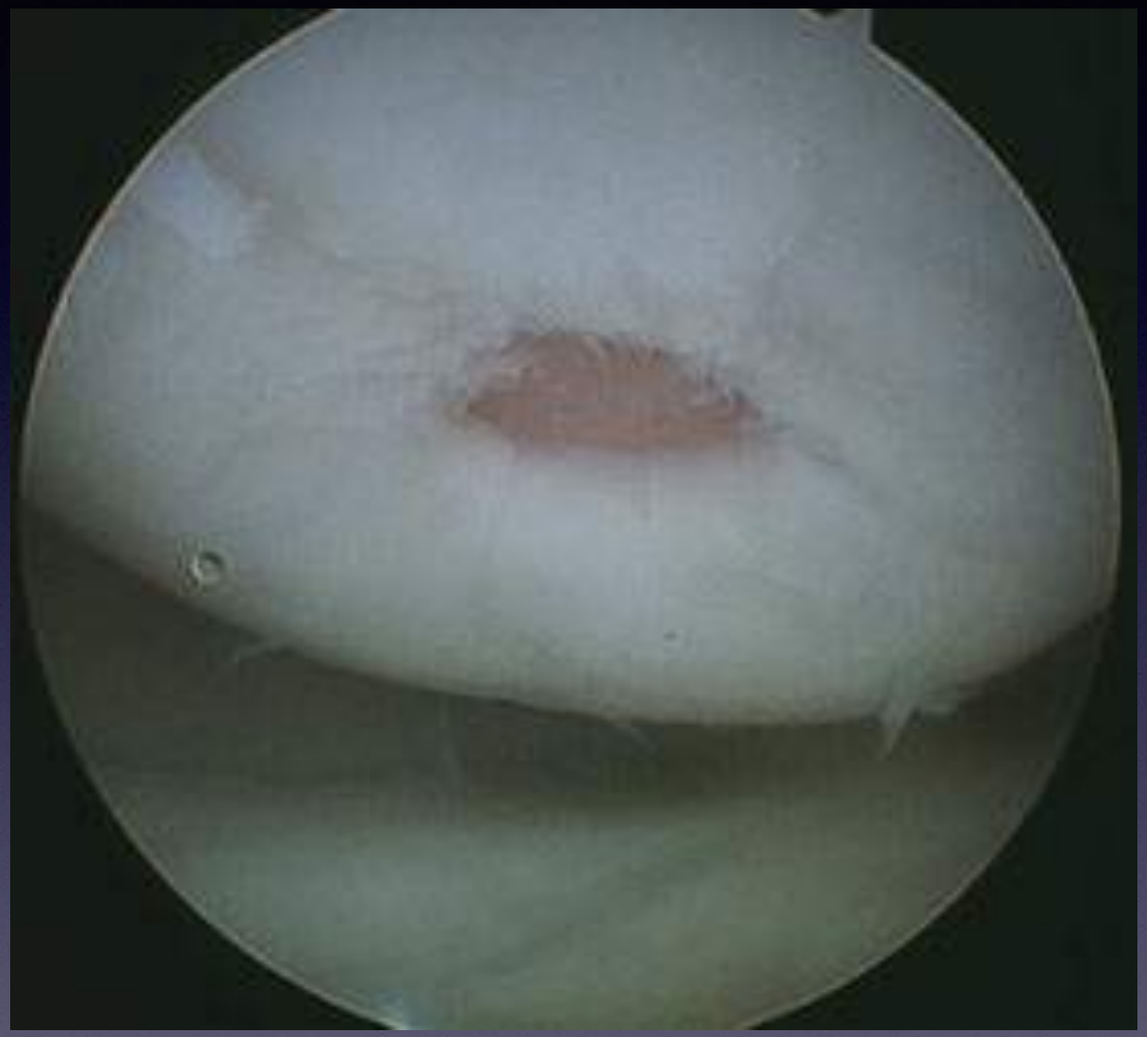
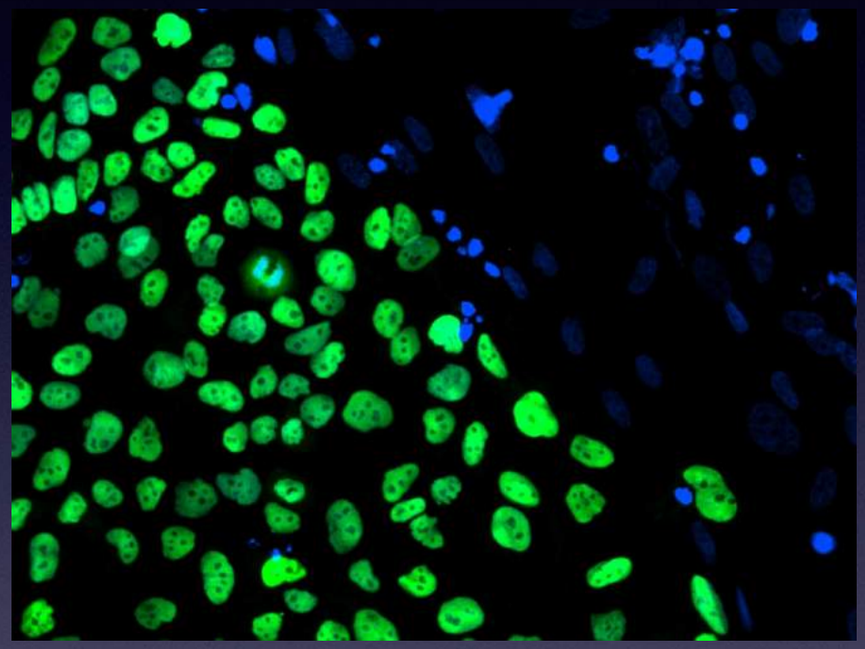
The Role of Orthobiologics for Articular Cartilage Damage in the Knee





Bryan Reuss, M.D.

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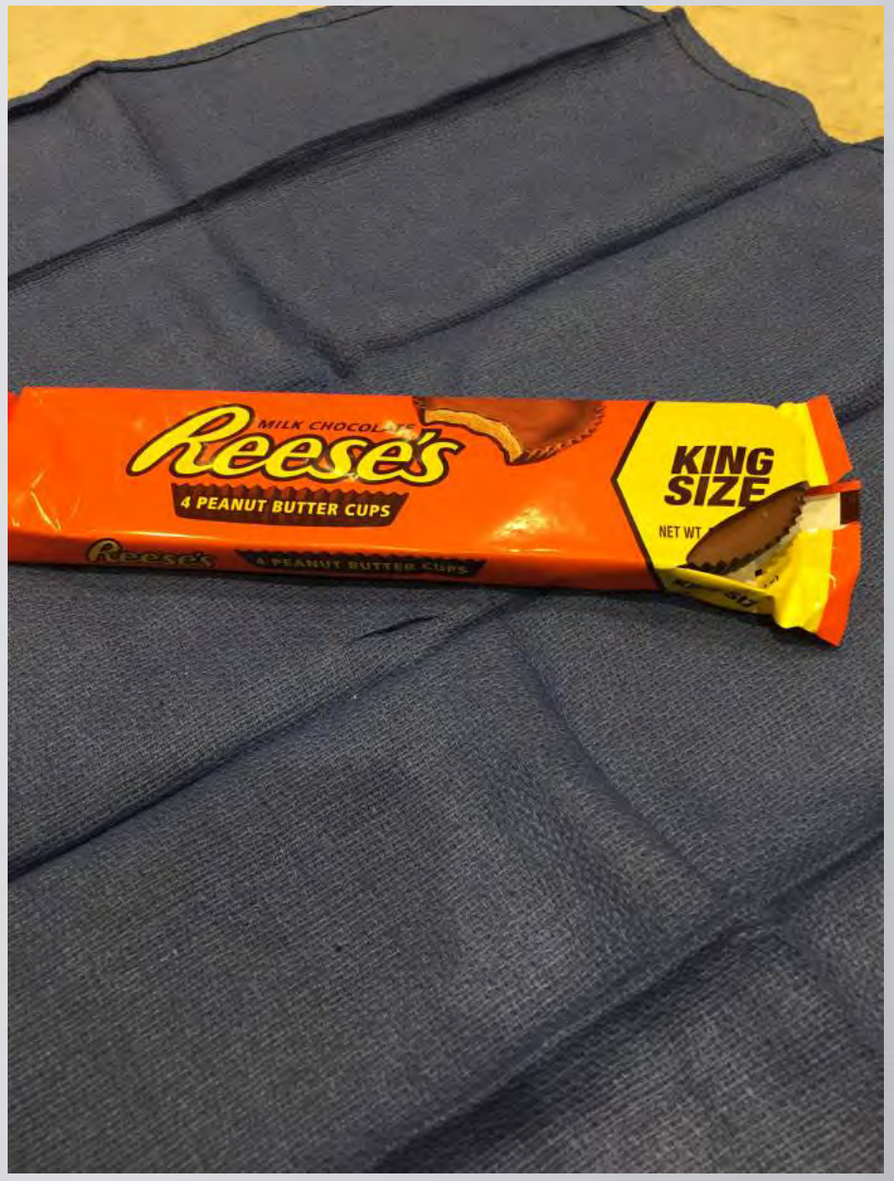
WHAT THE HELL
YOU SAY?
makeameme.org

Orthobiologics

- Bone Marrow Aspirate Concentrate
- Adipose-derived Mesenchymal Stem Cells
- Amniotic Fluid/membrane
- Platelet-Rich Plasma
- Umbilical Cord Tissue/Scaffold
- Micronized Allogeneic Cartilage

Chondral defect Demonstration…



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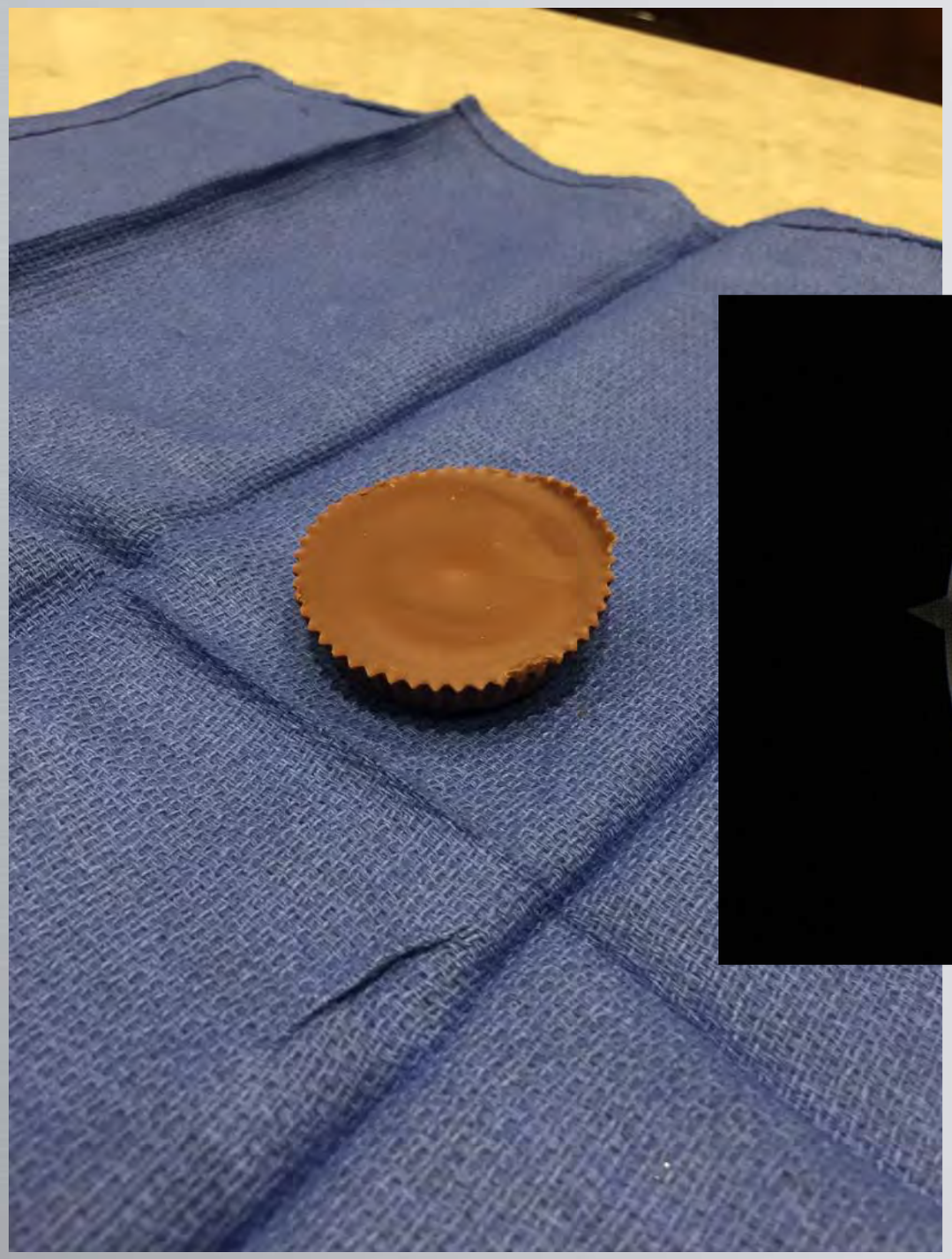

