

Platelet-Rich Plasma Devices Can Be Used to Isolate Stem Cells From Synovial Fluid at the Point of Care

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Disclosures

- Personal Disclosures:
 - No Relevant Disclosures
- Study Funding:
 - External support from Arthrex, Inc was provided for execution of this study.
 - No external funding was used/received for this study.

Location

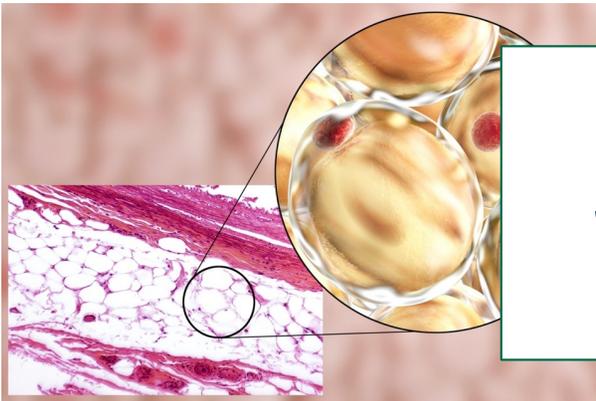
- Principle Investigation Site:
 - Andrews Research & Education Foundation, Gulf Breeze, Florida, U.S.A

Introduction

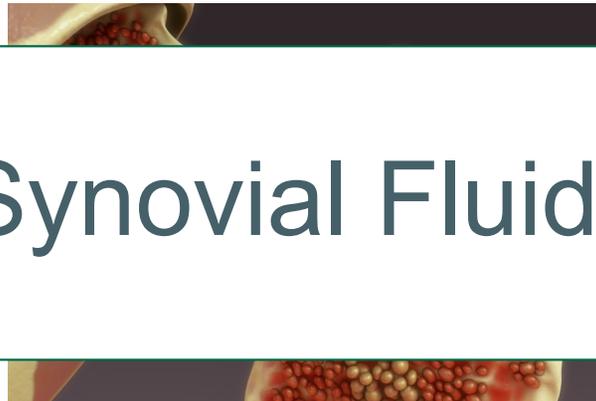
- Research Focus:
 - Investigate and define viable sources of autologous cellular products
- Goal:
 - Improve and augment the healing potential of orthopedic tissues.



Where have we looked?



Adipose Tissue



Bone Marrow

Synovial Fluid?



Whole Blood

What do we know?

- Resident stem cells are known to mobilize in the knee from the synovium and fat pad to the synovial fluid in the setting of:
 - Osteoarthritis (OA)
 - Anterior cruciate ligament (ACL) rupture
 - Meniscus tears
- Point-of-care processing of autologous products are becoming more frequently used in orthopaedics, with platelet-rich plasma (PRP) systems being one of the most prevalent.

Study Goals

- Purpose:
 - To assess whether point-of-care devices, designed for collecting cellular components from blood or bone marrow, could be used to isolate viable resident stem cells from synovial fluid.
- Hypothesis:
 - Synovial fluid could be mixed with whole blood, and a buffy coat based PRP processing device could be used to concentrate cells within the product.

Methods:

- Patients were recruited as they presented to clinic over a 24 month period.
- IRB approval was obtained.

Inclusion Criteria:

- ≥ 18 years old
- Dx:
 - Acute ACL tear confirmed with MRI, or
 - Knee OA and elected for PRP injection therapy
- Minimum estimated effusion of 20ml

Exclusion Criteria:

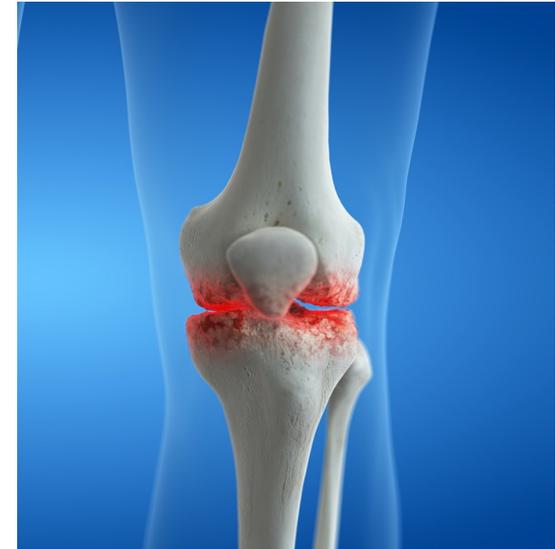
- Time between surgery and ACL injury >5 weeks
- Previous knee aspiration of the injury effusion
- Signs of local or systemic infection
- History of immunosuppression or use of chronic steroids

Patient Demographics

	ACL	OA	P Value
Age, y	18.8 ± 3.3	55.6 ± 14.4	<.001
Sex	3 F/6 M	2 F/8 M	N/A
Fluid volume, mL	36.0 ± 13.5	21.5 ± 9.8	.045

NOTE. Age = mean ± standard deviation; fluid volume = mean ± 95% confidence interval.

