Q12955	10,48491
UCUNDE ORTAK	Unmodified	_KSHGAHRAMR_	REV_Q8IZC7	8,53231
UCUNDE ORTAK	Unmodified	_KVRYRHFFVEDVPIQSEK_	REV_Q03701	13,96364
UCUNDE ORTAK	Phospho (STY)	_LGPKHT(Phospho (STY))MLKGCIAVAK_	REV_P05771	8,729305
UCUNDE ORTAK	Unmodified	_LLEIKVEIEPVRTSCYK_	REV_P18074	7,201904
11	Phospho (STY)	_LSLCGT(Phospho (STY))KIDPHRTK_	REV_Q8N110	10,18839
11	Phospho (STY)	_LSTLGS(Phospho (STY))KFWLKLQWAK_	REV_Q13607	8,729305
UCUNDE ORTAK	Unmodified	_NADGLSGSDKSR_	REV_Q5T5U3	10,23178
12	Phospho (STY)	_NS(Phospho (STY))GISPEDLPPYFSEIQPPK_	REV_O60934	8,088973
11+12	Phospho (STY),Phospho (ST)	_NSLQADSSKSIKVY(Phospho (STY))NAPVDST(Phospho (ST))TSQR_	REV_Q9NU19	8,912778
UCUNDE ORTAK	Phospho (STY),Phospho (ST)	_PEGT(Phospho (STY))HRT(Phospho (ST))WHVVLVLR_	REV_A6NHJ4	13,73083
11+12	Phospho (STY)	_PIATS(Phospho (STY))NLETLGEQLLR_	REV_Q9H9E3	14,15787
12	Phospho (STY)	_PS(Phospho (STY))HVTSPSAALGPWLAR_	REV_Q96M86	7,897146
11	Phospho (STY)	_PVGPS(Phospho (STY))AGPLAGPGRWAPVPAPR_	REV_A0PJZ3	13,32051
UCUNDE ORTAK	Phospho (STY),Phospho (ST)	_RWY(Phospho (STY))RIHYAMS(Phospho (ST))HCIYCR_	REV_P49286	9,921084
12	Phospho (STY)	_T(Phospho (STY))LHQKLEQNQLHTR_	REV_O95613	12,21349
11+12	Phospho (STY)	_TLPIDGLY(Phospho (STY))KVYK_	REV_Q5VYK3	15,57155
12	Phospho (STY)	_VAYIVDECMGLLIS(Phospho (STY))NLHDGK_	REV_Q12923	8,287891
UCUNDE ORTAK	Phospho (STY)	_VKTVS(Phospho (STY))DGEIHVLCTRSIR_	REV_Q9H0B3	9,163782
11+12	Phospho (STY)	_VQELRLELET(Phospho (STY))KQR_	REV_Q9H307	15,89069
11+12	Phospho (STY)	_WEEQNT(Phospho (STY))LK_	REV_Q8WXH0	10,87322
11+12	Phospho (STY),Phospho (ST)	_YAVQVGEDVT(Phospho (STY))LNLNADT(Phospho (ST))LHEQIRWPK_	REV_P18074	8,837771
13	Phospho (STY)	_YQFEISIPMSYKS(Phospho (STY))KLEAETNMR_	REV_Q5TKA1	13,18794