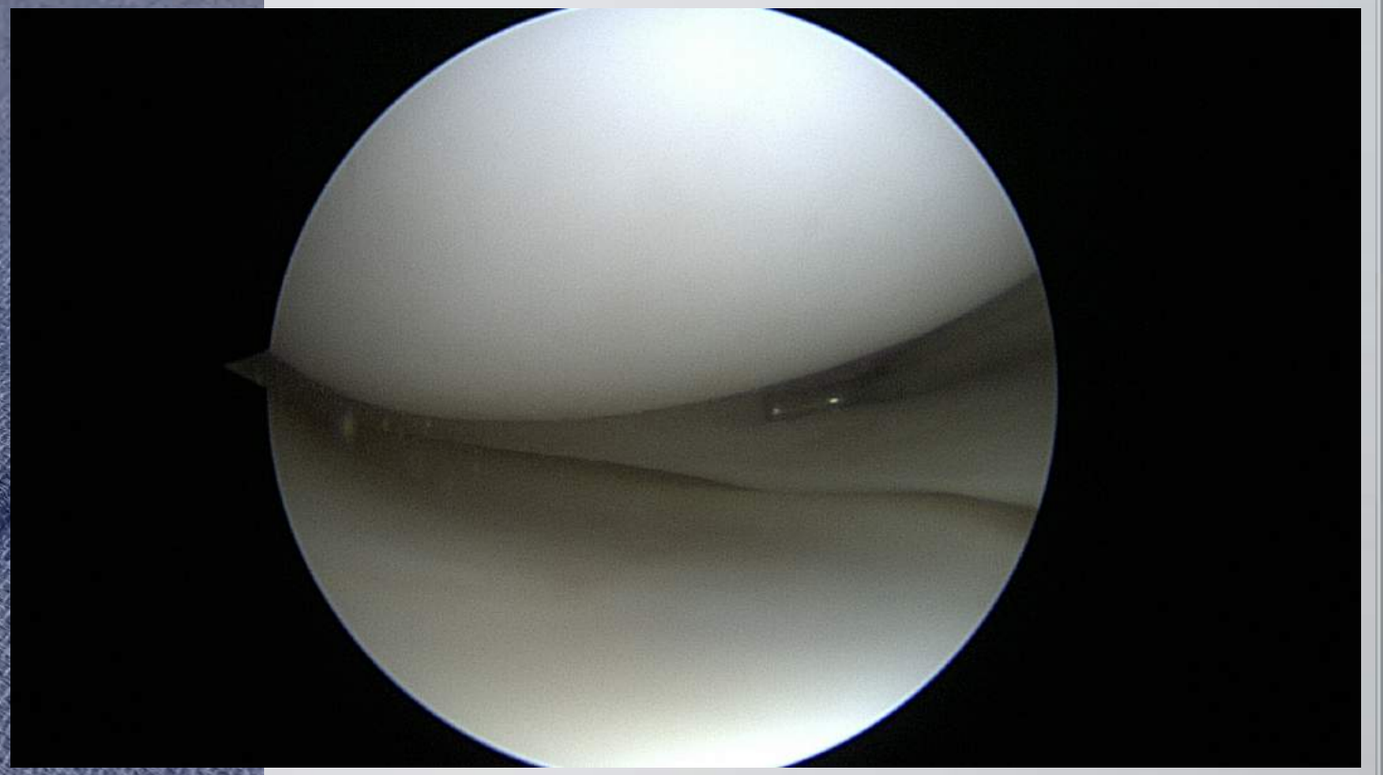

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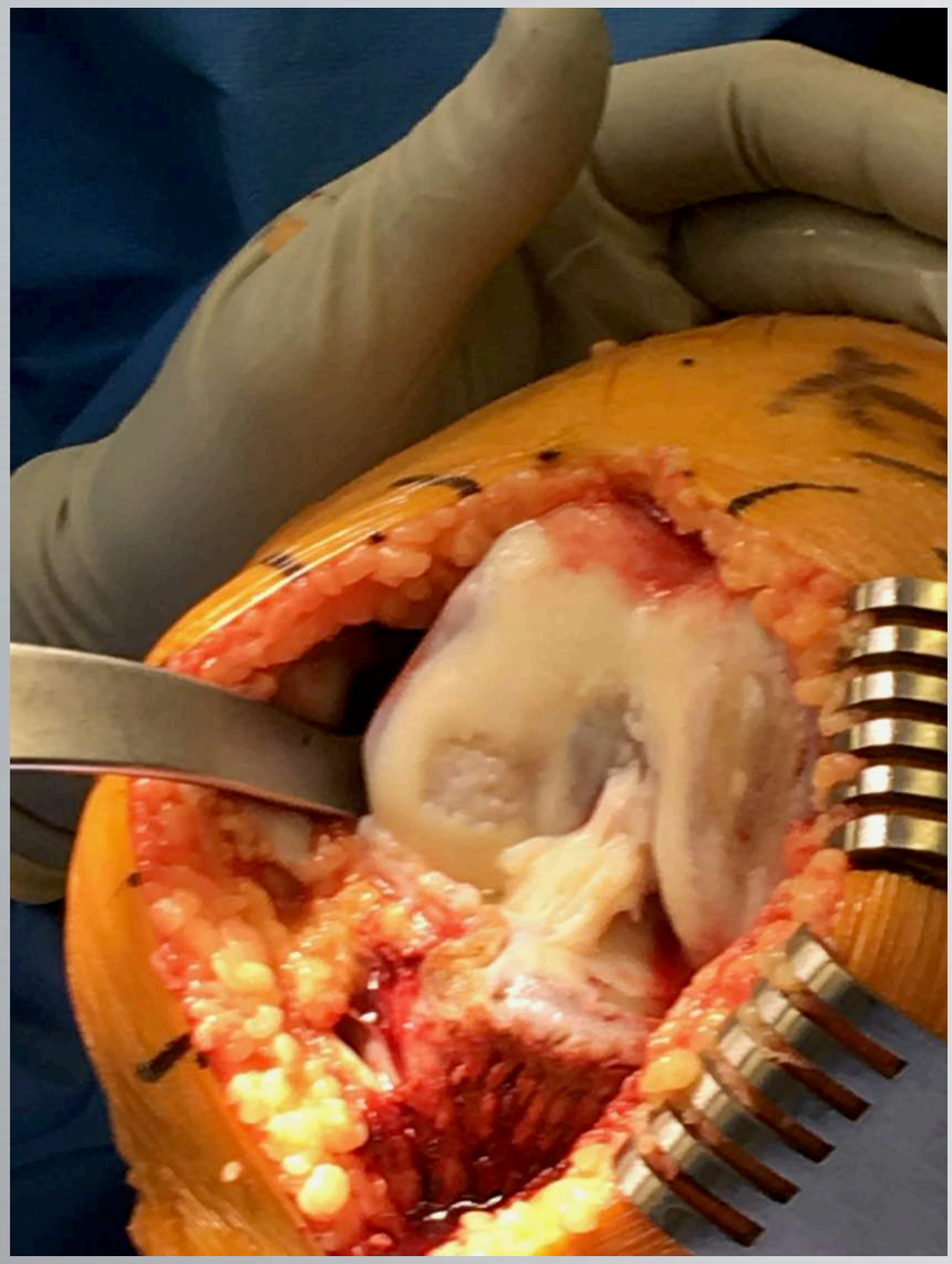

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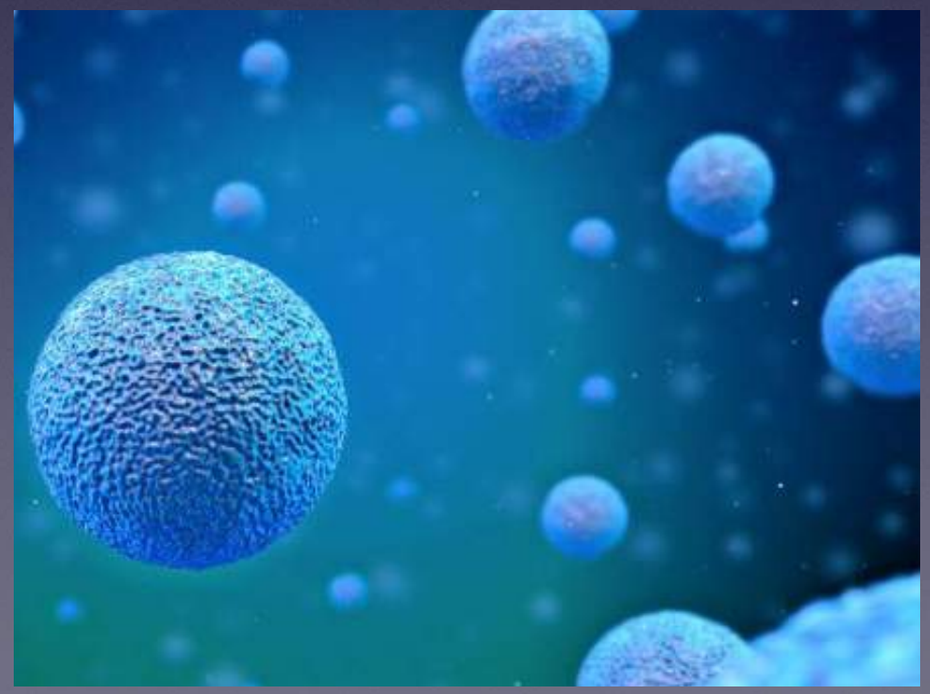
Bone Marrow Derived Stem Cells

Bone Marrow Derived Stem Cells

- Mesodermal Origin
- Precursors to Bone, Cartilage, fat, tendon, ligament



Mesenchymal Stem Cells

- Secrete Bioactive Molecules
  - Growth Factors
  - Cytokines
  - Chemokines
- In the Literature: Promising results with MSCs for Chondral defects and OA (AJSM, JBJS, Arthroscopy, etc)

References

Clinical Sports Medi

Intra-articular Mesenchymal Stem Cell Therapy for the Human Joint

A Systematic Review

James A. McIntyre,* BS, Ian A. Jones,† BA, Bo Han,‡ PhD, and C. Thomas Vangsn
Investigation performed at Department of Orthopaedic Surgery, Keck School of M
University of Southern California, Los Angeles, California, USA

Background: Stem cell therapy is emerging as a potential treatment of osteoarthritis (OA) and chondral defects there is a great deal of heterogeneity in the literature. The indications for stem cell use, the ideal tissue source, a outcome measures for stem cell–based treatments have yet to be determined.

Purpose: To provide clinicians with a comprehensive overview of the entire body of the current human literature i safety and efficacy of intra-articular mesenchymal stem cell (MSC) therapy in all joints.

Methods: To provide a comprehensive overview of the current literature, all clinical studies investigating the safety intra-articular MSC therapy were included. PubMed, MEDLINE, and Cochrane Library databases were searched human clinical trials involving the use of MSCs for the treatment of OA and CDs in all joints. A total of 3867 pub screened.

Results: Twenty-eight studies met the criteria to be included in this review. Fourteen studies treating osteoarthritis a treating focal chondral defects were included. MSCs originating from bone marrow (13), adipose tissue (12), synovia peripheral blood (2) were administered to 584 distinct individuals. MSCs were administered into the knee (523 knee (61), and hip (5). The mean follow-up time was 24.4 months after MSC therapy. All studies reported improvement fro at least 1 clinical outcome measure, and no study reported major adverse events attributable to MSC therapy.

Discussion: The studies included in this review suggest that intra-articular MSC therapy is safe. While clinical and, in radiological improvements were reported for both OA and CD trials, the overall quality of the literature was poor, an neity and lack of reproducibility limit firm conclusions regarding the efficacy of these treatments.

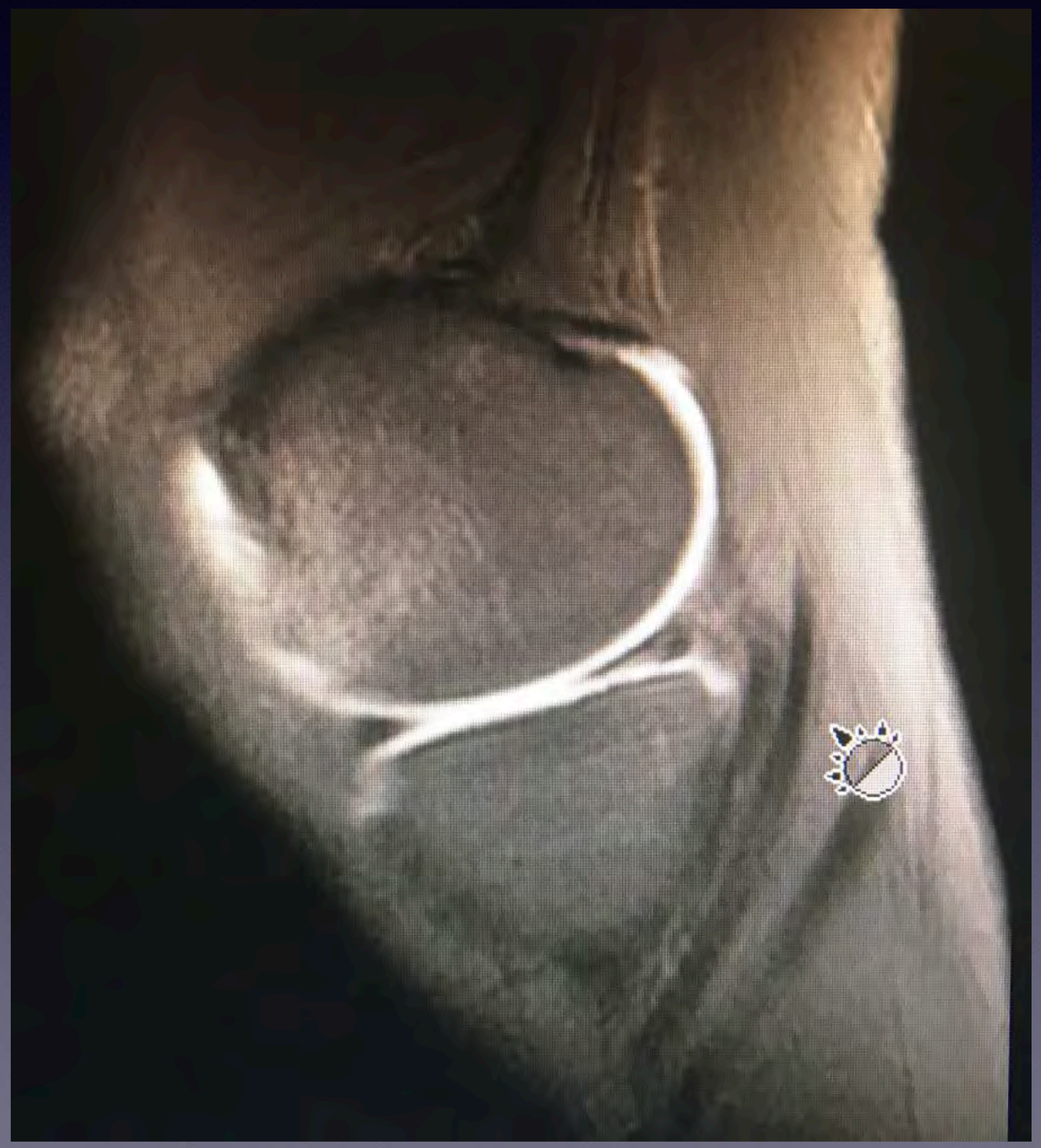
Conclusion: This review provides strong evidence that autologous intra-articular MSC therapy is safe, with generally p ical outcomes.

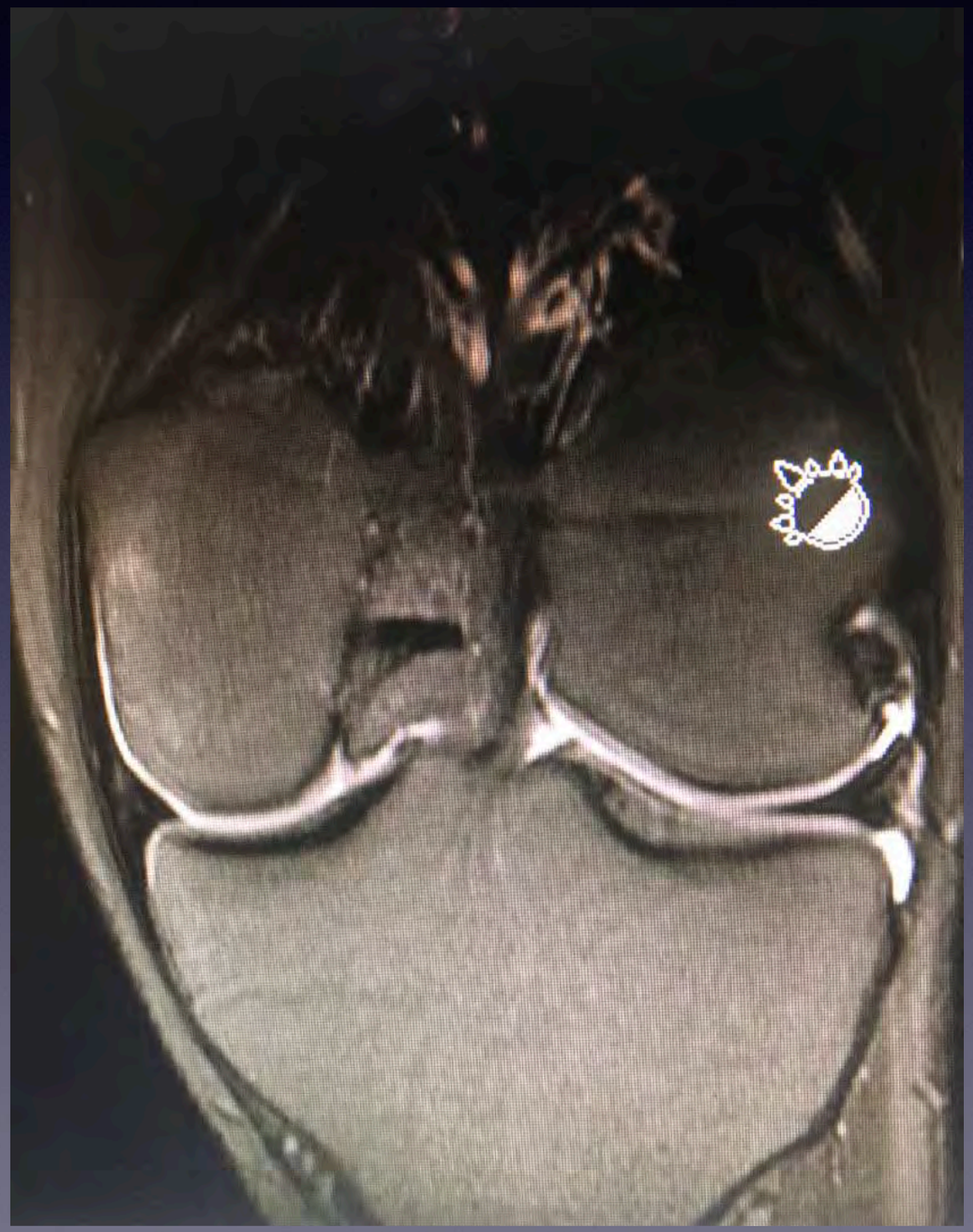
Keywords: mesenchymal stem cell; MSC; intra-articular; stem cell; human

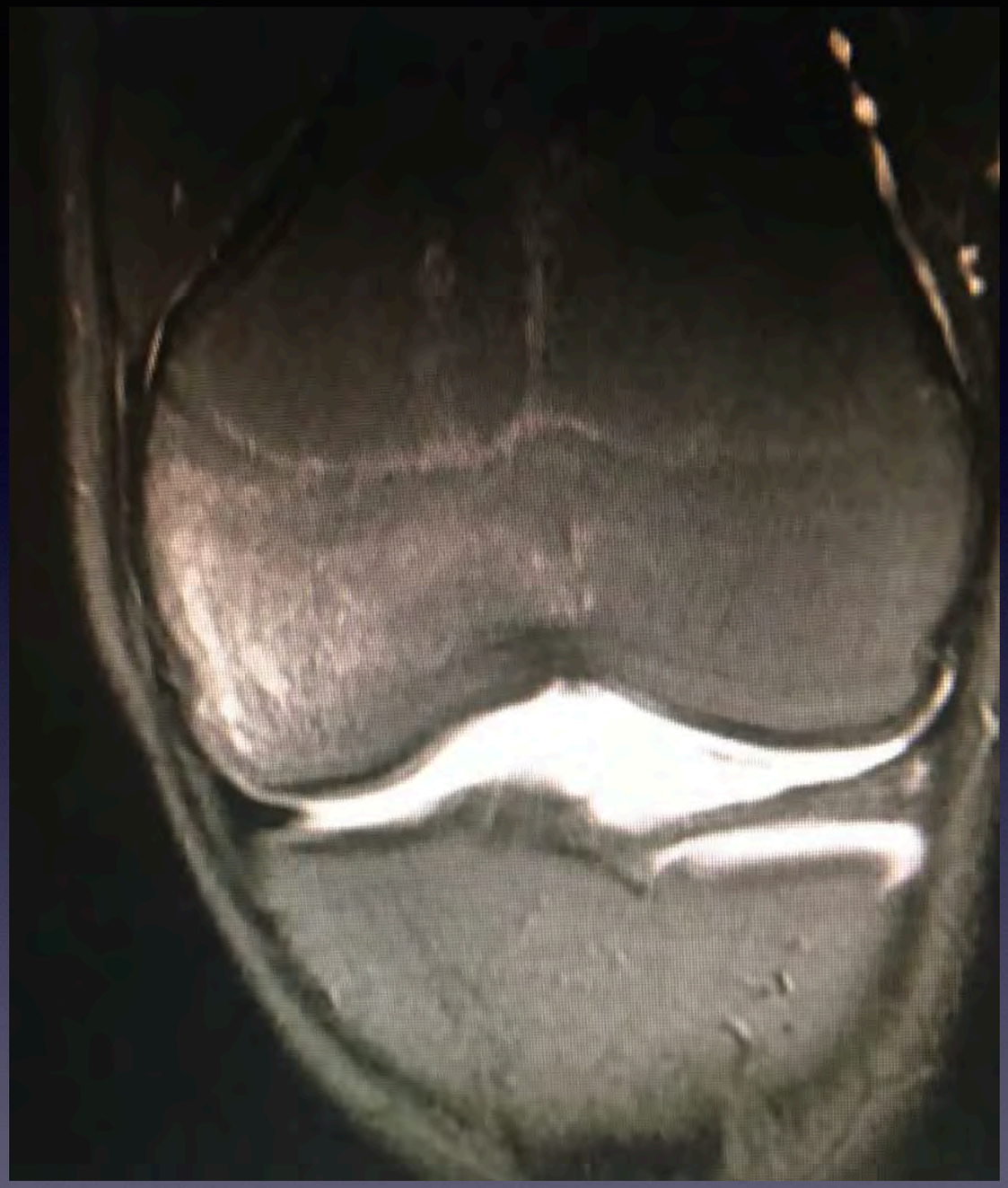
Adult cartilage is characterized by a limited intrinsic repair capacity after injury, owing to the sparse distribution of highly differentiated chondrocytes, the low sup genitor cells, and the lack of vascular supply.[44] or pathologic injury to articular hyaline cartilage leads to progressive damage and irreversible joi ation. Osteoarthritis (OA) affects an estimated world's population and is the most common joi in the United States.[27]

