



Modern Indications for the Use of Cellular Therapy: Allogenic Tissue

(Amnion/Placenta/Cord/Exosomes)

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Disclosures

Keralink International

Parcus Medical

Cellular Biomedicine Group

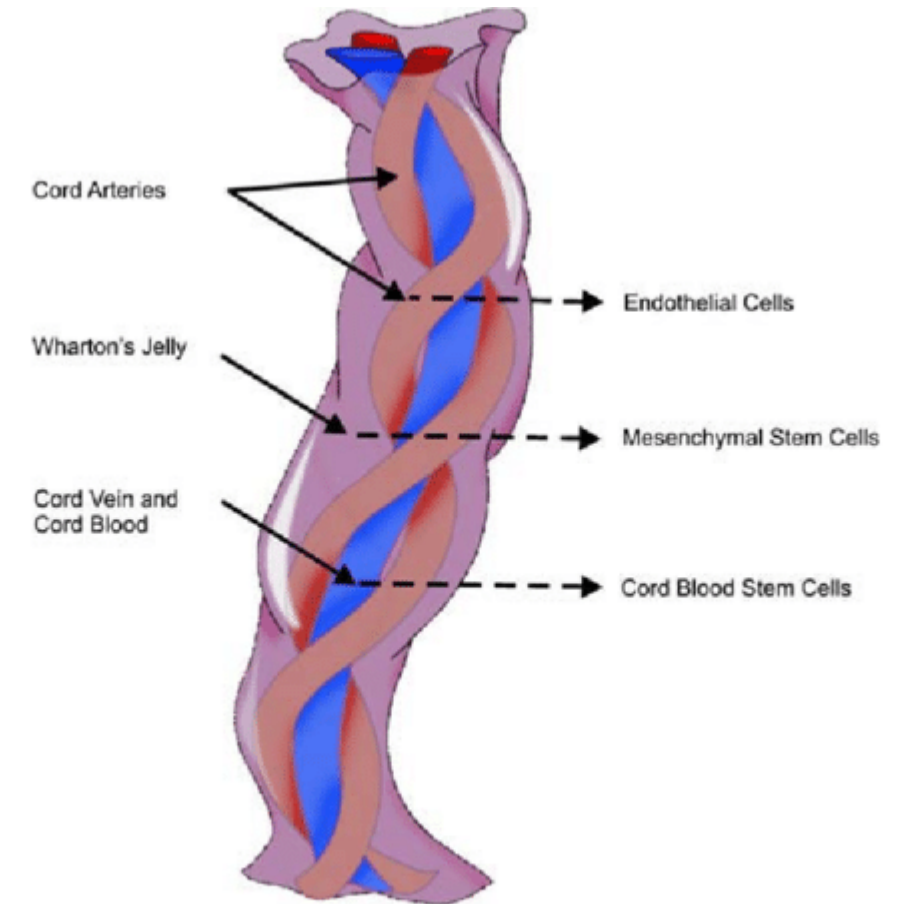
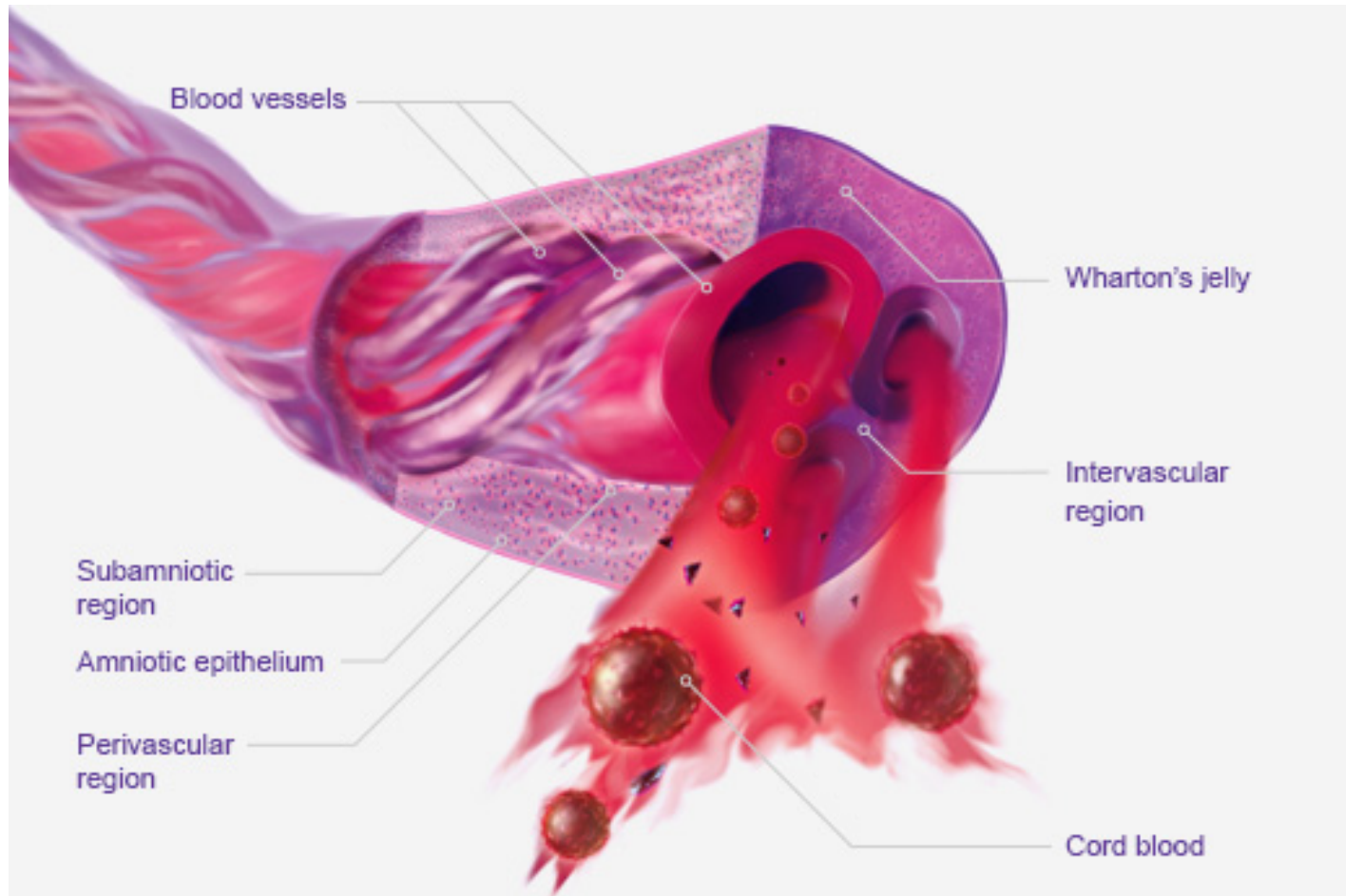
CarthroniX