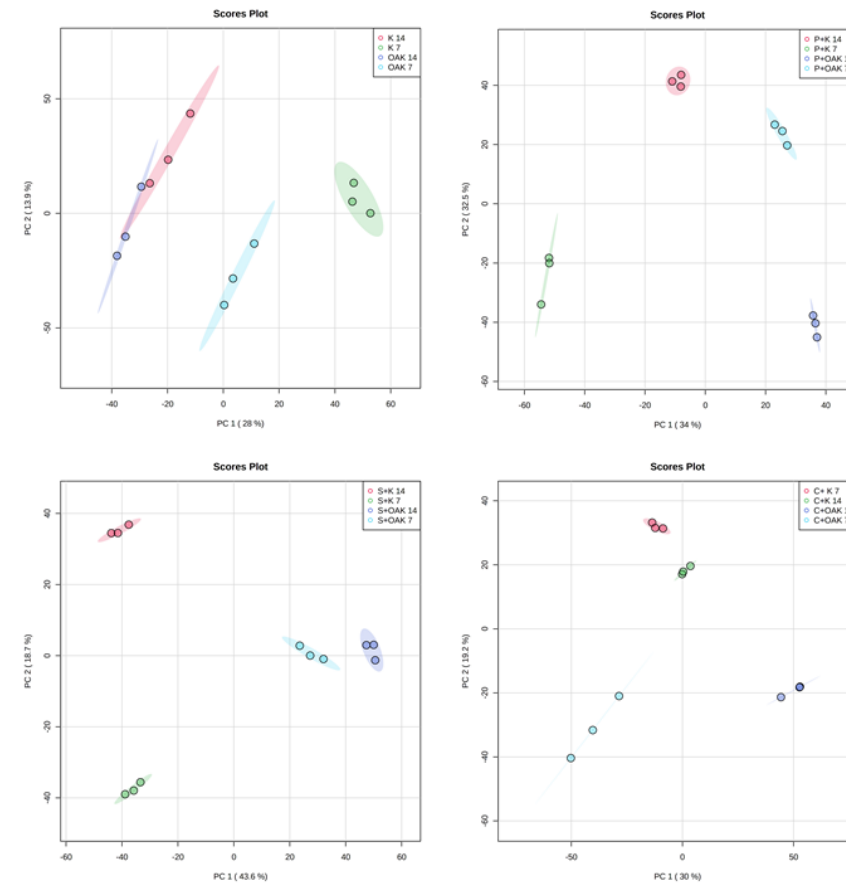
Stromal Cells (SVF)



Device-Specific



Tissue-Specific



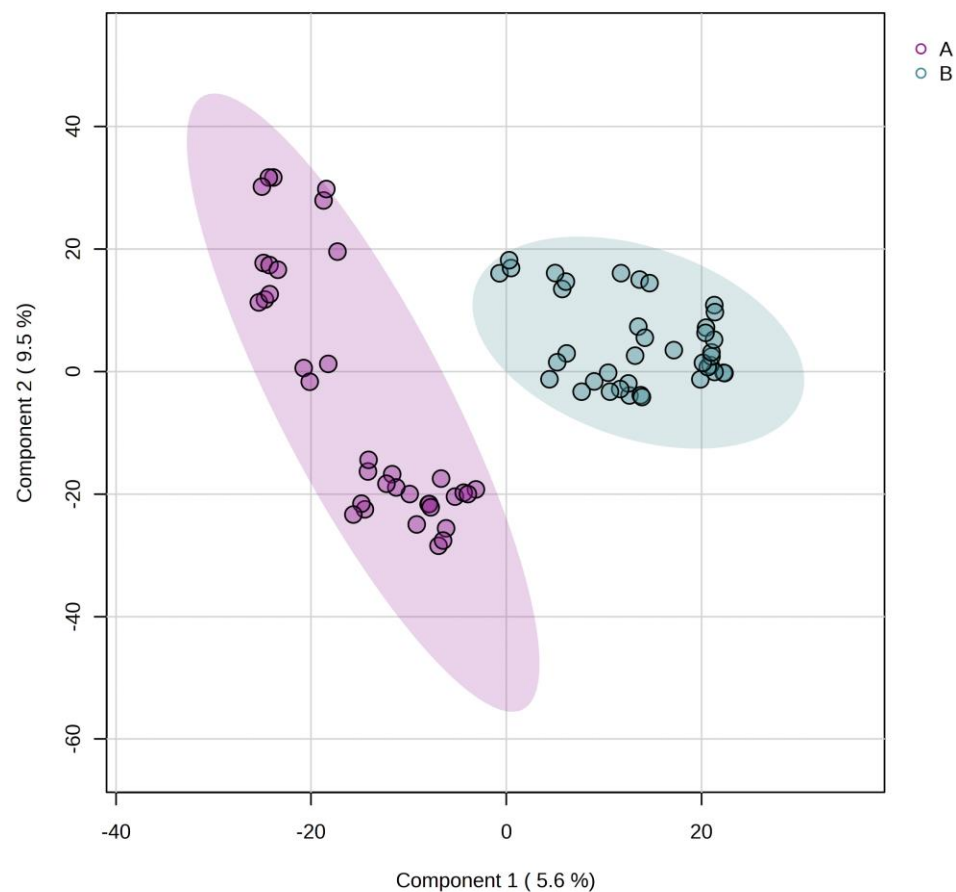
Time-Specific (in vitro)