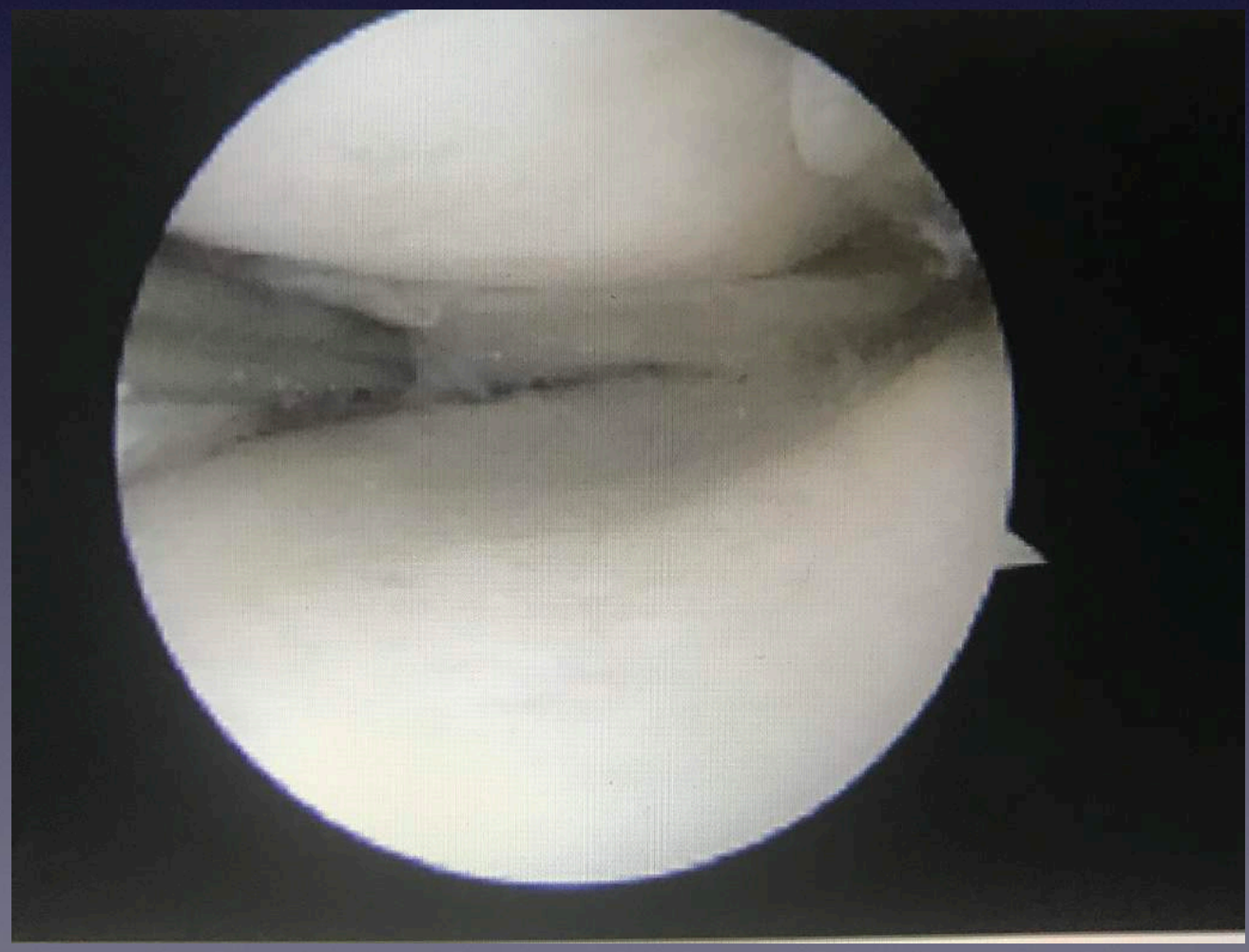
§Address correspondence to C. Thomas Vangsness Jr, MD, Department of Orthopaedic Surgery, Keck School of Medicine of USC, HCT 1520 San Pablo Street, Suite 2000, Los Angeles, CA 90033, USA (email:

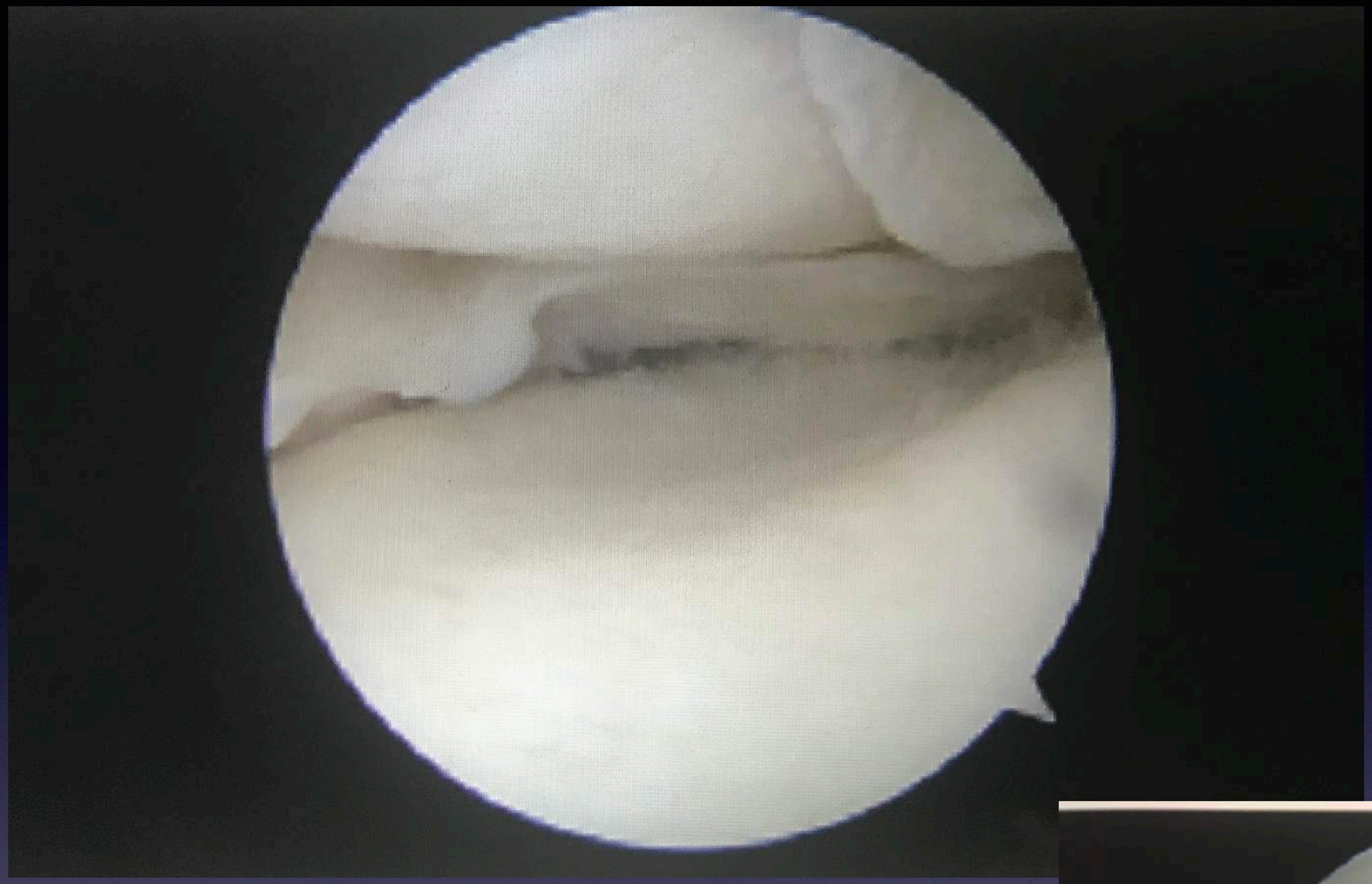
19-y/o with medial chondromalacia s/p meniscectomy