Inheritor Cell Technology USA

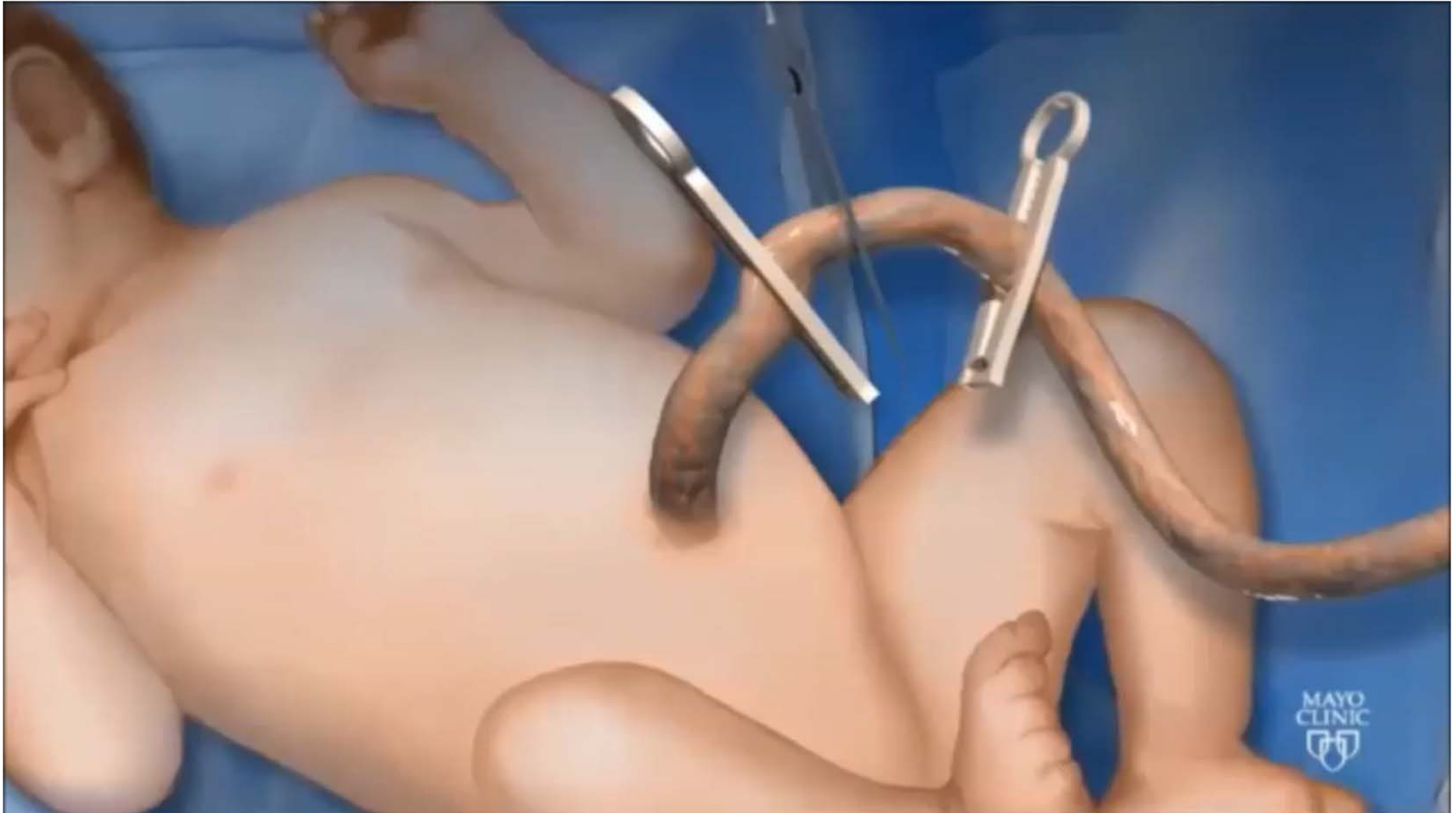
This research has been supported by a generous gift by the Wilton Webster Foundation