ACL, anterior cruciate ligament; F, female; M, male; N/A, not applicable; OA, osteoarthritis.



Fluid Collection

- 52ml of whole blood was collected in 60ml syringe preloaded with 8ml of citrate dextrose solution.
- 5ml of anticoagulated whole blood was set aside for CBC analysis and culture.

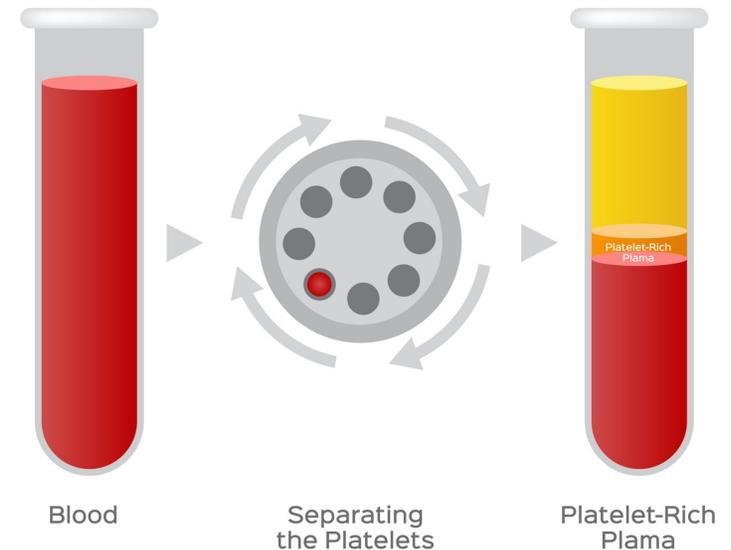
Synovial Fluid Aspirate:

- 2ml was set aside for CBC analysis and culture
- Remainder was combined with the 55ml of anticoagulated whole blood.



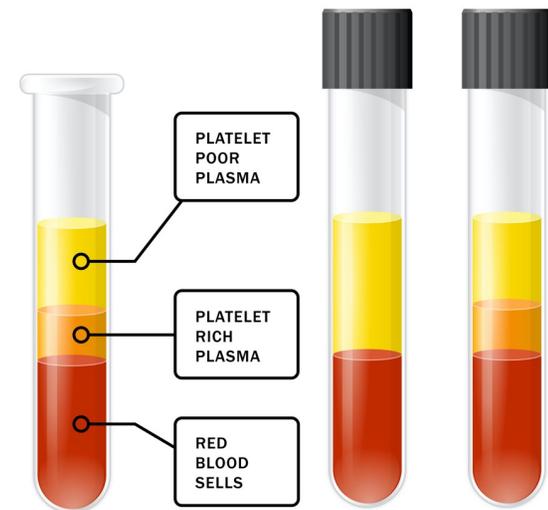
PRP Processing

- Combined effusion and whole blood solution was processed to create a platelet-poor plasma (PPP) and a buffy coat-based PRP product.
- PRP system used for this study utilized automated valve actuation for sequential aspiration of plasma, platelet, white blood cell (WBC), and red blood cell (RBC) layers based on the wavelength of the product.



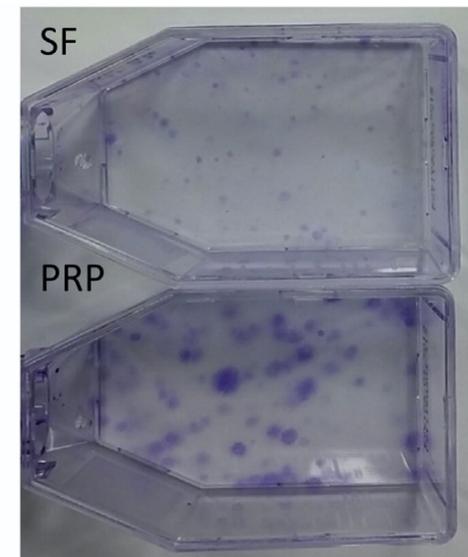
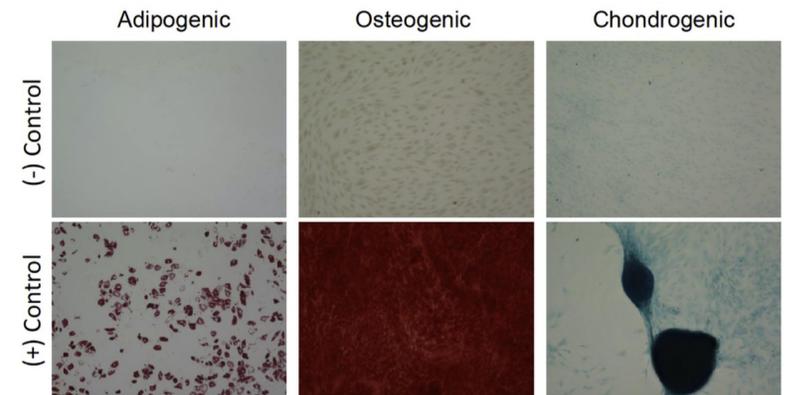
PRP Processing