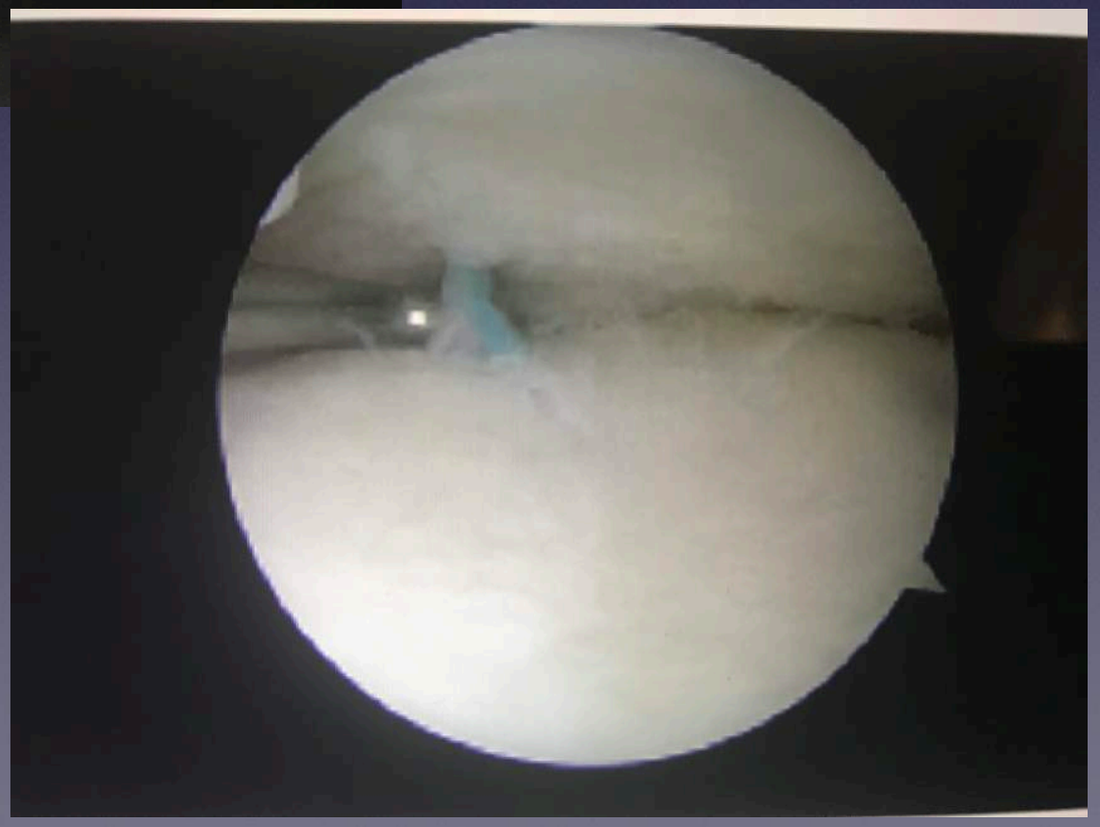


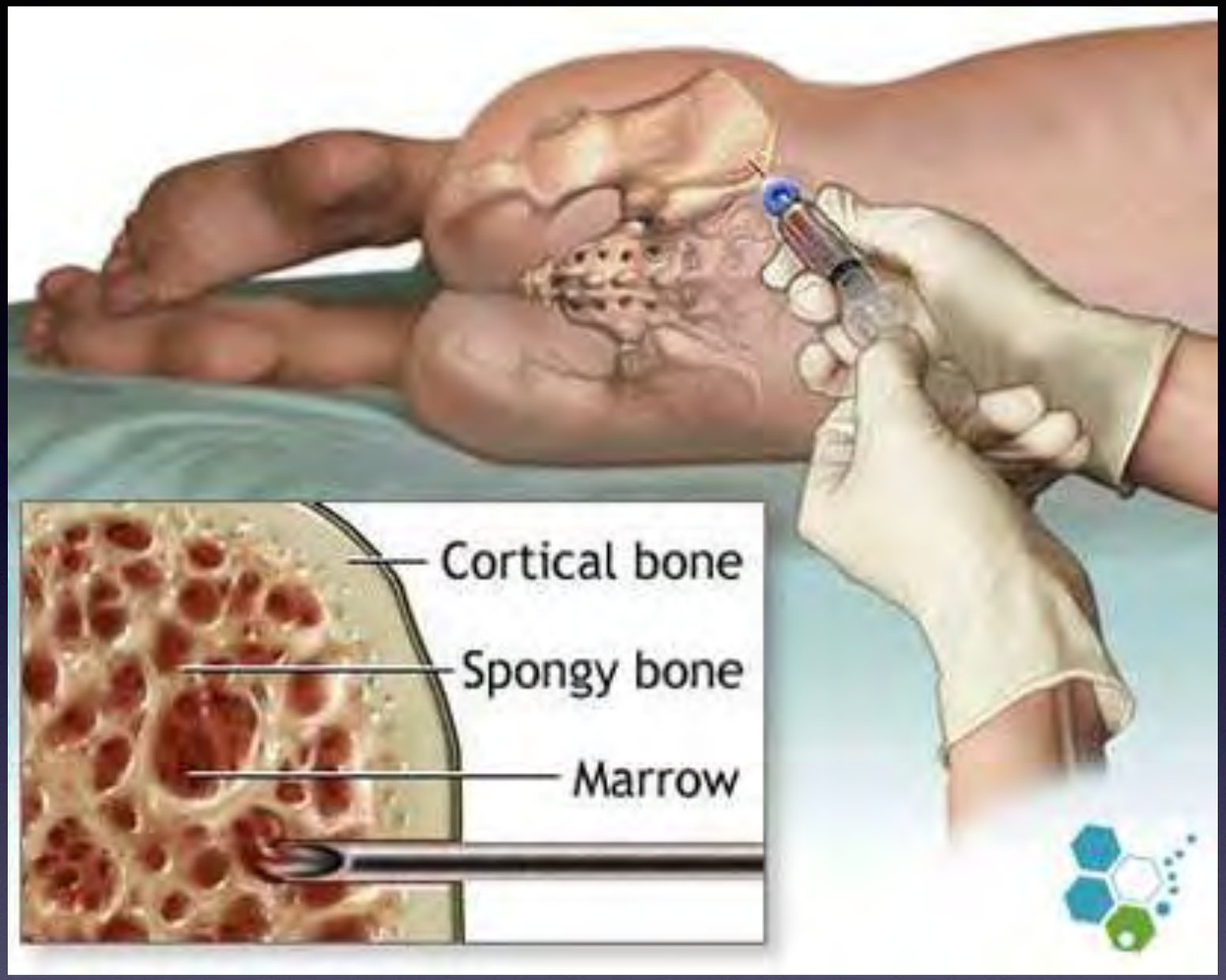


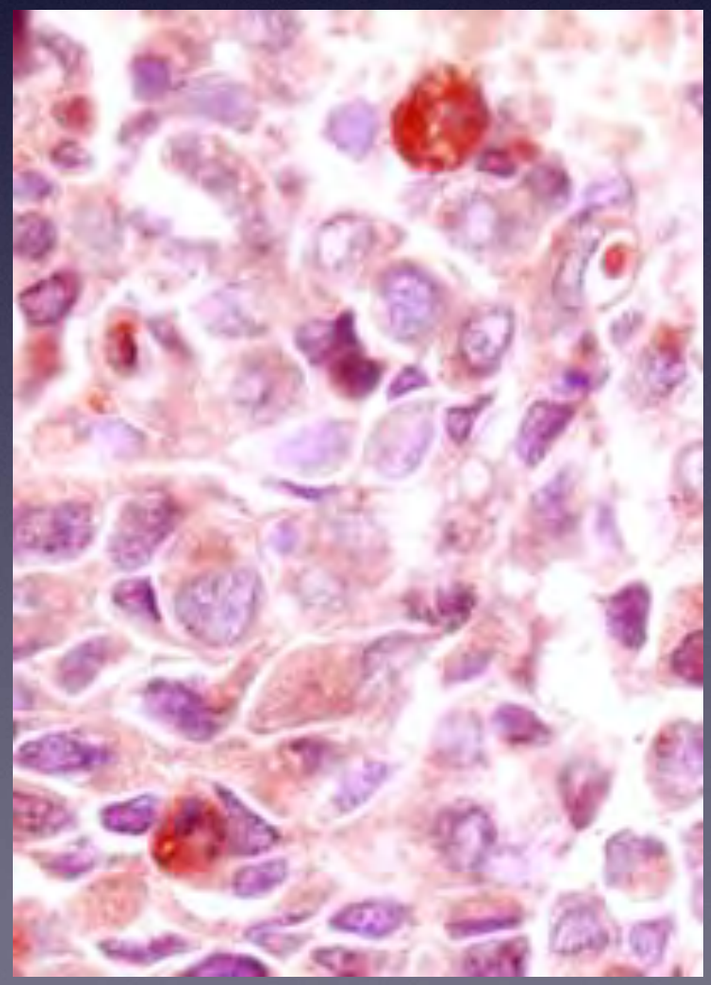


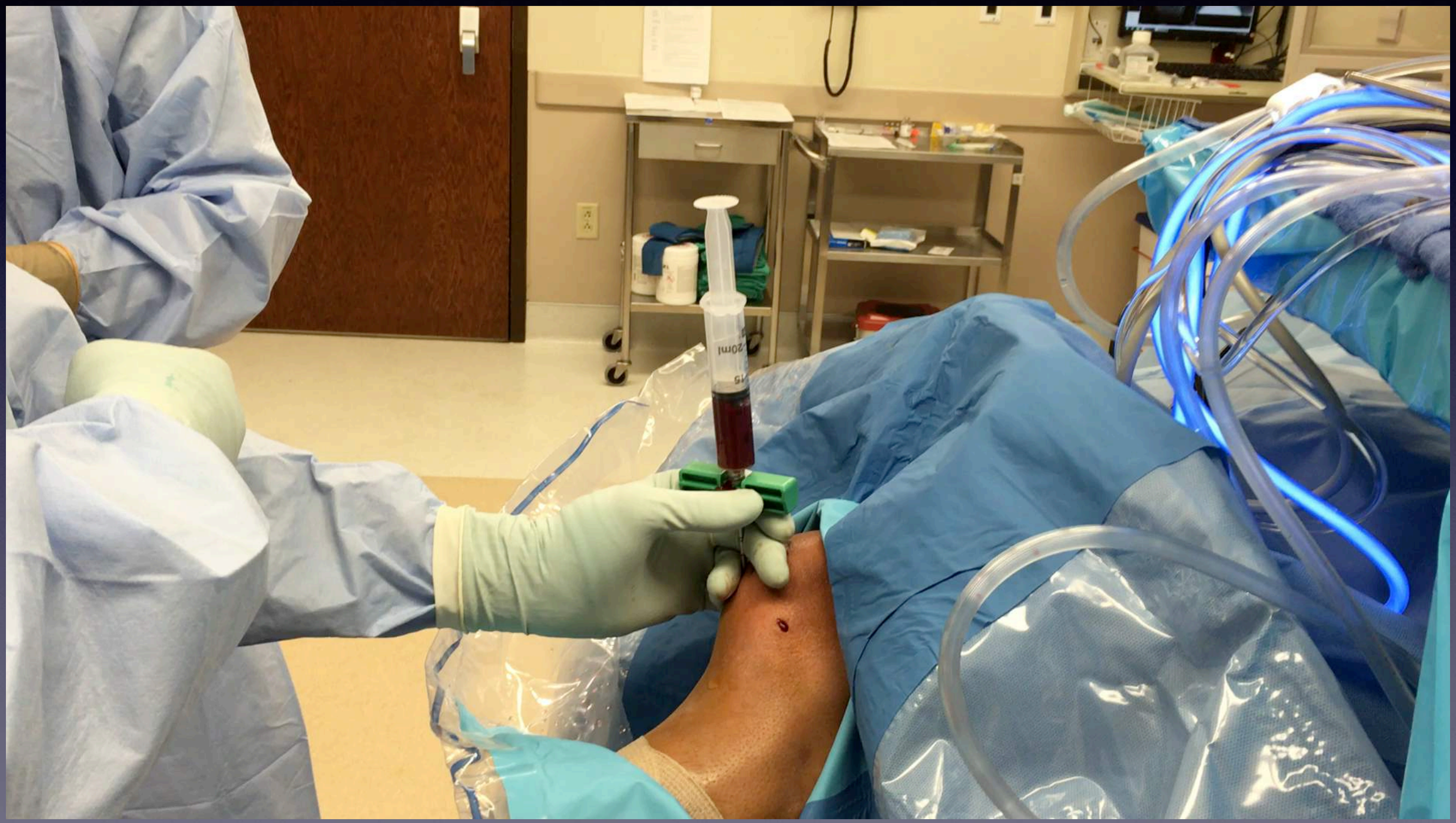







Cortical bone
Spongy bone
Marrow





Adipose-derived MSCs

Adult Stem Sources

	Bone Marrow	Adipose Tissue
Surgical Isolation Method	**Bone Marrow Aspiration**	**Lipoaspiration**
Method of Extraction	Centrifugation	Centrifugation
Enzymatic Digestion	**No**	**Depends**
Stem Cell Yield per Gram	100 – 1,000 cells	~5,000 cells
Abundance in Nucleated Cells	**0.001% - 0.01%**	**~2%**

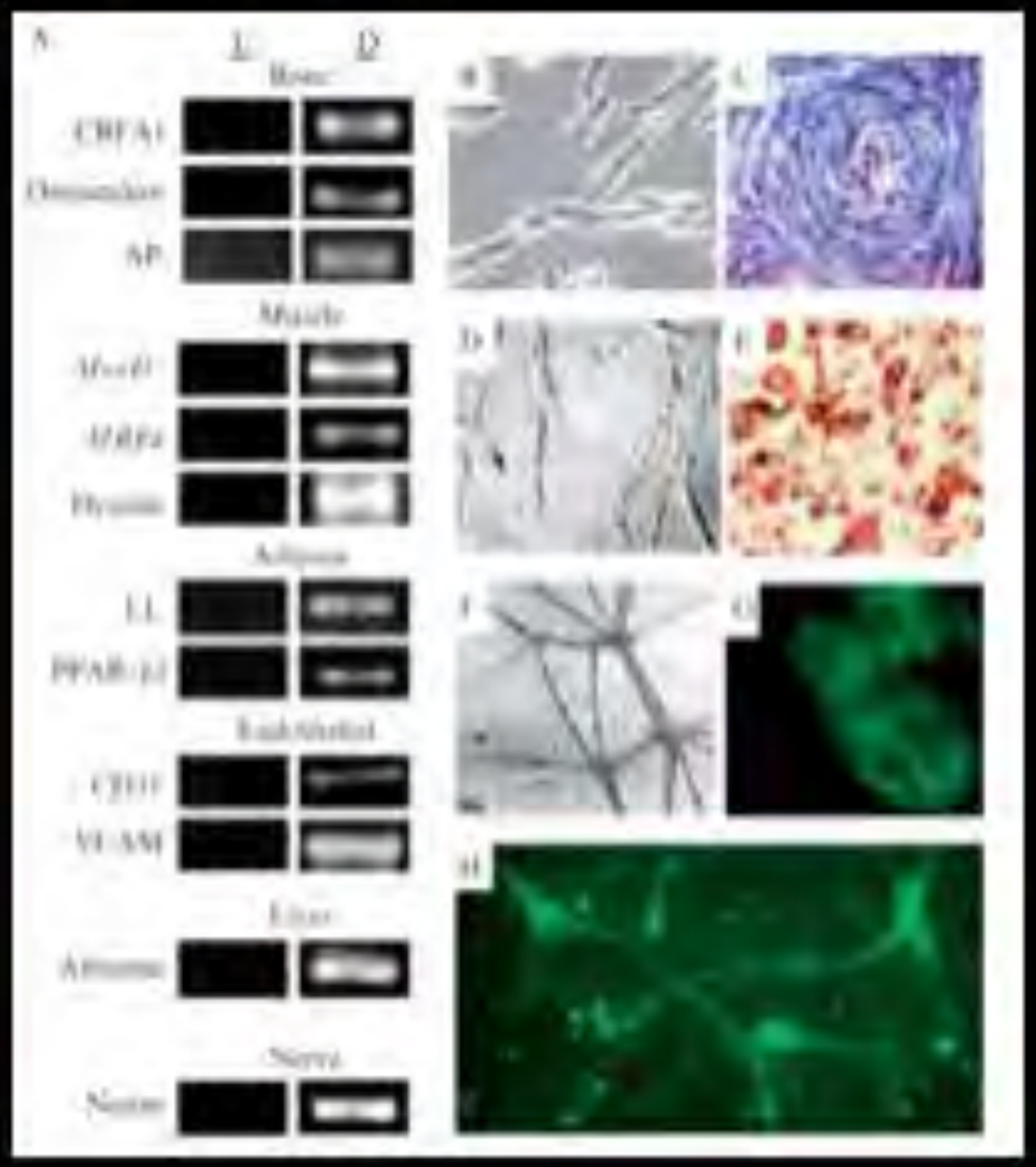
Bone Marrow Derived or Lipoaspirate Derived Adult Stem Cells are Mesenchymal Stem Cells (MSCs).

Amniotic Fluid/Membrane

Amniotic Fluid Cells

Amniotic fluid cells produce high quantities of growth factor.

They have tremendous capacity for the production of growth and anti-inflammatory factors, and can differentiate into multiple tissues including bone, cartilage, and muscle among other cell types



Growth factors found in Amniotic Fluid Concentrate

HGF: Hepatocyte Growth Factor → **Myogenesis, Wound Healing, Organ Regeneration**

EGF: Epidermal Growth Factor → **Cell Growth, Proliferation, Differentiation**

TNF-α: Tumor Necrosis Factor-Alpha → **Apoptosis, Angiogenesis**

GRO-α: Chemokine → **Angiogenesis, Wound Healing**

MCP-1: Monocyte Chemoattractant Protein-1 → **Immune Modulation**

TIMP (1,2,3,4): Tissue Inhibitor of Metalloproteinases (2,3,4) → **Growth Promotion**

IGF-1: Insulin-Like Growth Factor-1

IGF-2: Insulin-Like Growth Factor-2

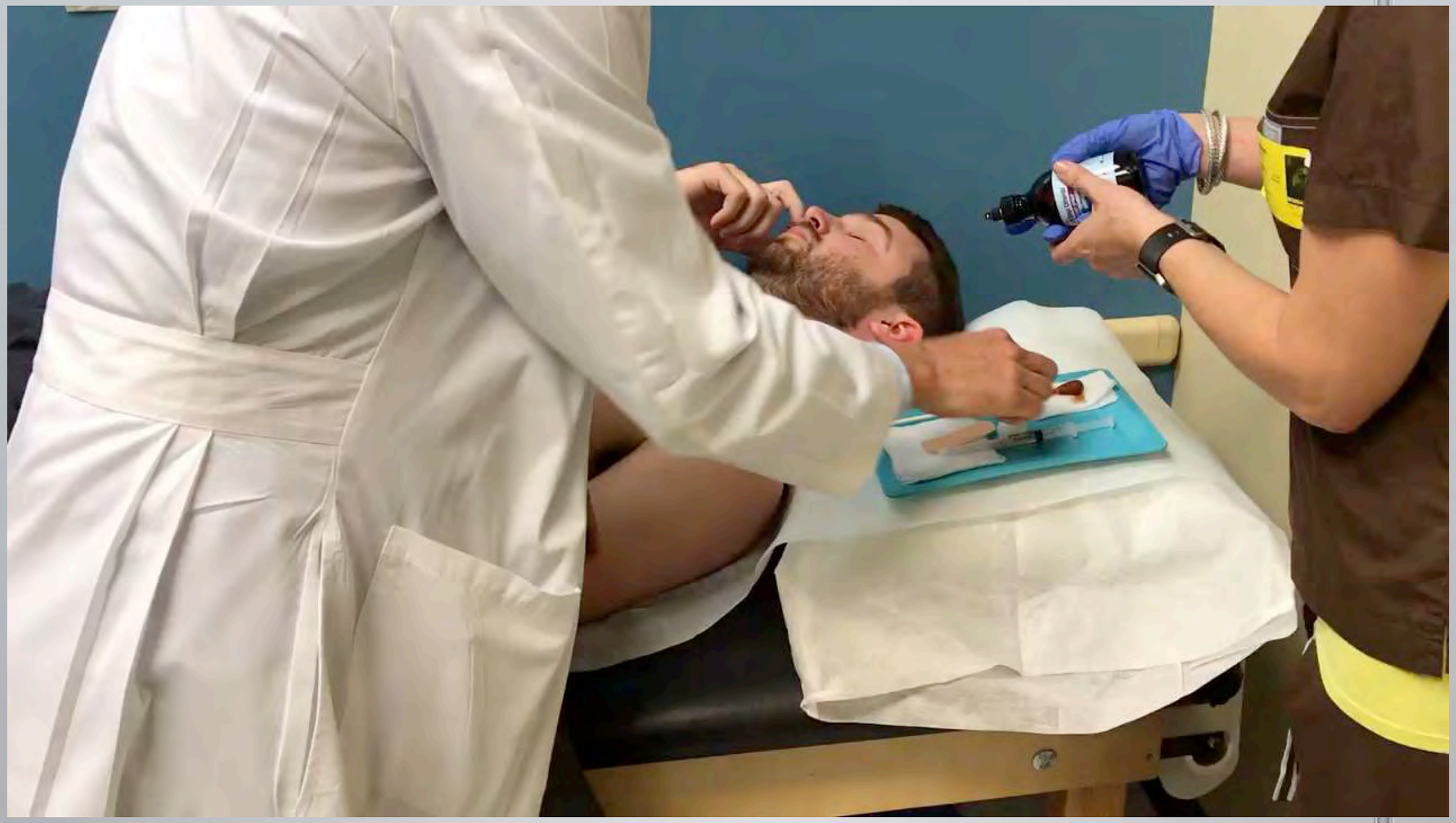
IL1-RA: IL1-Receptor Antagonist => **Anti-inflammatory**

TGF-α: Transforming Growth Factor-Alpha

TGF-β1: Transforming Growth Factor-Beta 1

TGF-β2: Transforming Growth Factor-Beta 2

IL6: Interleukin 6 => **Immune modulation**



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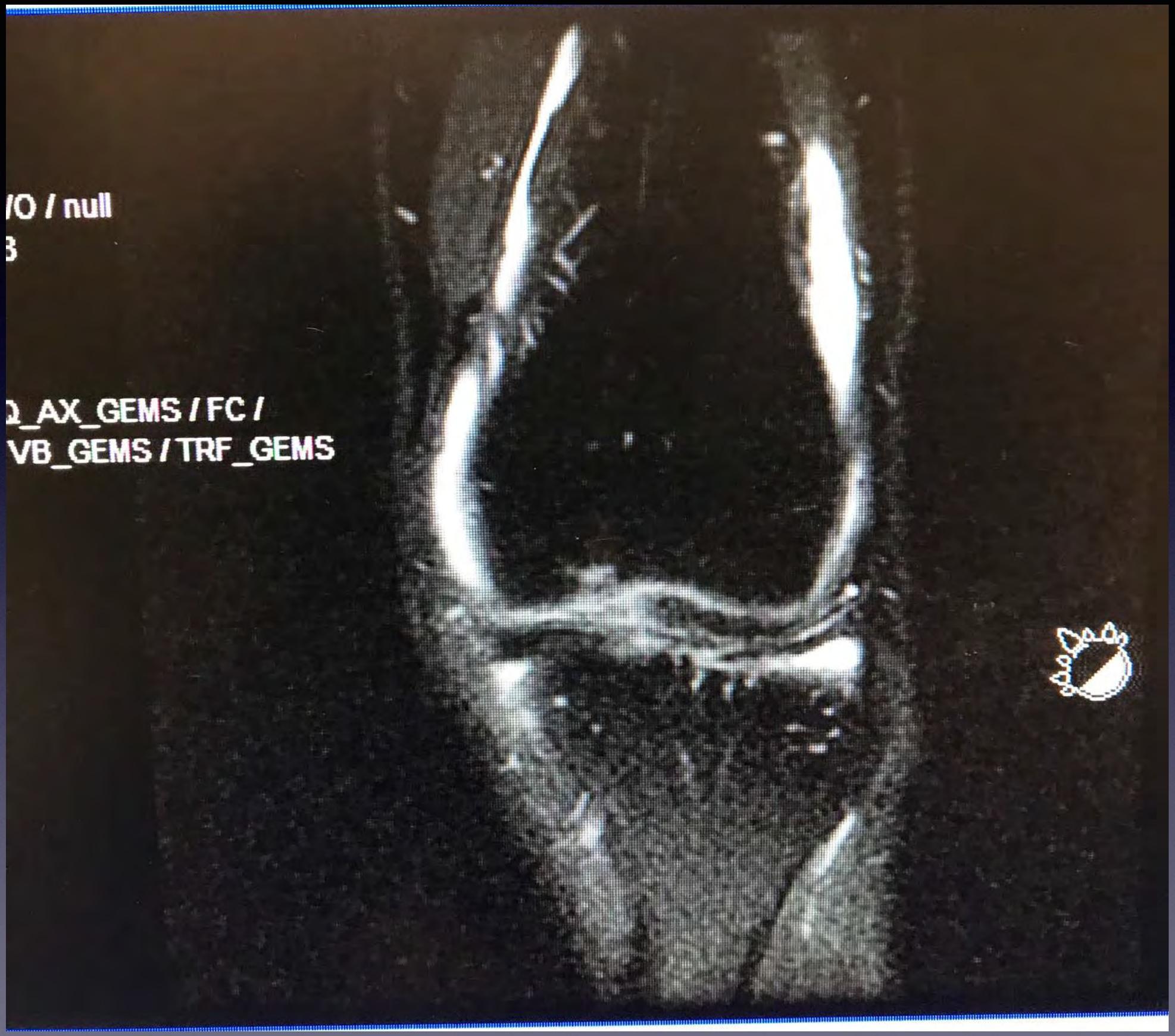
56-y/o with pain s/p PMM