Anatomy of the Umbilical Cord



Harvesting Cord Blood





Clinical Issues Placenta Tissue

The American Journal of Sports Medicine, Vol. 44, No. 9
DOI: 10.1177/0363546515612750
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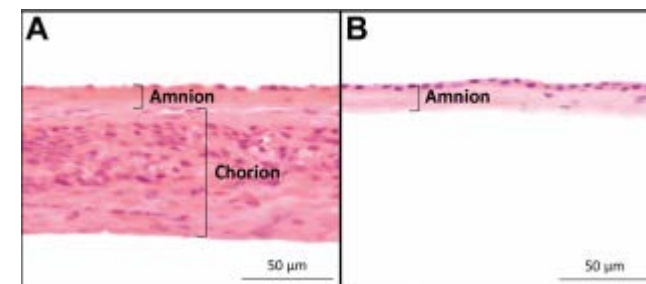
Human Amniotic Membrane–Derived Products in Sports Medicine

2015

Jonathan C. Riboh,^{*†} MD, Bryan M. Saltzman,[†] MD,
Adam B. Yanke,[†] MD, and Brian J. Cole,[†] MD, MBA

*Investigation performed at Rush University Medical Center, Division of Sports Medicine,
Chicago, Illinois, USA*

- As of February 2015, there were 14 companies or distributors that provide commercially available AM-derived products
- Current body of evidence is heavily biased toward in vitro and animal studies, with little to no human clinical data
- 40 ongoing clinical trials investigating the use of AM (3 in the field of orthopaedics)





Placental Tissue

The American Journal of Sports Medicine



The American Orthopaedic
Society for Sports Medicine®

5.673 Impact Factor 5.673
5-Year Impact Factor 6.255
[more »](#)

The Placenta: Applications in Orthopaedic Sports Medicine

[James Alexander McIntyre](#), BA, [Ian A. Jones](#), BA, [Alla Danilkovich](#), PhD, [C. Thomas Vangsness](#), MD§

First Published April 4, 2017 | Research Article

- Minimal published reports
- Lack of clinical data
- Level V Evidence





TABLE 2
Literature Review Results^a

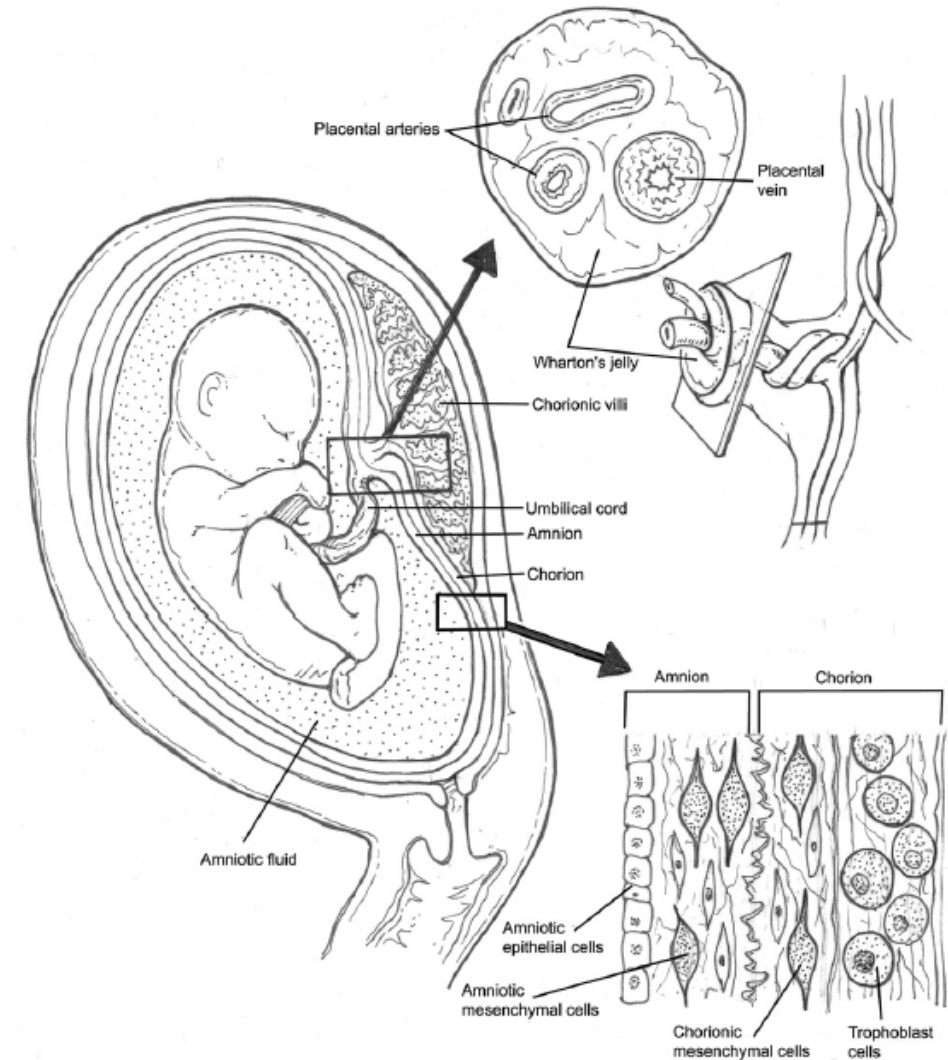
Tissue Type	Indication							Total
	Orthopaedic	Sports Medicine	Musculoskeletal Injury	Knee	Shoulder	Elbow	Ankle	
Placenta	68	21	21	34	2	131	160	437
Wharton's jelly	7	0	1	3	0	11	18	40
Amnion	3	1	0	1	0	4	2	11
Amniotic membrane	36	2	1	8	5	1	15	68
Amniotic fluid	23	3	8	18	2	75	55	184
Chorion	60	1	4	34	1	57	111	268
Chorionic membrane	8	0	0	2	1	1	4	16
Umbilical cord	4	0	1	1	1	4	11	22
Umbilical cord blood	4	1	7	6	2	24	18	62
Total	213	29	43	107	14	308	394	1108