- The percentage of RBCs collected in the PRP syringe is determined by the hematocrit setting (15%).
- This setting isolates cells from a deeper portion of the buffy coat, which would result in capturing more resident stem cells.



Results

- Cultured cells showed tri-lineage differentiation potential
- Flow cytometry: >95% of cells expressed surface markers typical of known resident stem cell populations (CD45-, CD73+, CD29+, CD44+, CD105+, and CD90+)

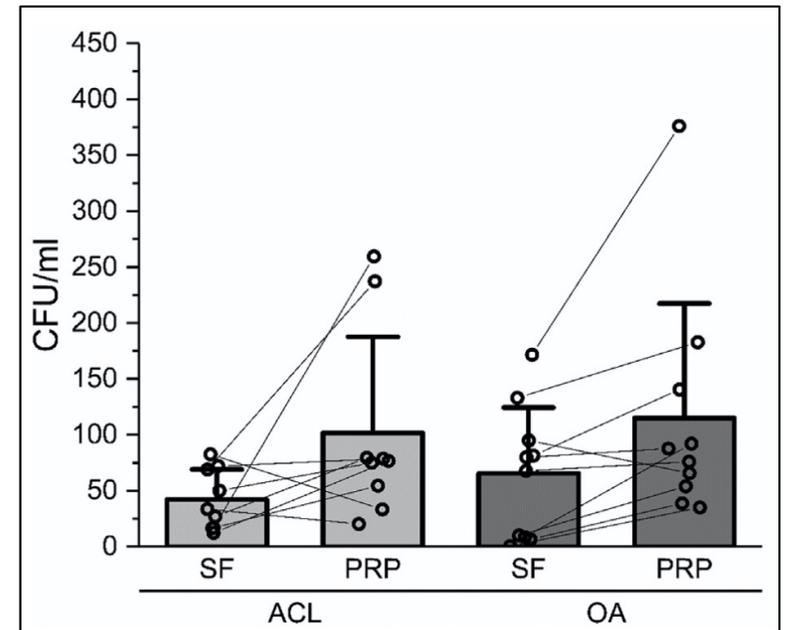


Results

	ACL, CFU/mL	OA, CFU/mL	<i>P</i> Value (ACL vs OA)
Synovial fluid	42.1 ± 20.7	65.4 ± 42.1	.283
Platelet-rich plasma	101.6 ± 66.1	114.8 ± 73.4	.713
<i>P</i> value (SF vs PRP)	.076	.014	

NOTE. This table captures the results of the CFU assays (mean ± 95% confidence interval.)

ACL, anterior cruciate ligament; CFU, colony-forming unit; OA, osteoarthritis; PRP, platelet-rich plasma; SF, synovial fluid.



Note: Unmixed whole blood and PPP were also cultured to evaluate the presence of CFU and none were detected.

How does it compare to prior study?

Viabie Stem Cells Are in the Injury Effusion Fluid and Arthroscopic Byproducts From Knee Cruciate Ligament Surgery: An In Vivo Analysis



Adam W. Anz, M.D., Eric A. Branch, M.D., John Rodriguez, M.D., Fellipo Chillemi, M.D.,

The similar counts suggest that point-of-care processing can be used with similar results compared to off-site processing

Byproduct fluid		
Small	2,357 ± 339	14 ± 6.14
Large	515 ± 157.4	0.39 ± 0.34

utilizing off-site laboratory processing.

NOTE. This table captures the results of the colony-forming unit of fibroblast (CFU-F counts) assays (± standard deviation).

How does it compare to other sources?

Umbilical Cord Tissue Offers the Greatest Number of Harvestable Mesenchymal Stem Cells for Research and Clinical Application: A Literature Review of Different Harvest Sites

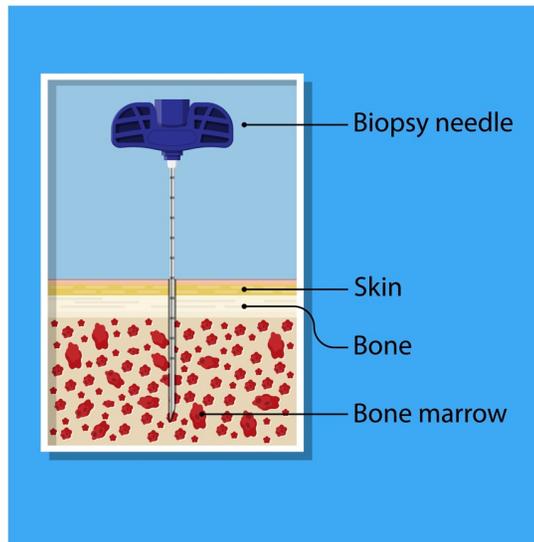
C. Thomas Vangsness Jr., M.D., Hal Sternberg, M.D., and Liam Harris, B.S.