- Prior surgery by outside surgeon (PMM)…continued pain

- No relief with PT/cortisone/meds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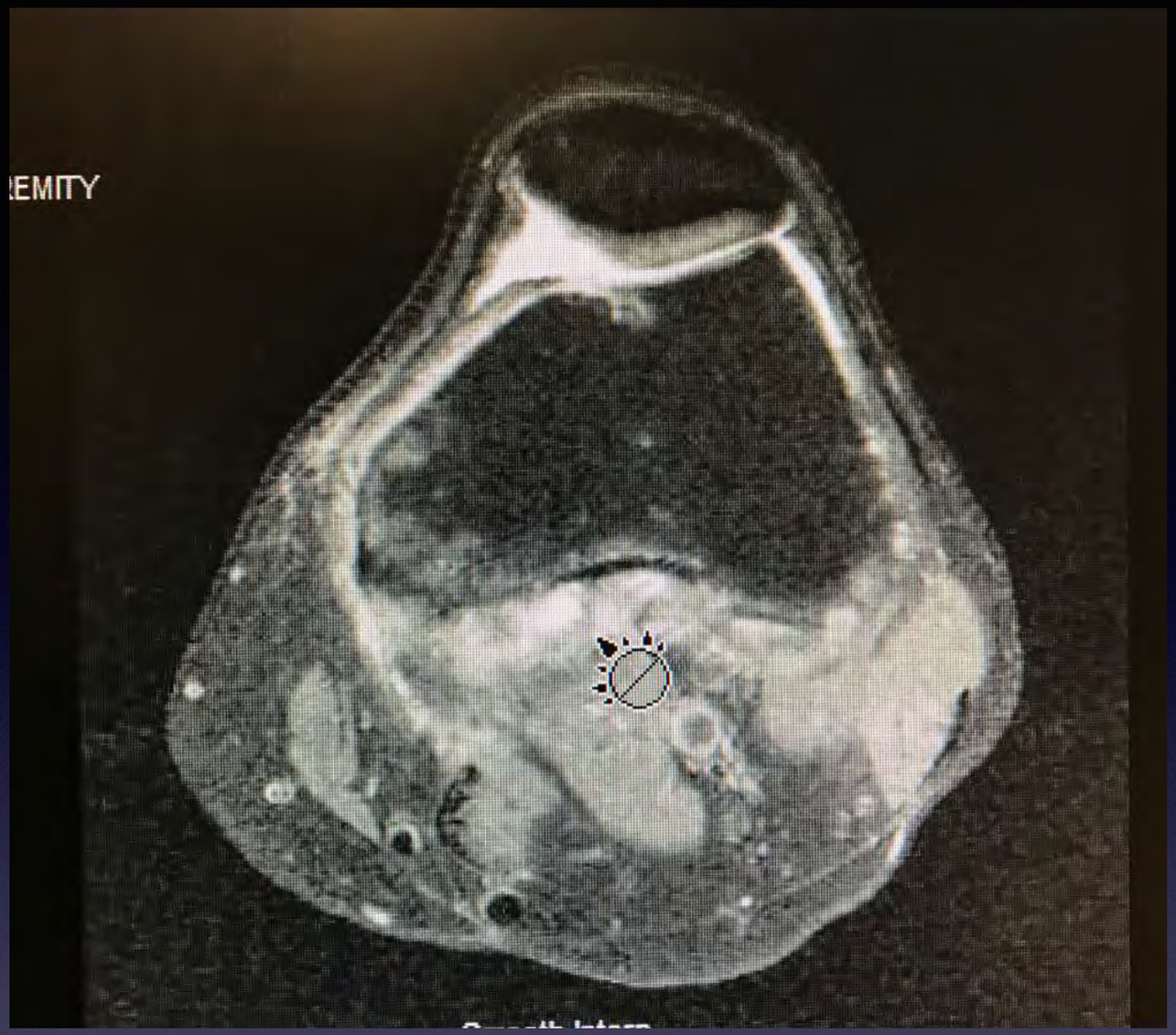
Q_AX_GEMS / FC /
VB_GEMS / TRF_GEMS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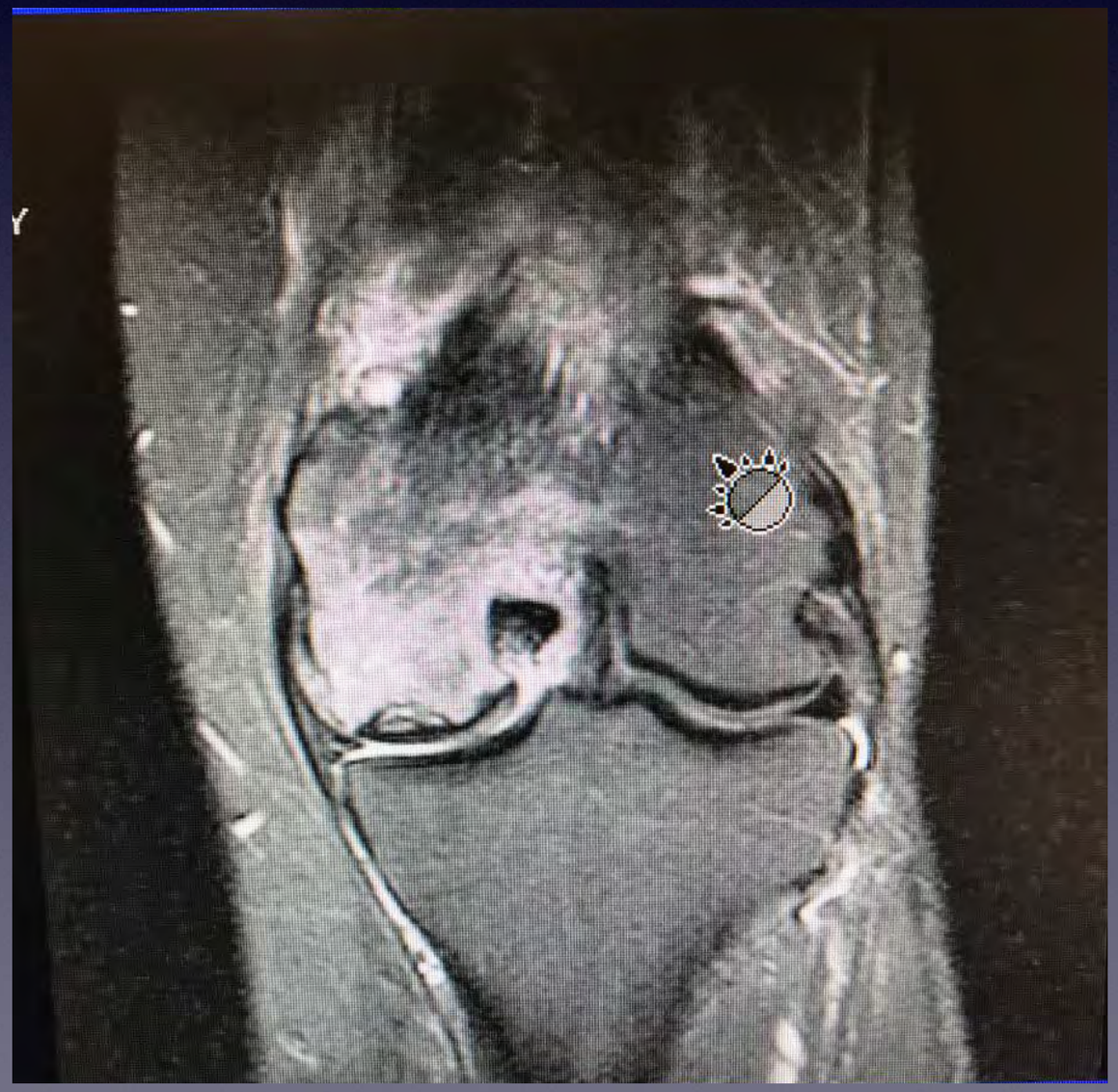
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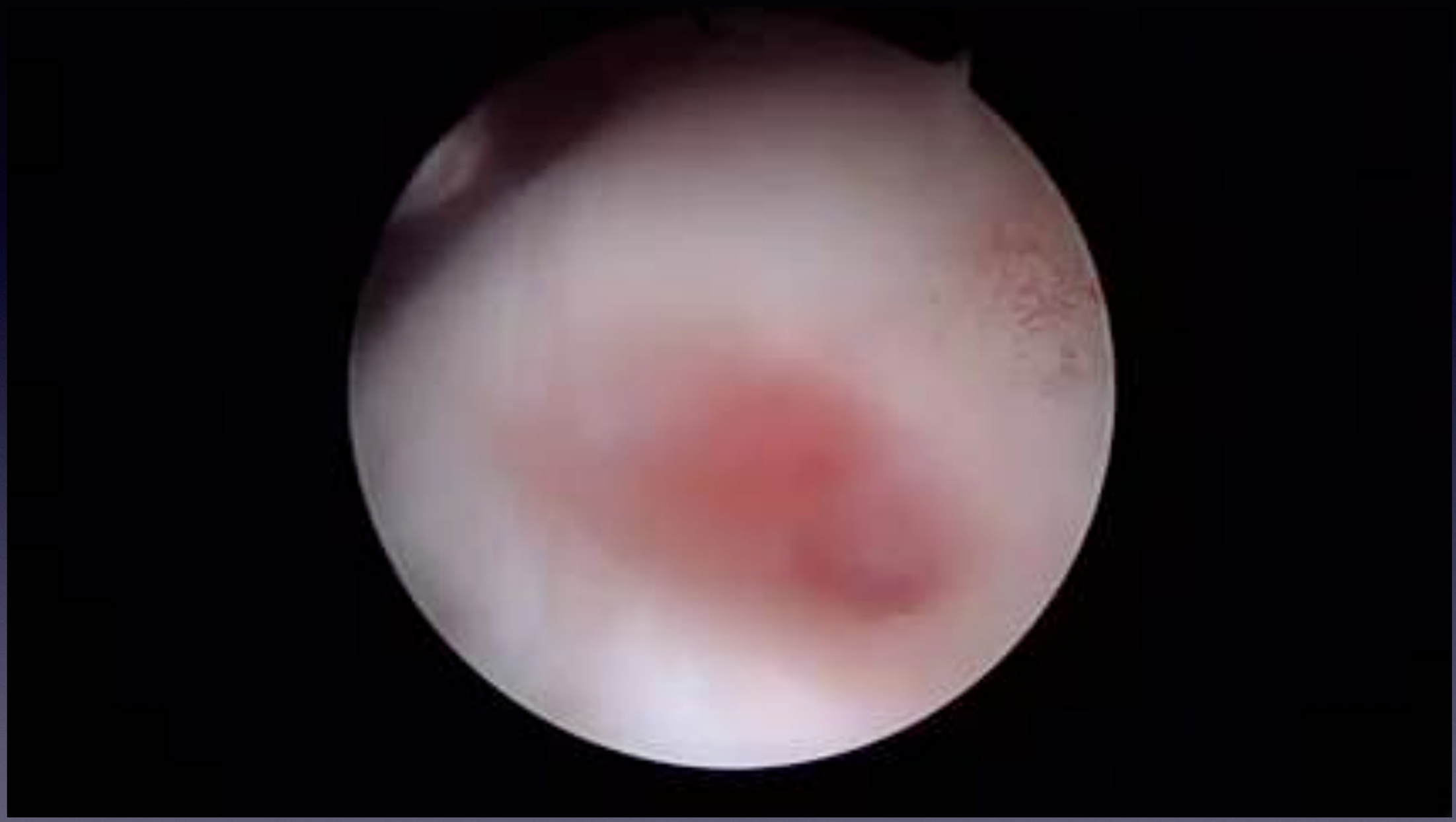

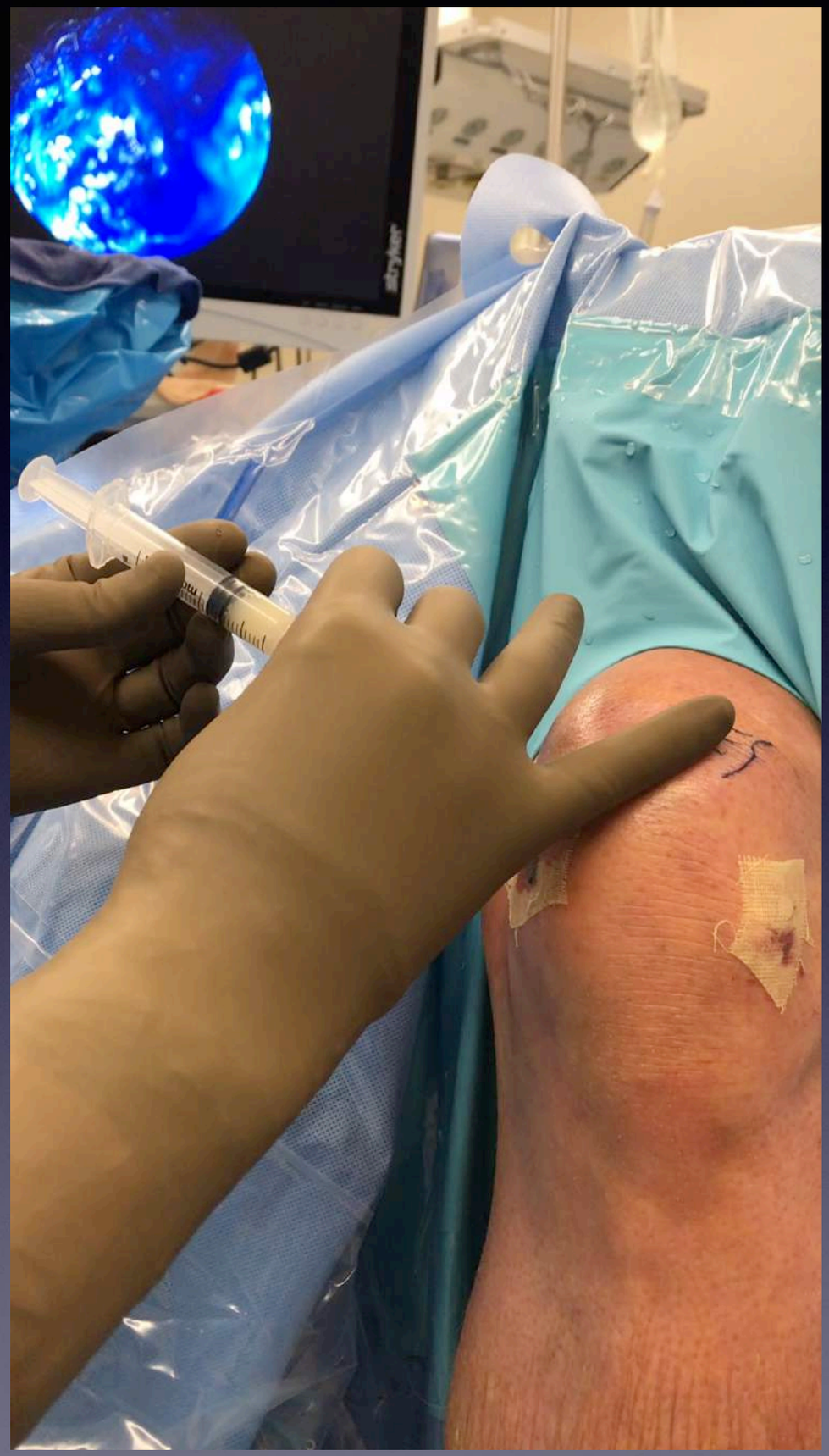

PRP (Platelet-Rich Plasma)

- When soft tissue is injured, platelet cells are delivered to the site of injury
  - These platelets contain growth factors that help to heal the tissue
- PRP therapy amplifies this response
- The platelet-derived growth factors:
  - Increase collagen production
  - Stimulate blood flow
  - Enhance Stem cell proliferation

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Platelet-Rich Plasma

- Growth factors (Alpha granules storage units)
  - Transforming Growth Beta Factor
  - Vascular Endothelial Growth Factor
  - Platelet Derived Growth Factor
  - Epithelial Growth Factor
  - Fibroblast Growth Factor
- Stimulate Cell Replication, angiogenesis, epithelialization, granulation tissue formation, extracellular matrix formation and regulation of bone cell metabolism