^aThe literature search was conducted by selecting 1 term from the indication group, followed by 1 term from the tissue type group. Values represent the total number of publications obtained using the 2 search terms.

- The majority of placenta tissue allografts available contains no living cells because of processing, preservation, and sterilization methods

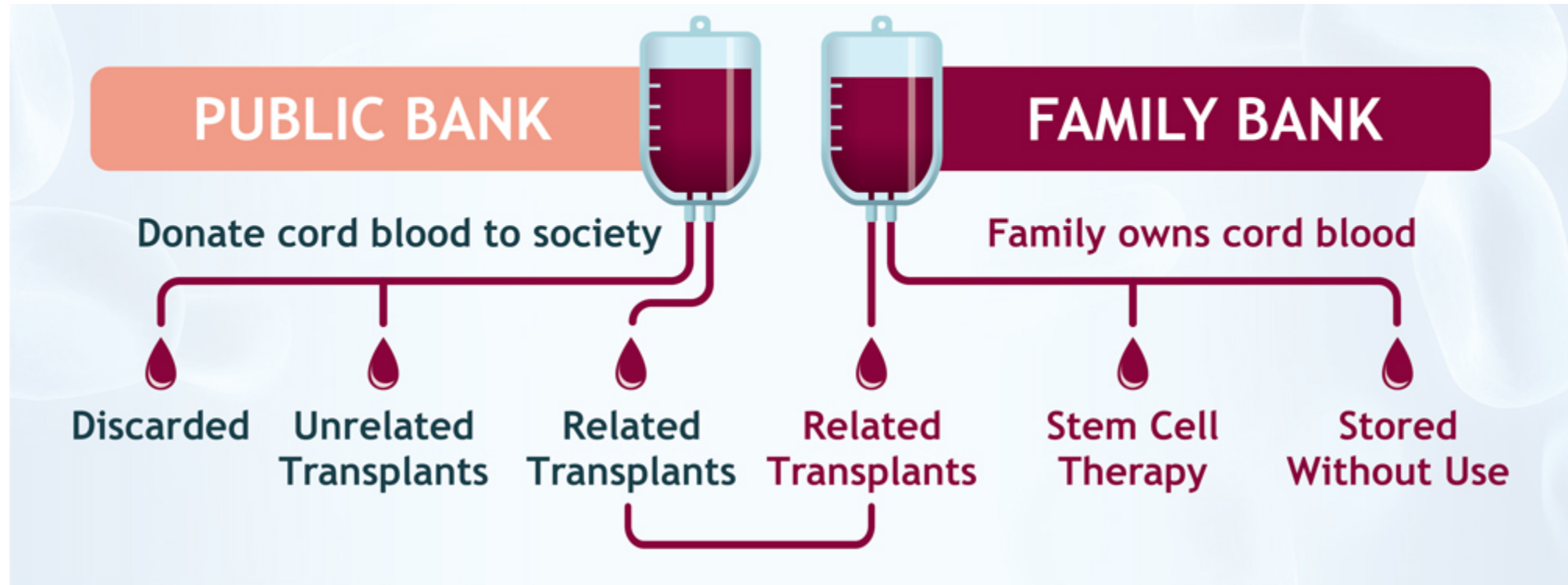
Levels of MSC:

- ❖ Umbilical Cord: 2%
- ❖ Amniotic Fluid: 0.3%
- ❖ Amniotic Membrane: 1.4-8%





Cord Blood Banking



- Huge Industry valued at \$29.7 billion in 2016 and expected to only grow
- Approximately 5.1 million cord blood and tissue units in storage
- “Biological Insurance” against future diseases
- Possible Ethical Concerns?