Metabolites	Mass	HMDB code	KEGG code
L-Fukoz	147,0657702	HMDB0000174	C01019
Alfa-Tokotrienol	224,165375	HMDB0006327	C14153
Koproporfirin	328,1429424	HMDB0000643	C05769
Nikotinamid	393,0224517	HMDB0000229	C00455
Bilirubin	469,1707788	HMDB0003325	C05787
Taurodeoksikolik asit	472,3106687	HMDB0000896	C05463
Galaktosfingozin	500,296839	HMDB0000648	C01747

3913 peaks > 187 peaks 1.5-fold change ($p < 0.05$) > **51 metabolites matched > 21 common metabolites > 7 metabolites** were considered significant.

Rotator Cuff Tendons

Scores Plot



Metabolites	HMDB	rt (min)	m/z	p Value	Fold Change
Benzamide	HMDB0004461	0.58	140.07	1E-06	1.59
Pyridoxal	HMDB0001545	0.58	140.07	1E-06	1.59
4-Pyridoxic acid	HMDB0000017	0.58	140.07	1E-06	1.59
Alpha-Pinene-oxide	HMDB0003667	5.36	153.13	3E-02	2.90
(-)-trans-Carveol	HMDB0003450	5.36	153.13	3E-02	2.90
Arachidonic acid	HMDB0001043	5.36	153.13	3E-02	2.90
4-Imidazolone-5-propionic acid	HMDB0001014	0.57	156.05	4E-08	1.78
Nonadeca-10(Z)-enoic acid	HMDB0013622	0.59	160.13	2E-05	1.72
Aniline	HMDB0003012	0.59	162.05	6E-05	1.69
2-Aminomuconic acid semialdehyde	HMDB0001280	0.58	164.03	4E-03	1.50
4-Hydroxycinnamic acid	HMDB0002035	0.62	165.05	1E-03	1.62
Enol-phenylpyruvate	HMDB0012225	0.62	165.05	1E-03	1.62
Phenylpyruvic acid	HMDB0000205	0.62	165.05	1E-03	1.62
3-Methoxy-4-hydroxyphenylglycolaldehyde	HMDB0004061	0.62	165.05	1E-03	1.62
Homovanillic acid	HMDB0000118	0.62	165.05	1E-03	1.62
L-2,3-Dihydrodipicolinate	HMDB0012247	0.65	169.04	1E-07	3.58
17alpha,21-Dihydroxypregnenolone	HMDB0006762	0.52	175.12	2E-03	1.52
11b,21-Dihydroxy-5b-pregnane-3,20-dione	HMDB0006757	0.52	175.12	2E-03	1.52
3a,21-Dihydroxy-5b-pregnane-11,20-dione	HMDB0006755	0.52	175.12	2E-03	1.52
Normetanephrine	HMDB0000819	0.57	184.10	2E-10	1.80
Epinephrine	HMDB0000068	0.57	184.10	2E-10	1.80
2-amino-3-oxo-hexanedioic acid	HMDB0060273	0.58	214.01	2E-03	1.57
N-Formyl-L-glutamic acid	HMDB0003470	0.58	214.01	2E-03	1.57
N-Acetyl-L-aspartic acid	HMDB0000812	0.58	214.01	2E-03	1.57
Galactosylglycerol	HMDB0006790	0.54	219.09	3E-03	1.51
Galactosylglycerol	HMDB0006790	7.71	255.11	2E-02	1.66

Take Home Message

- Omic Technology may aid to;
 - Accurate diagnosis of diseases and conditions.
 - Inflammation – Degeneration – Regeneration
 - Monitor outcomes of treatment modalities.
- Targeted omics will influence our diagnoses and treatments.
- **Not all the treatment modalities have the same metabolomic profiles.**
- **Freezing and thawing changes metabolomic profiles.**
- **Metabolomic profiles change by tissue, device and time.**



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