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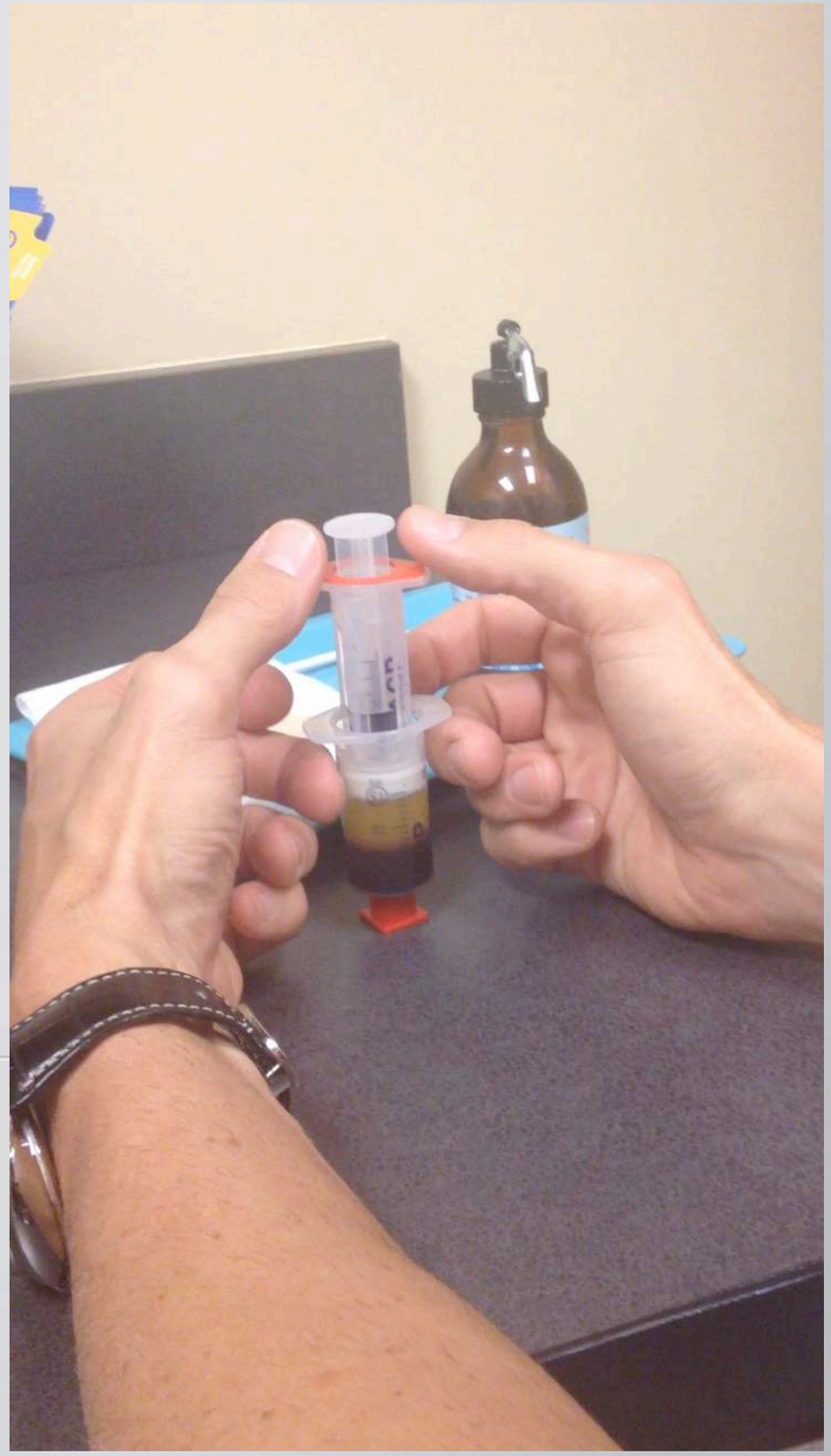
PRP Extraction



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Umbilical Cord Tissue/Stem Cells

- **Stem cells are found in the Wharton's Jelly (smooth muscle cells surrounding the cord vessels)**
- **PolyCyte: growth factors, HA, cytokines**
- **CoreCyte: same + 1,000,000 MSC/cc**
- **Hypoimmunogenic, homologous, younger/higher proliferation**
- ****Predictive Technologies**

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PREDICTIVE BIOTECH

A PREDICTIVE TECHNOLOGY GROUP COMPANY

MEMORANDUM

Subject: Memo on File

From: Doug Schmid, PhD

Date: 01/15/2017

Re: Ohio State University Viability Study

3 samples of varying concentration were sent on dry ice, overnight via FedEx, to Ohio State University for viability testing. Using a dye similar to DAPI, cellular counts and viability were obtained on a Countess II cell counter. The data are as follows:

A. **Sample 1:** (our count was 2 million cells/mL)
   1.82 million total cell count per mL (average of 2 counts), 78% viability
B. **Sample 2:** (our count was 5 million cells/mL)
   4 million total cell count per mL (average of 2 counts), 73% viability
C. **Sample 3:** (our count was 500,000 cells/mL)
   555,000 total cell count per mL (average of 2 counts), 64% viability

In storing cells, cellular concentration is a key component of viability-if the cell density is too low (less than 1 million cells per mL) or too high (more than 10 million cells per mL), cellular viability upon thaw will be affected. These numbers can vary by cell type; some cell types can survive the freeze/thaw cycle better than others.

From these viability measurements (and others made in our lab), we have determined that the optimal freezing density for the CoreCyte™ product is 2 million cells/mL. This concentration consistently yields approximately 80% viability and at least 1 million cells/mL in the freshly thawed product. These numbers were again confirmed in-house.

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Cat#	Vendor	Antibody	Fluor	ul/test
11-0909-41	ebioscience	CD 90	FITC	2.5ul
11-4714-41	ebioscience	IgG	FITC	2.5ul
12-1057-41	ebioscience	CD105	PE	2.5ul
12-4714-41	ebioscience	IgG	PE	5ul
25-9459-41	ebioscience	CD45	PECY7	2.5ul
25-4714-41	ebioscience	IgG	PECY7	2.5ul
17-0739-41	ebioscience	CD73	APC	2.5ul
17-4714-41	ebioscience	IgG	APC	0.625
		viability	DAPI	5ul

Reagents

Wash Buffer- 2.5%BSA in PBS (Core)

ASSAY RESULTS

Fresh Sample ID

Excluding RBC contamination, cellularity consisted of 9.7X10^5 viable cells per mL. Viability based on exclusion of DAPI equals 67.6%. Cellular phenotype consisted of:
CD90= 59.2%
CD105= 55.4%
CD73= 86.7%
CD45= 3.19%
Total Viable Meschencymal Stem Cell Count: 1,200,000 per mL. (This number is an approximation based on the use of bead markers. There is to be expected normal cell loss based on the steps to process the sample before Flow Cytometry testing. The 1,200,000 per mL number is the cell count that remains after testing protocol preparations.)

Frozen Sample ID

Excluding RBC contamination, cellularity consisted of 7.8X10^5 viable cells per mL. Viability based on exclusion of DAPI equals 43.1%. Cellular phenotype consisted of:
CD90= 28.0%
CD105= 35.1%
CD73= 84.4%
CD45= 4.06%
Total Viable Meschencymal Stem Cell Count: 800,000 per mL. (This number is an approximation based on the use of bead markers. There is to be expected normal cell loss based on the steps to process the sample before Flow Cytometry testing. The 800,000 per mL number is the cell count that remains after testing protocol preparations.)

James Marvin
Director, Flow Cytometry SRL

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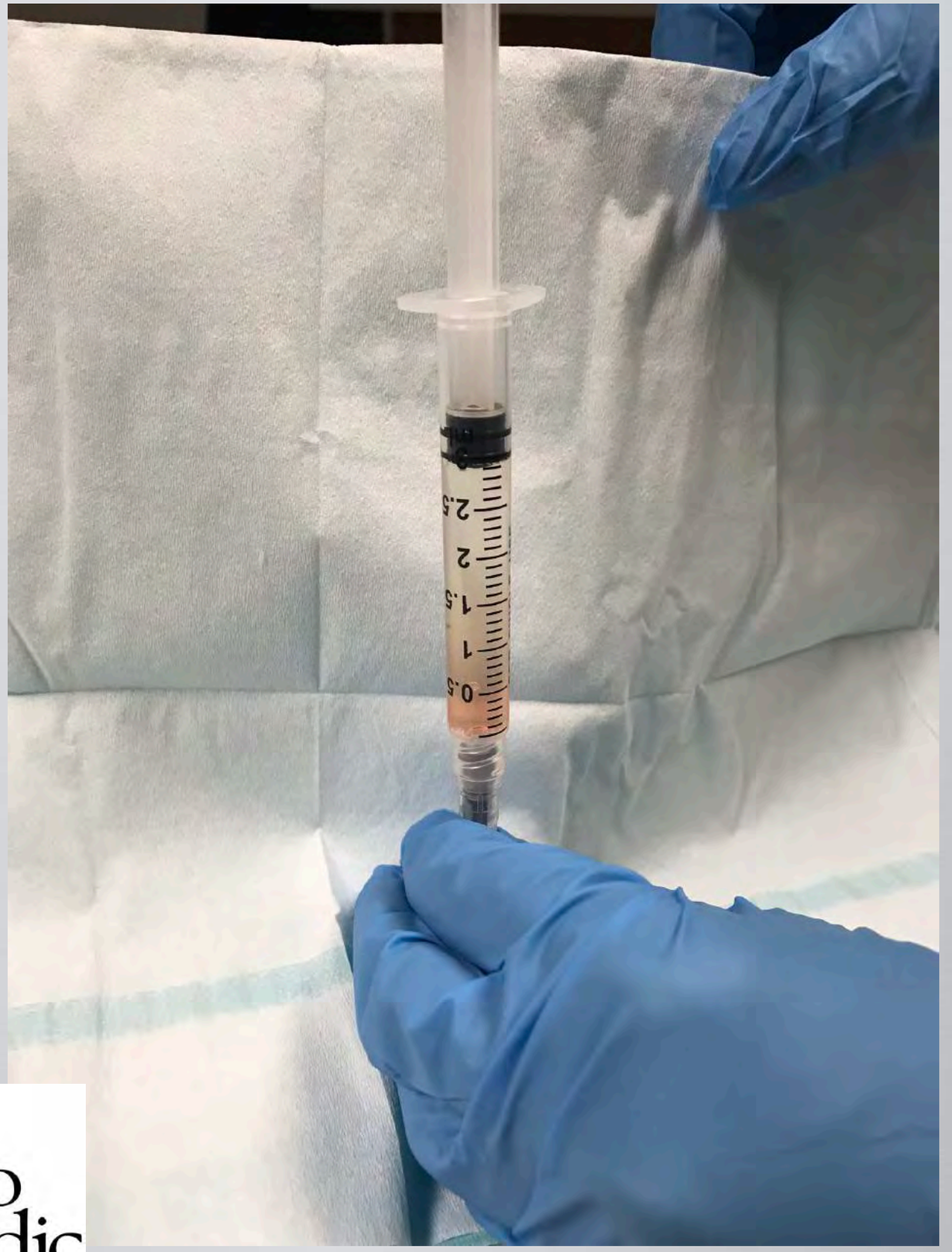

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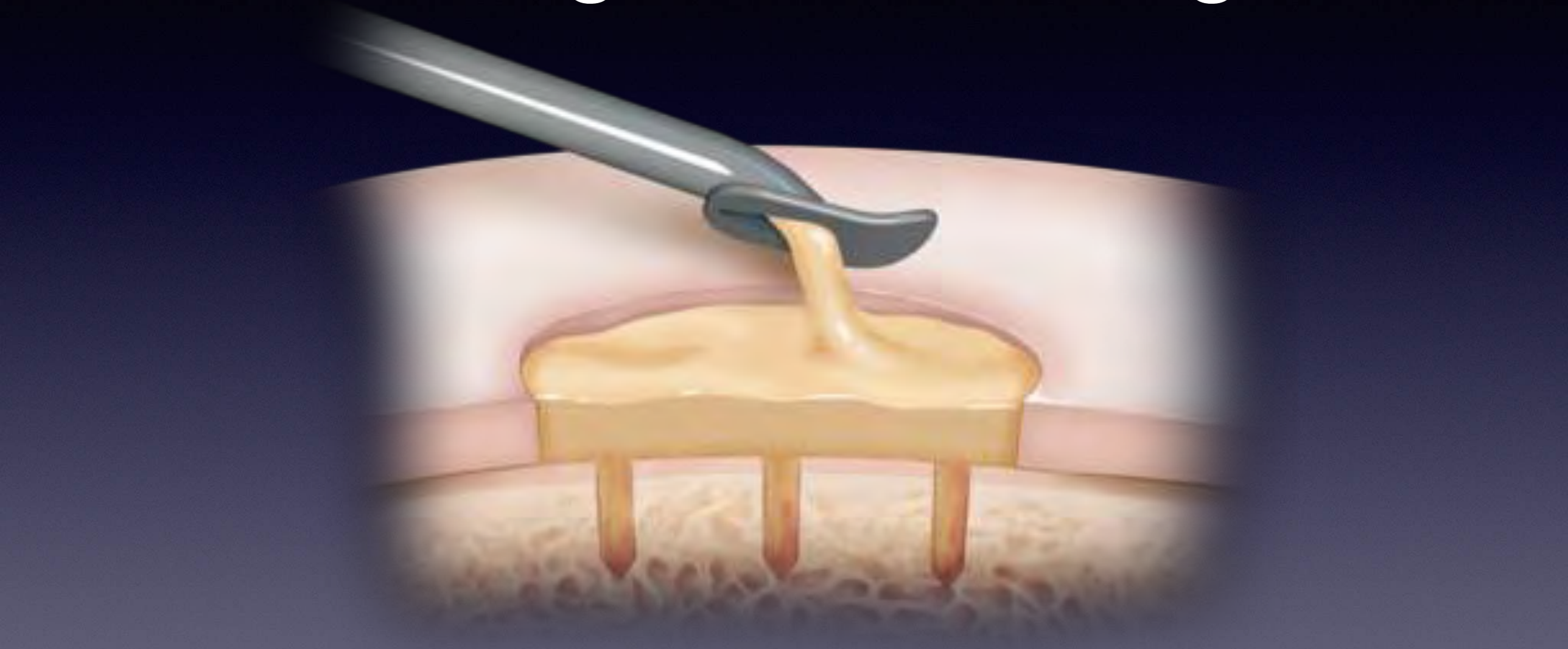
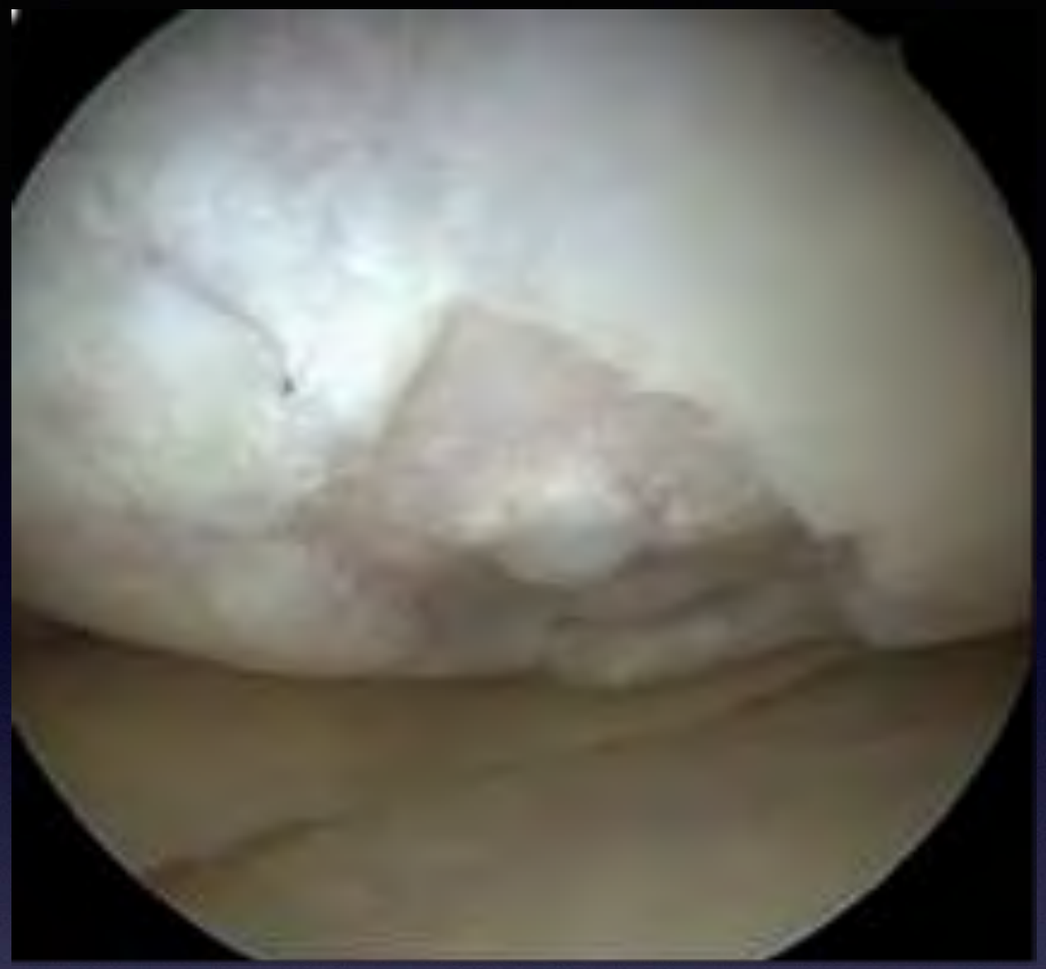
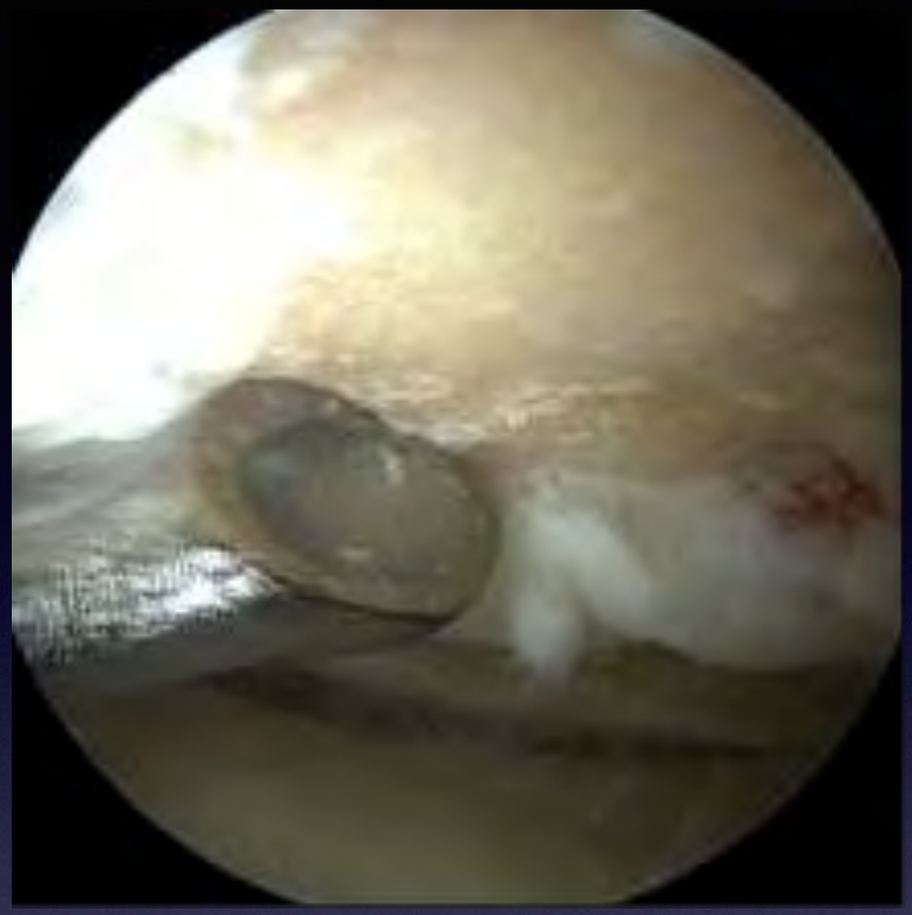
Micronized Allogeneic Cartilage- BioCartilage