Expert Opin. Biol. Ther. (2015) 15(9):1293-1306

Clinical Review

EXPERT
OPINION
ON BIOLOGICAL THERAPY

Human umbilical cord mesenchymal stem cells: an overview of their potential in cell-based therapy

2015

Tan Li, Mingxu Xia, Yuanyuan Gao, Yanting Chen & Yun Xu[†]

[†]Medical School of Nanjing University, Drum Tower Hospital, Department of Neurology, Nanjing, China

- Collection of human umbilical cord MSCs (HUC-MSCs) is noninvasive
- HUC-MSCs display higher proliferation potential than BM-MSCs in vitro
- 3 main isolation methods: collagenase digestion method, collagenase/trypsin digestion method, tissue explant method
 - No standard protocol
- Little to no clinical trials done in the field of Orthopedics



Received March 13, 2018;
accepted for publication
October 20, 2018; first
published December 28, 2018.

Umbilical Cord-Derived Mesenchymal Stromal Cells (MSCs) for Knee Osteoarthritis: Repeated MSC Dosing Is Superior to a Single MSC Dose and to Hyaluronic Acid in a Controlled Randomized Phase I/II Trial

JOSE MATAS,^a MARIO ORREGO,^a DIEGO AMENABAR,^a CATALINA INFANTE,^b
2018 RAFAEL TAPIA-LIMONCHI,^{b,c} MARIA IGNACIA CADIZ,^b FRANCISCA ALCAYAGA-MIRANDA,^{b,c,d}
PAZ L. GONZÁLEZ,^d EMILIO MUSE,^e MAROUN KHOURY,^{b,c,d} FERNANDO E. FIGUEROA^{ib},^{c,d,f}
FRANCISCO ESPINOZA^{ib},^{b,c,f}

- N=40 patients between 40-65 with grade 1-3 Kellgren-Lawrence
- HA Control
- 20×10^6 UC-MSCs in 3 cc of saline
 - Wharton's Jelly; 5 passages
- No serious adverse events
- MSC-treated patients experienced significant pain and function improvements from baseline ($p = .001$). At 12 months (WOMAC-A; pain subscale) reached significantly lower levels of pain in the MSC treated group (1.1 ± 1.3) as compared with the HA group (4.3 ± 3.5 ; $p = .04$)

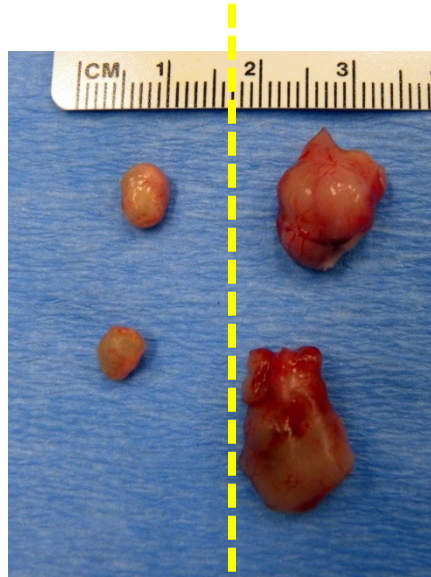


All That Glitters...



MSCs promote tumor growth

28 days



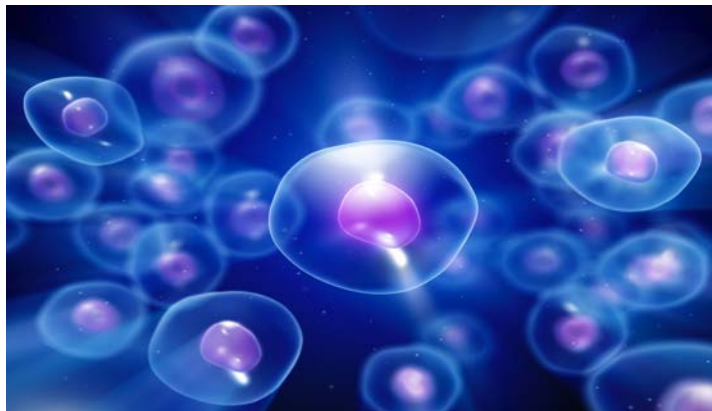
Cancer cells	+	+
Scaffold	+	+
MSC		+

- Prostate cancer cells were injected subcutaneously in nude mice.
- Gel matrix was used as the cell carrier
- MSCs were premixed with cancer cells before injection



Cell Injections- Where are we now?