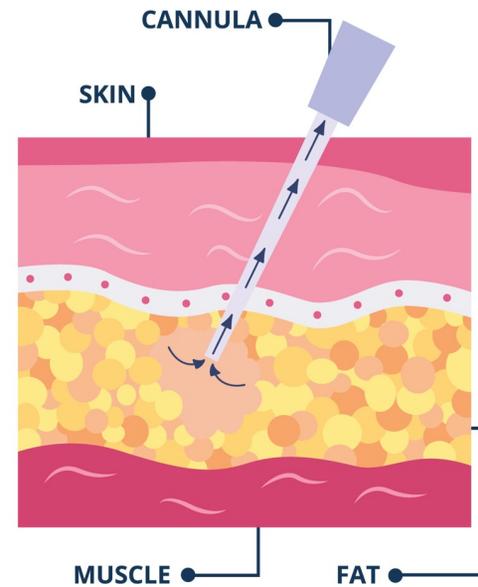
Arthroscopy
The Journal of Arthroscopic
and Related Surgery



- Bone marrow aspirate:
 - 1 - 300,000 stem cells per ml of tissue.



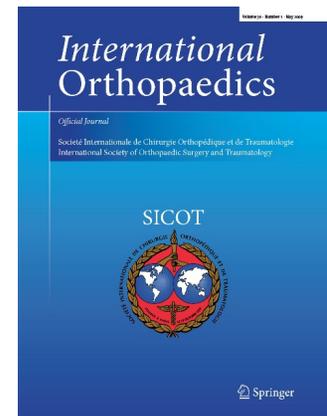
- Lipoaspirate:
 - 5,000 - 1,500,000 stem cells per ml of tissue.



Benefits of small volume and small syringe for bone marrow aspirations of mesenchymal stem cells

**Philippe Hernigou • Yasuhiro Homma •
Charles Henri Flouzat Lachaniette •
Alexandre Poignard • Jerome Allain •
Nathalie Chevallier • Helene Rouard**

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Bone marrow aspiration:
 612 ± 134 progenitor cells/ml

Comparison of Mesenchymal Stem Cells (Osteoprogenitors) Harvested From Proximal Humerus and Distal Femur During Arthroscopic Surgery

Knut Beitzel, M.A., M.D., Mary Beth R. McCarthy, B.S., Mark P. Cote, D.P.T.,
Thomas J.S. Durant, P.T., David M. Chowaniec, B.S., Olga Solovyova, B.S.,
Ryan P. Russell, M.A., Robert A. Arciero, M.D., and Augustus D. Mazzocca, M.S., M.D.

Arthroscopy
The Journal of Arthroscopic
and Related Surgery



Bone marrow aspiration proximal humerus/tibia:
 766 ± 545.3 progenitor cell/ml

Limitations

- Small sample size.
- Unable to perform a power analysis, as the number of cells mobilized with a knee injury has not been previously evaluated, and the therapeutic dosage of stem cell technologies has not been established.
- Demographic differences between OA group and ACL group exist; therefore, conclusions between the populations are susceptible to type II error.
- Number of CFU colonies present in a particular culture is highly dependent on the culturing techniques, conditions, and investigators' definition of a colony.

Conclusion

- Viable Resident Stem Cells can be harvested from knee synovial fluid, associated with an ACL injury or OA, and concentrated with a buffy coat-based PRP processing device.
- Synovial fluid yielded significantly fewer CFU/ml compared to bone marrow and adipose sources.
- While Resident Stem Cells can be harvested and concentrated with the techniques studied here, it is unclear the number of cells required for clinical application.

Thank You



Disclaimer

- It is important to mention that obtaining tissue at the point of care and preparing with minimal manipulation, as in this study, for homologous use is considered low risk by regulatory agencies.
- However, obtaining tissue and processing it to alter its relevant structural properties, such as enzymatically digesting or culturing, is considered high risk by regulatory agencies and not currently available for patient care in most countries.
- While cells can be harvested and concentrated with the techniques studied here, it is unclear if the number of cells available with these techniques have any clinical utility to augment healing.
- Clinical application studies have suggested that success of clinical application is dependent upon the number of stem cells harvested and used, although a threshold value for efficacy has not been defined.