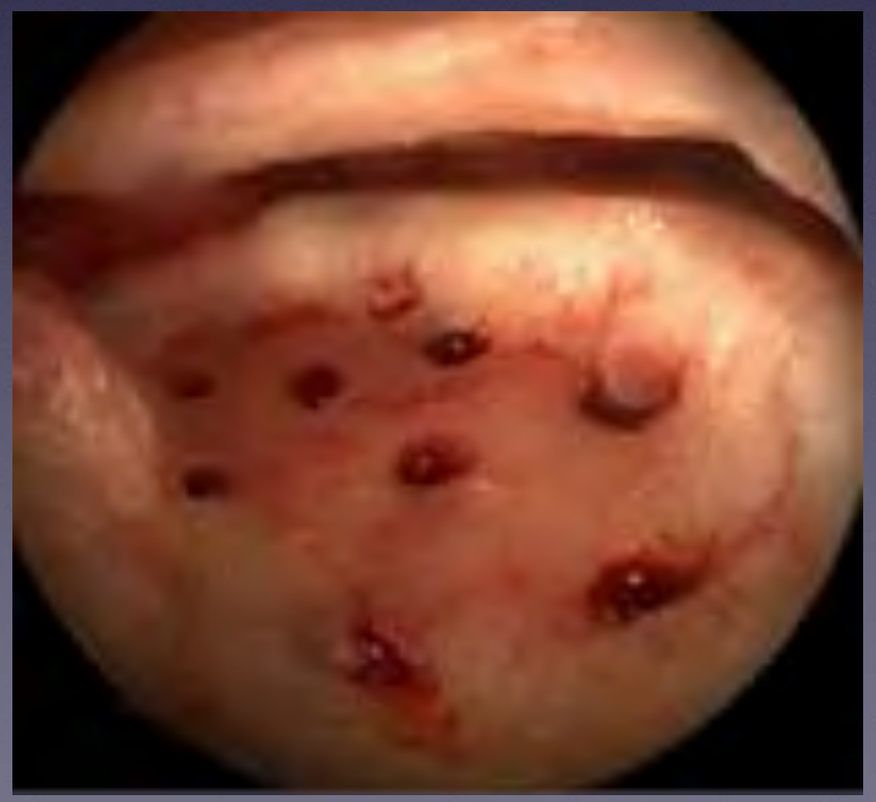


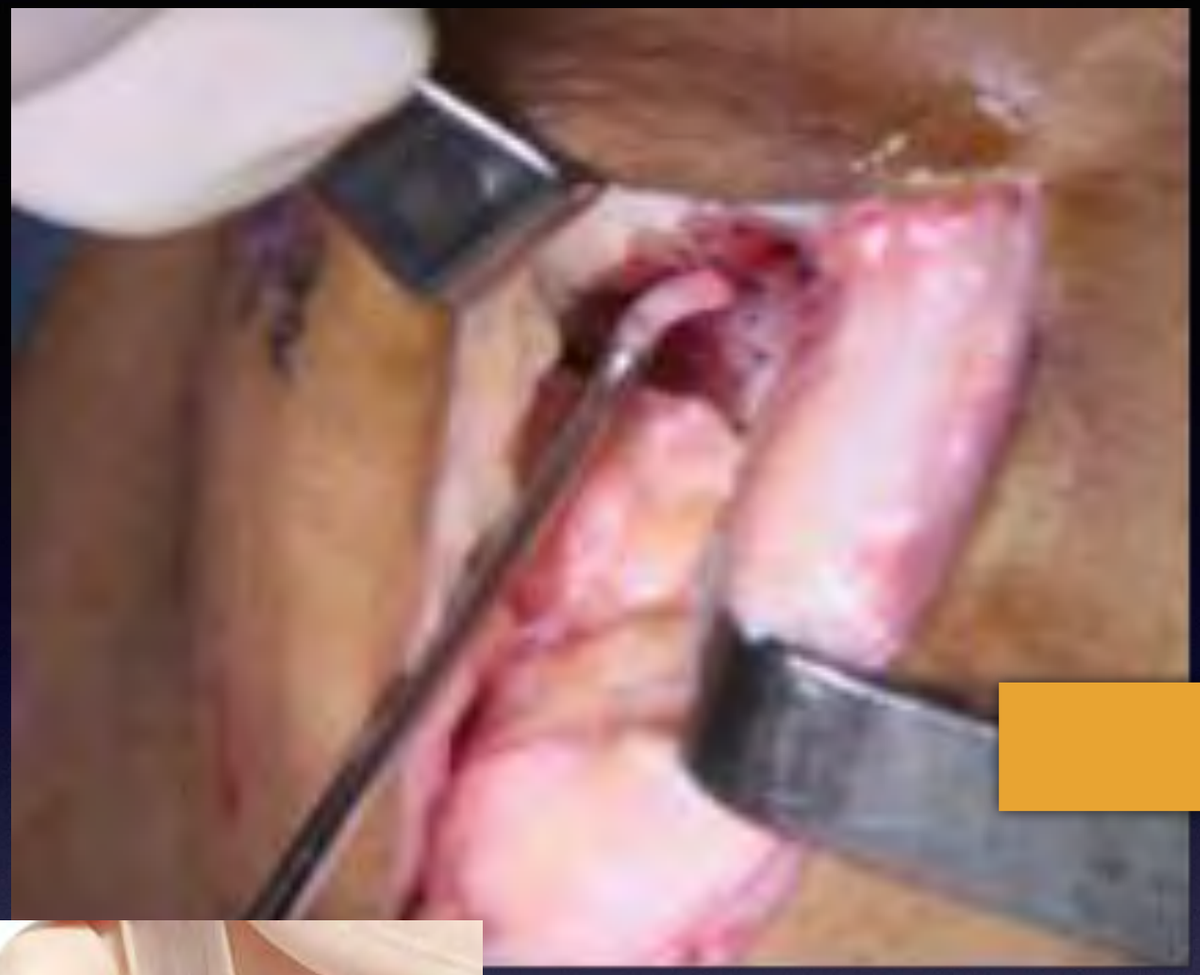
BioCartilage

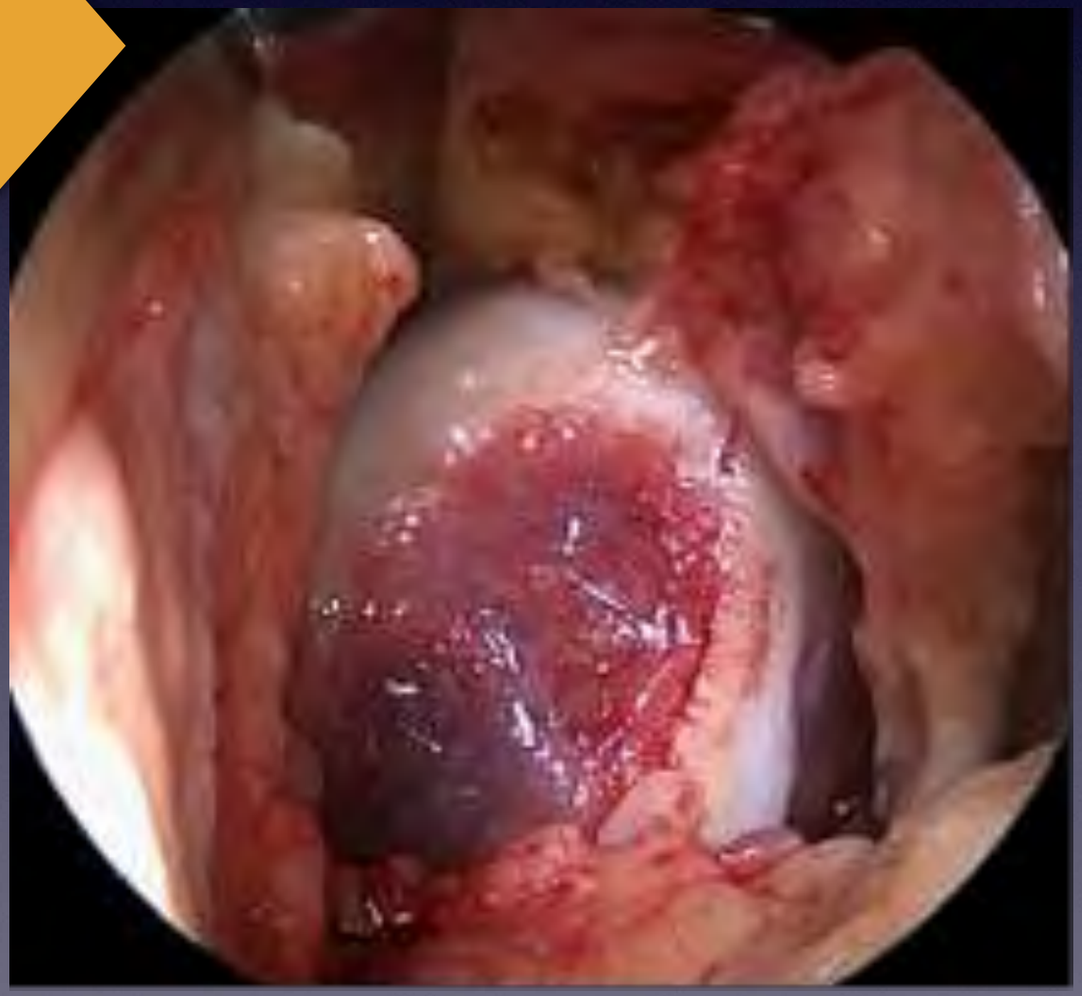
- Allograft cartilage with extracellular matrix (Type 2 Collagen, proteoglycans, growth factors)
- Serves as a scaffold over the Microfractured defect
- More closely resembles native hyaline cartilage than microfx alone (AJSM, 2016)