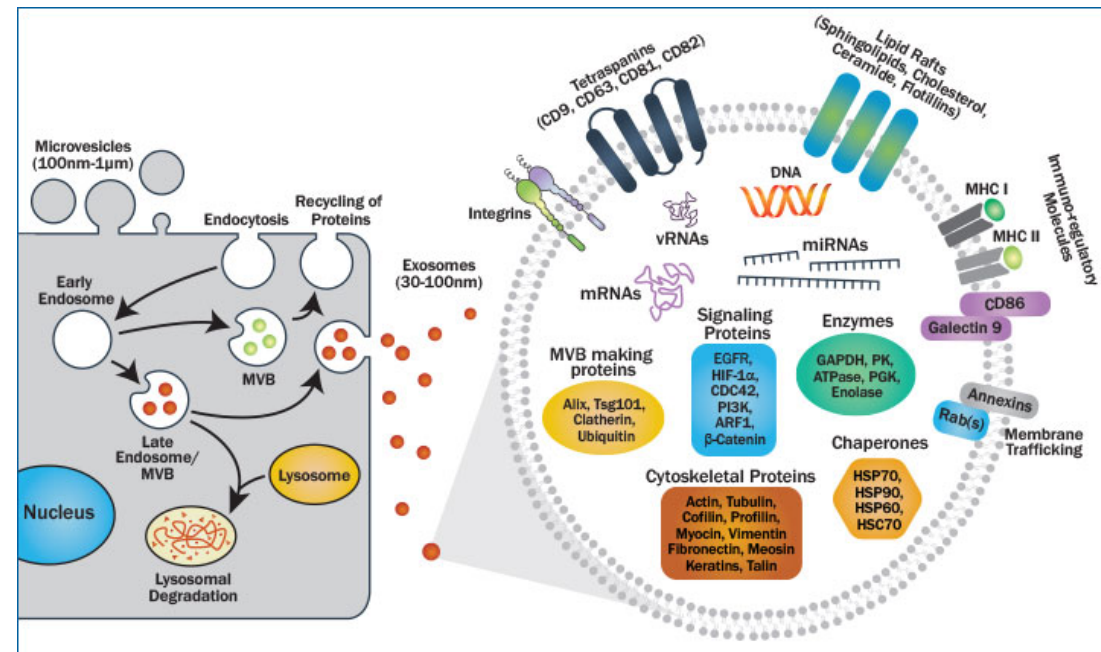
1. No cell drug has been approved to date in the US for musculoskeletal medicine
2. Very little Orthopaedic literature has been published
3. Extensive public curiosity / Misinformation
4. Injections are currently being given at hundreds of clinics illegally
5. Early results favorable and **safe**
6. Other biological products in the pipeline





Exosome

1. Communication of macromolecules
2. Spread proteins, lipids, mRNA, miRNA, and DNA
3. Drug delivery vector

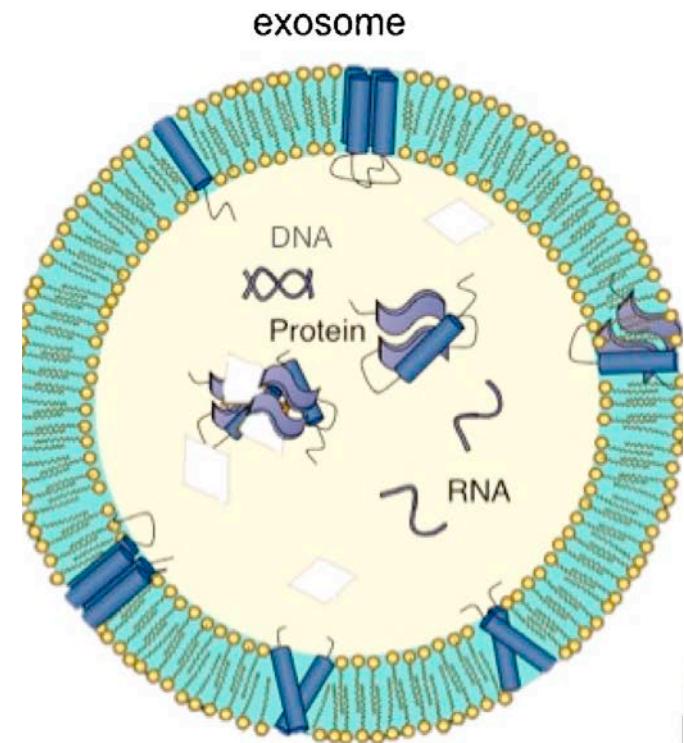


<https://www.novusbio.com/research-areas/cell-biology/Exosome-research-tools>



Signaling Factors in Blood/Tissue/Fluids

- Extracellular Vesicles
- Growth Factors
- Cytokines
- Hormones
- Lipids
- Other





Exosomes: *Not Mentioned on FDA website* *Probable 351*

Dozens of Exosome companies
Over 3,000 patents

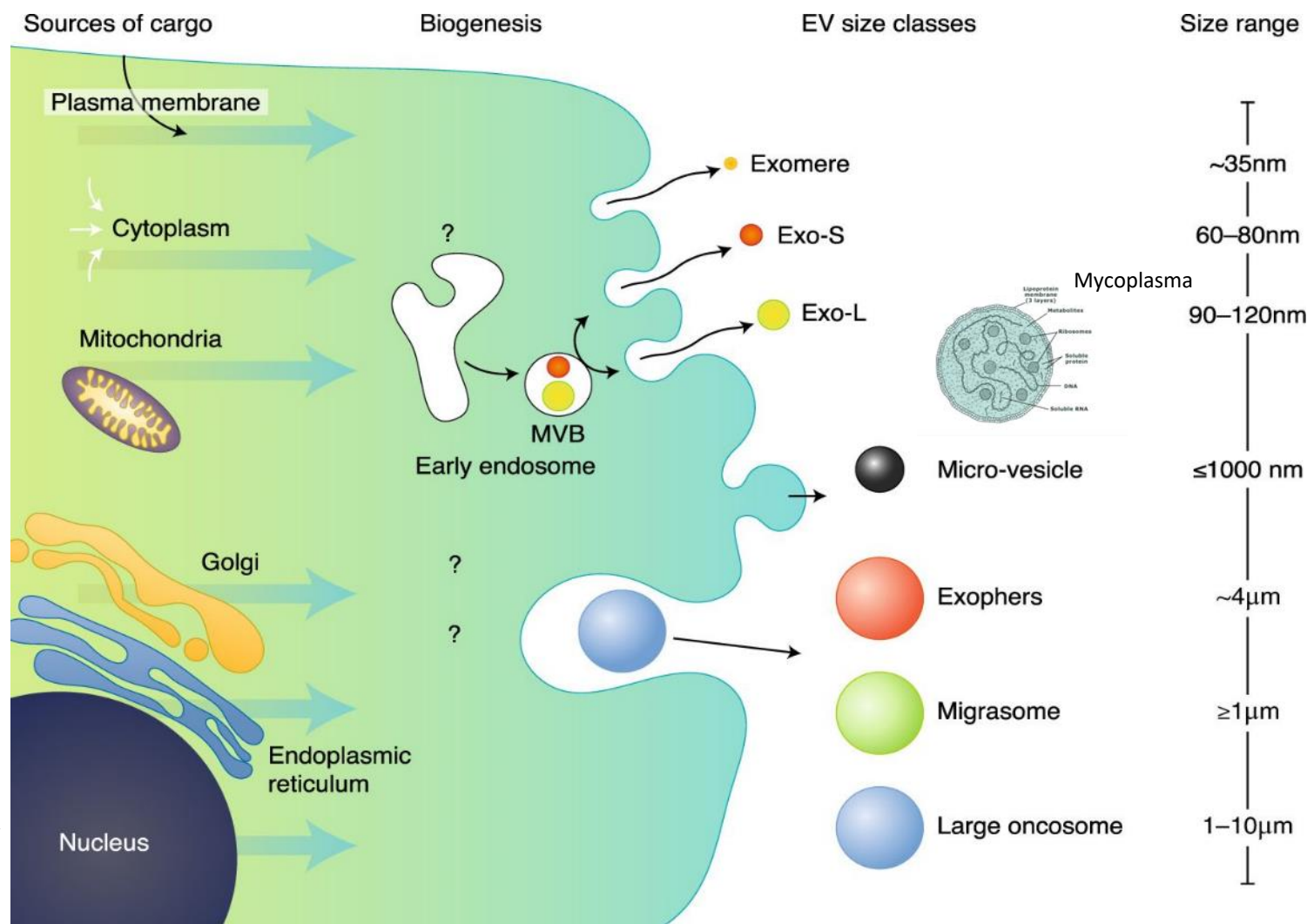




Types of Extracellular Vesicles

- Exosomes
- Microvesicles
- Apoptotic bodies
- Mycoplasma (150-300nm)