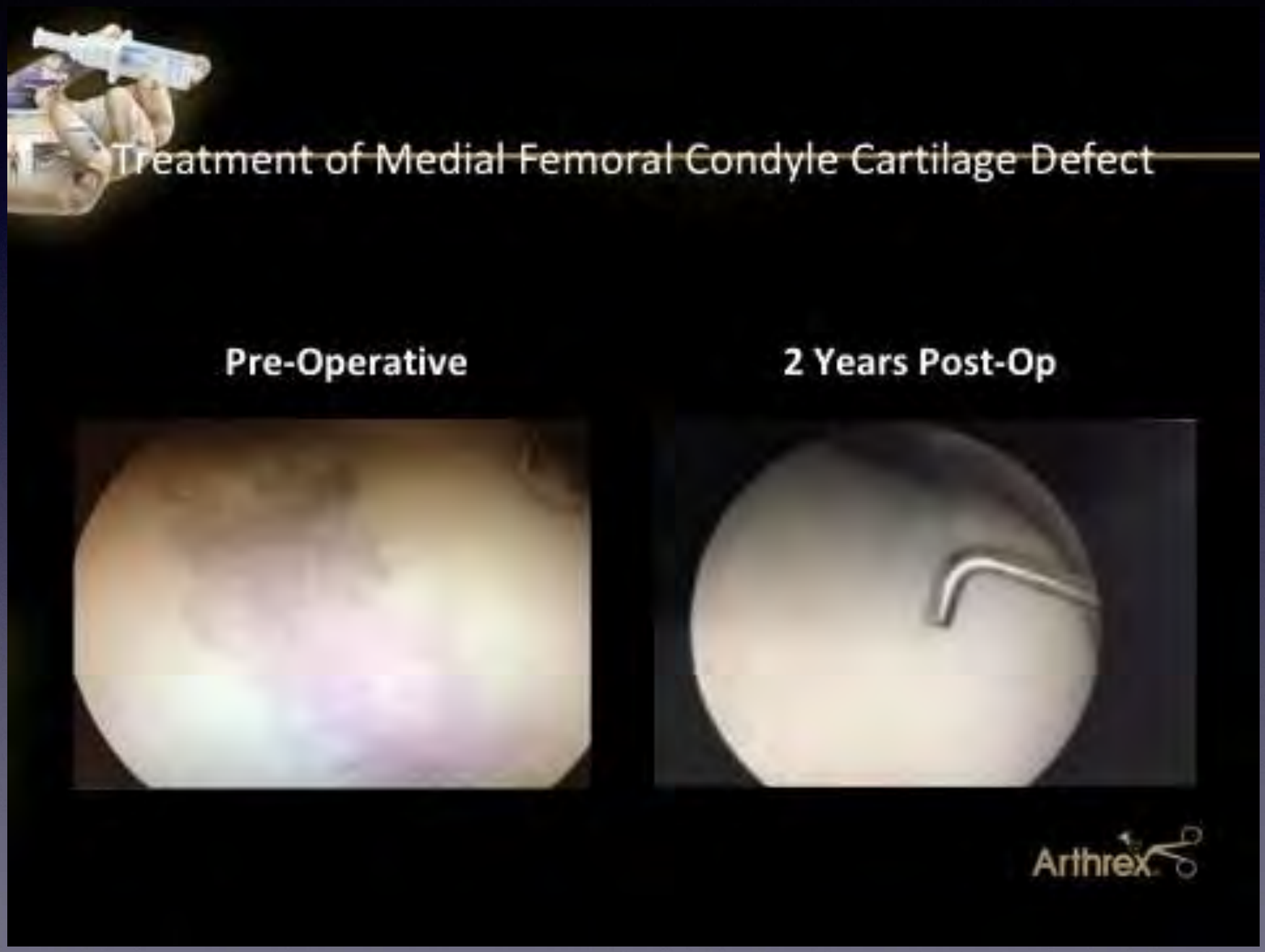








BioCartilage

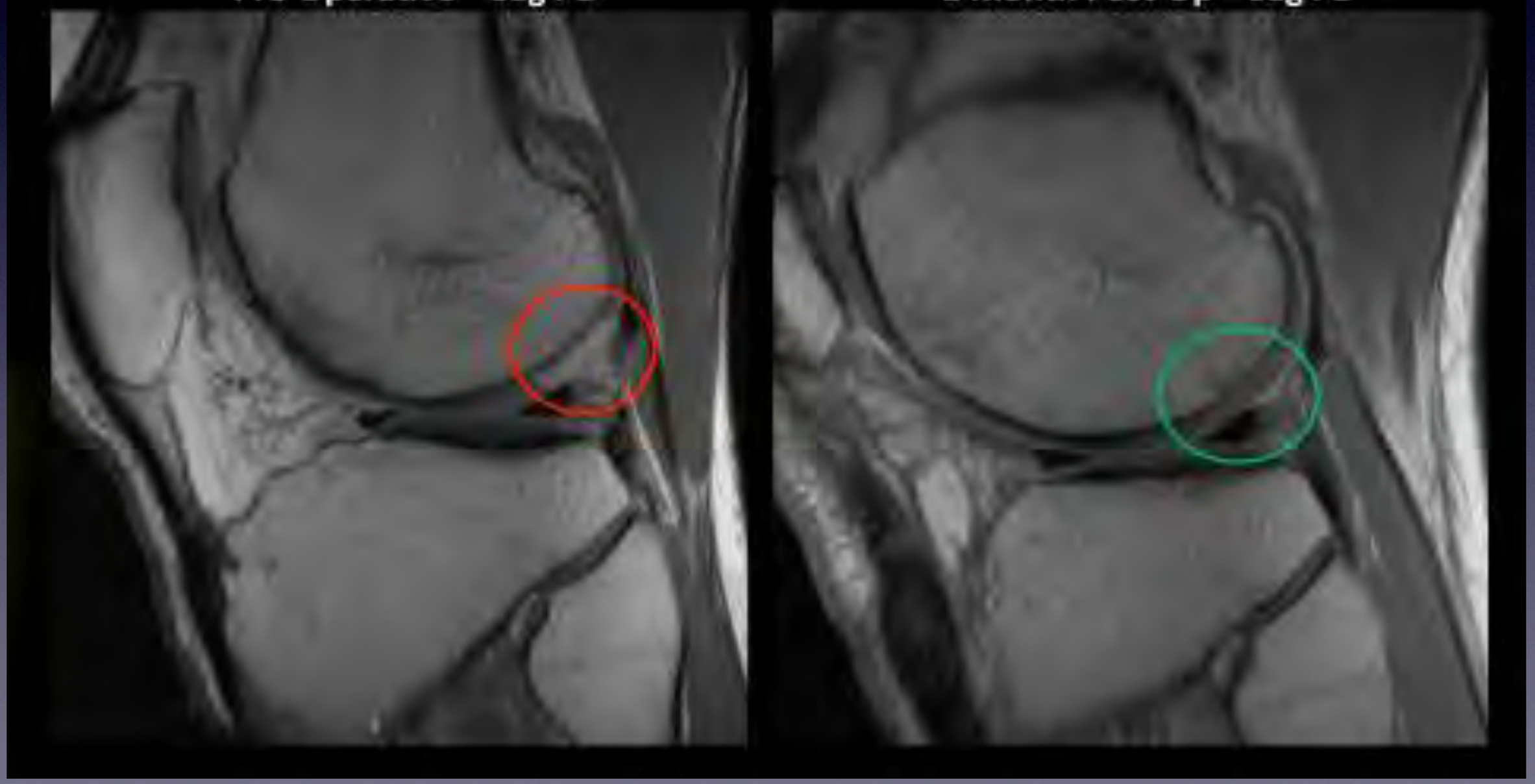


BioCartilage™

Treatment of Lateral Femoral Condyle Cartilage Defect

Pre-Operative – Sag PD

1 Month Post-Op – Sag PD

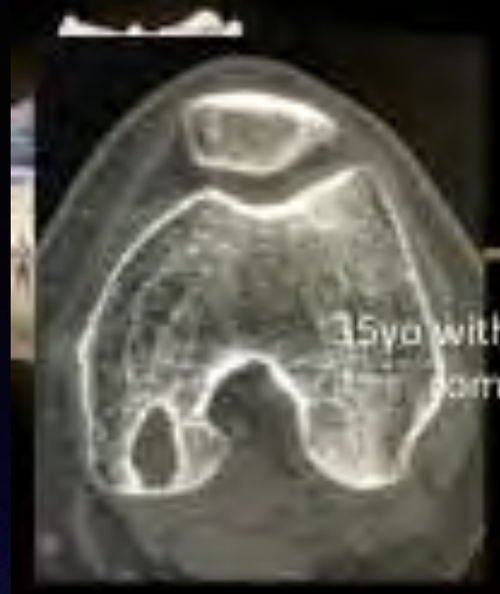


BioCartilage™

Treatment of Lateral Femoral Condyle Cartilage Defect

Pre-Operative – Sag T2 FS

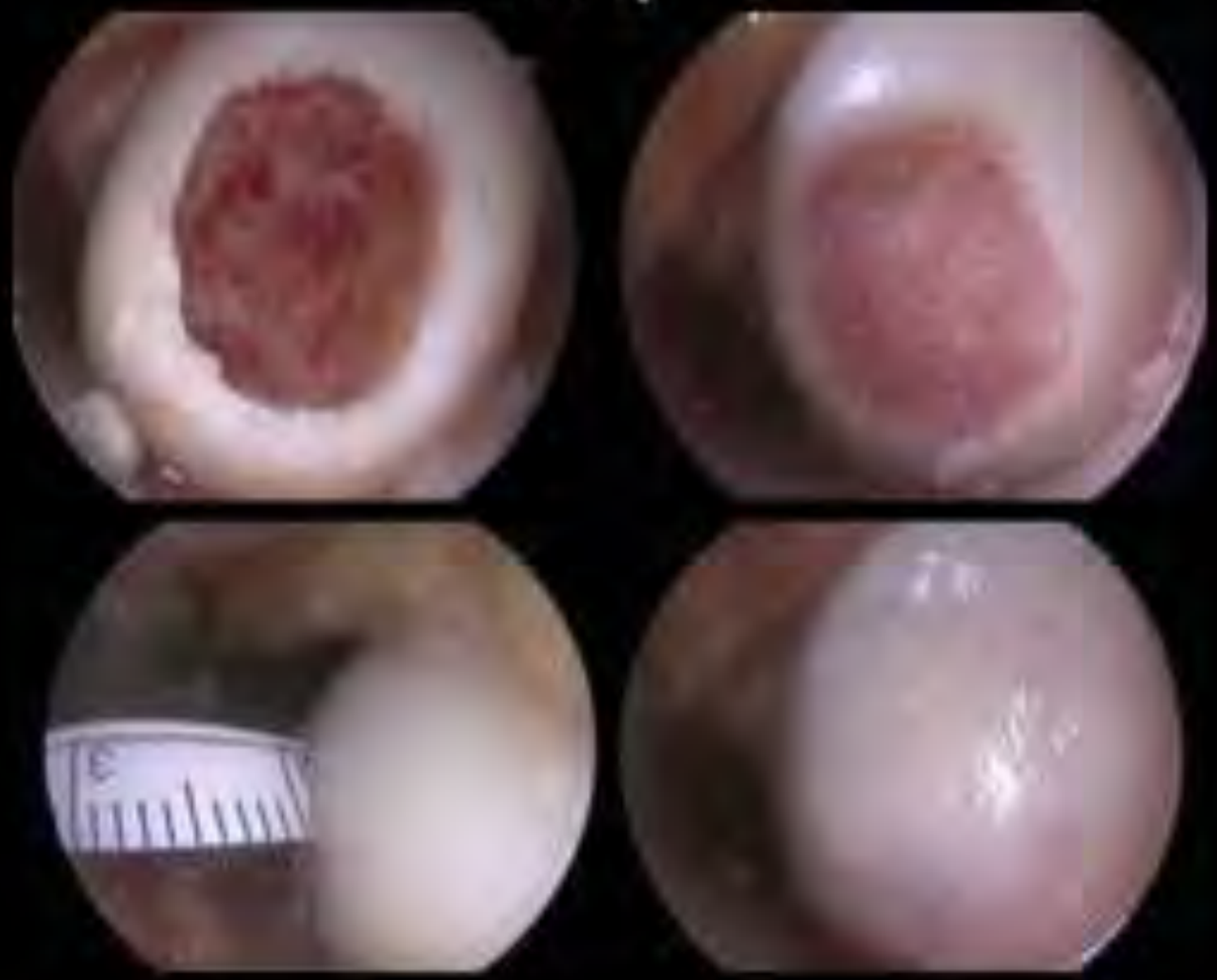
1 Month Post-Op – Sag T2 FS



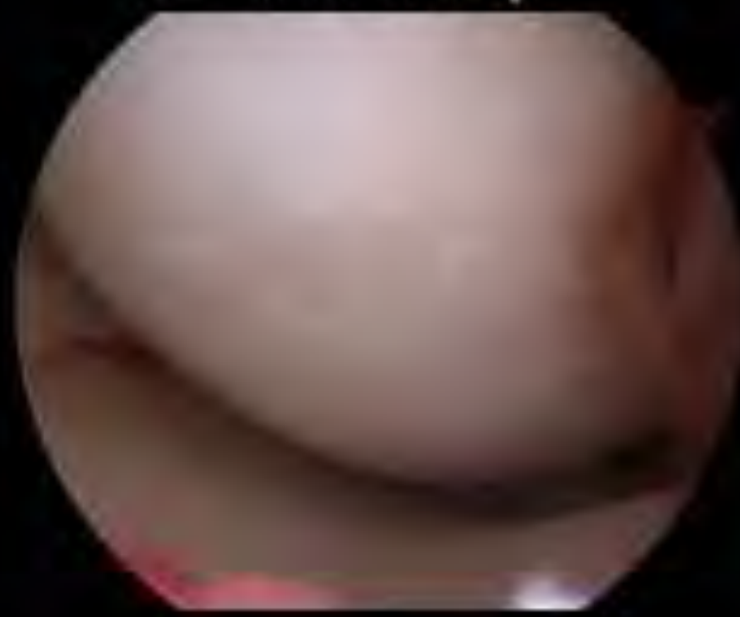
MFC

35yo with large cyst of MFC. Autologous bone graft topped with BioCartilage + ACP. Non-compliant patient, patient became stiff. 3mo post-op, manipulation performed.

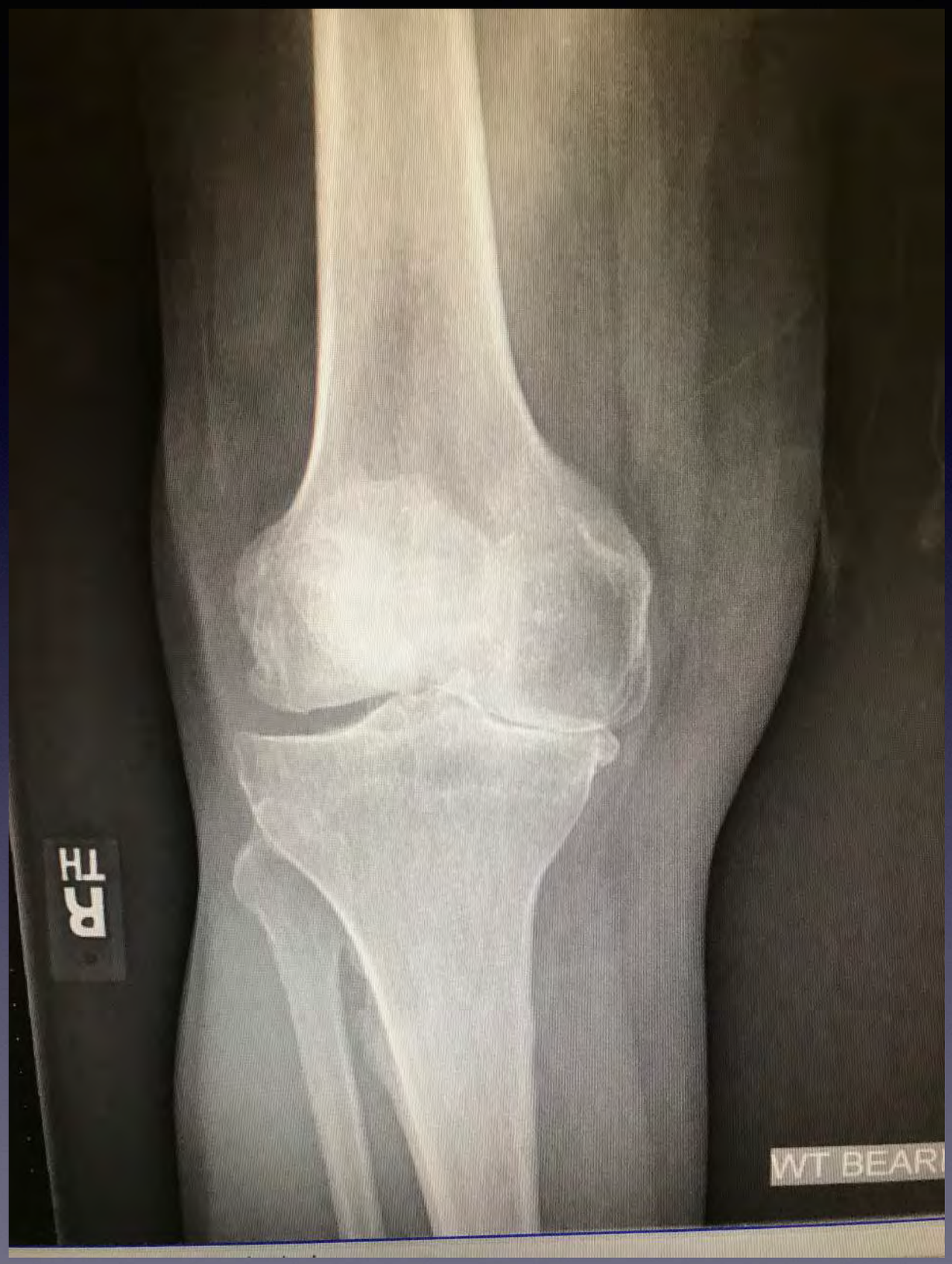
Intra-op



3mo Post-op



Arthrex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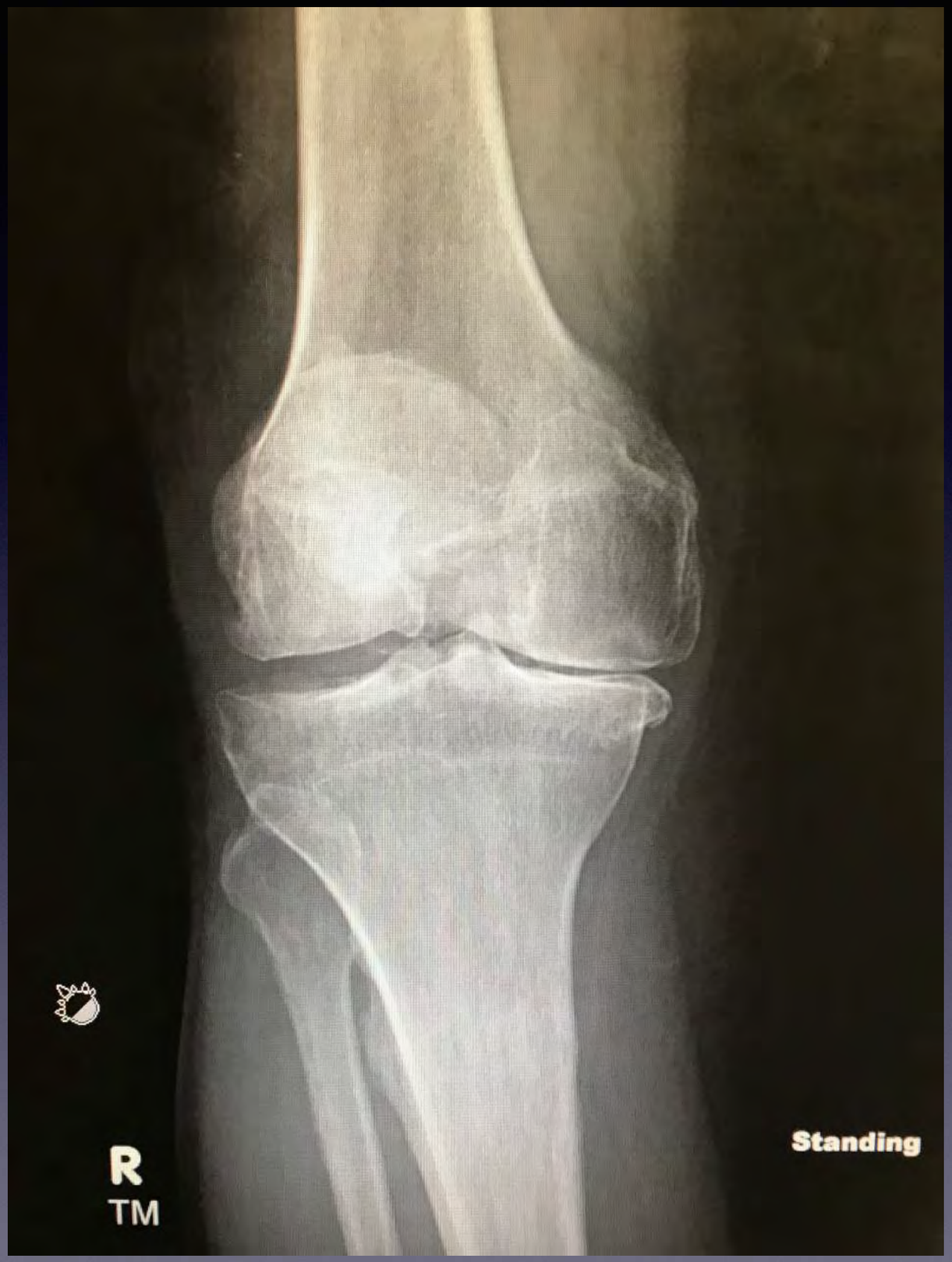
R
TM
Standing

Thank-you

Orlando Orthopædic Center

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