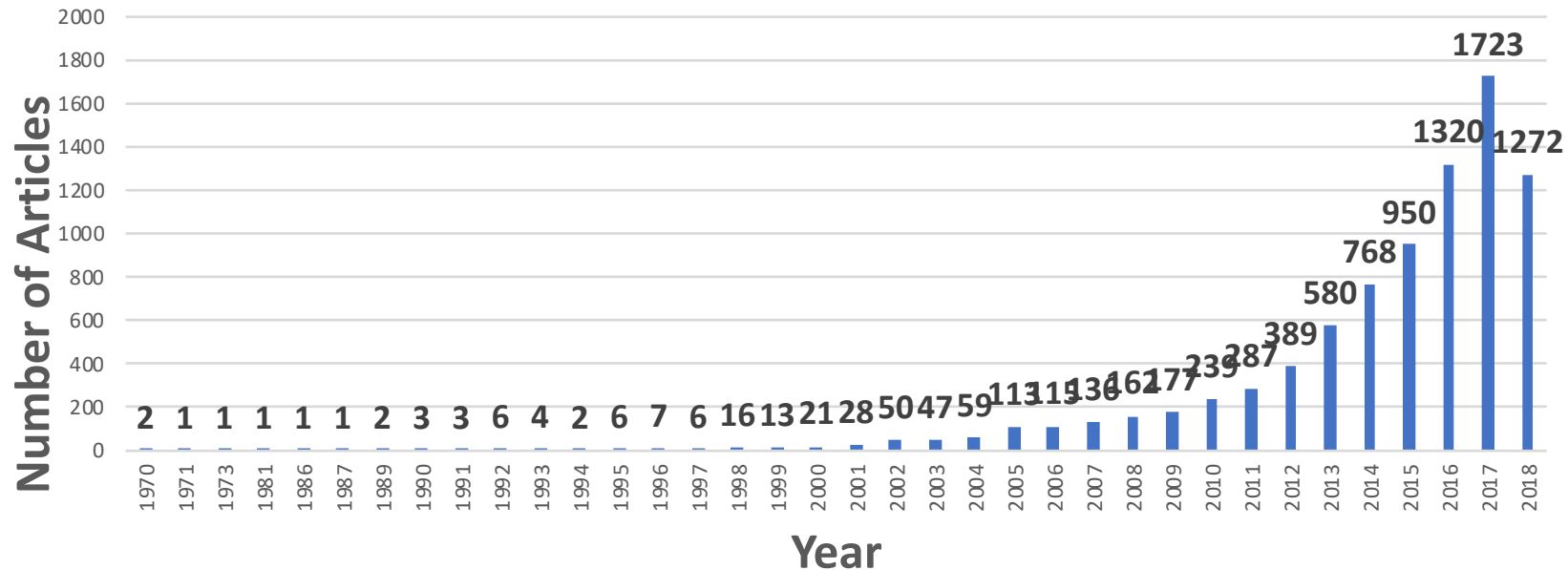
Journal of Endocrinology
228 R57-R71





Pubmed Search Exosome

Exosome Research by Year



Total Exosome Articles:	8,511
Exosome Bone:	408
Exosome Muscle:	318
Exosome Cartilage:	23
Exosome Ligament:	4
Exosome Tendon:	3



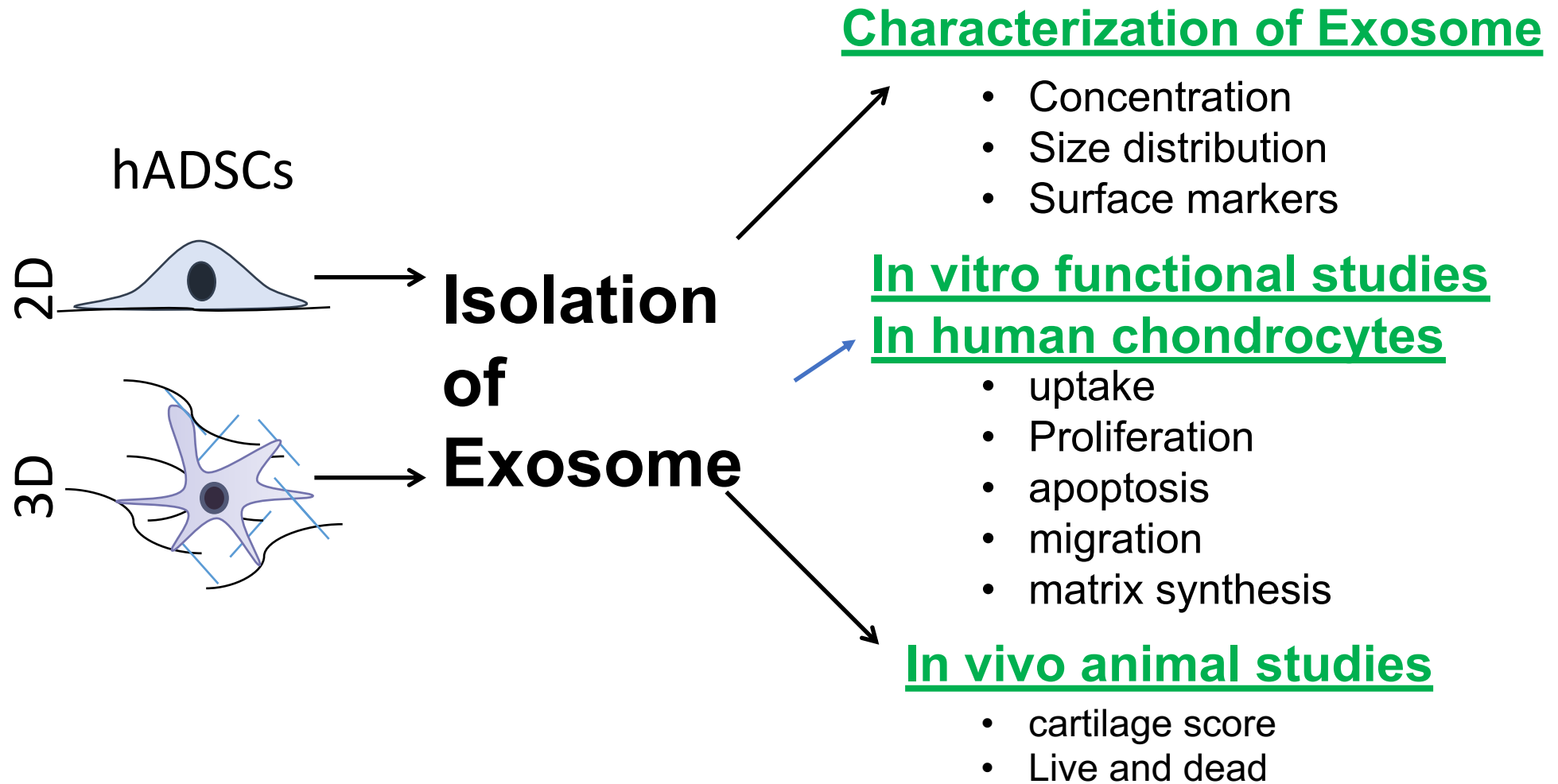
Hypothesis

Cell growth conditions determine their
exosome cargo content that elicit
distinguished functions in recipient cells

The Niche



Methods





hADSCs Growth Conditions

2D



Cell culture flask

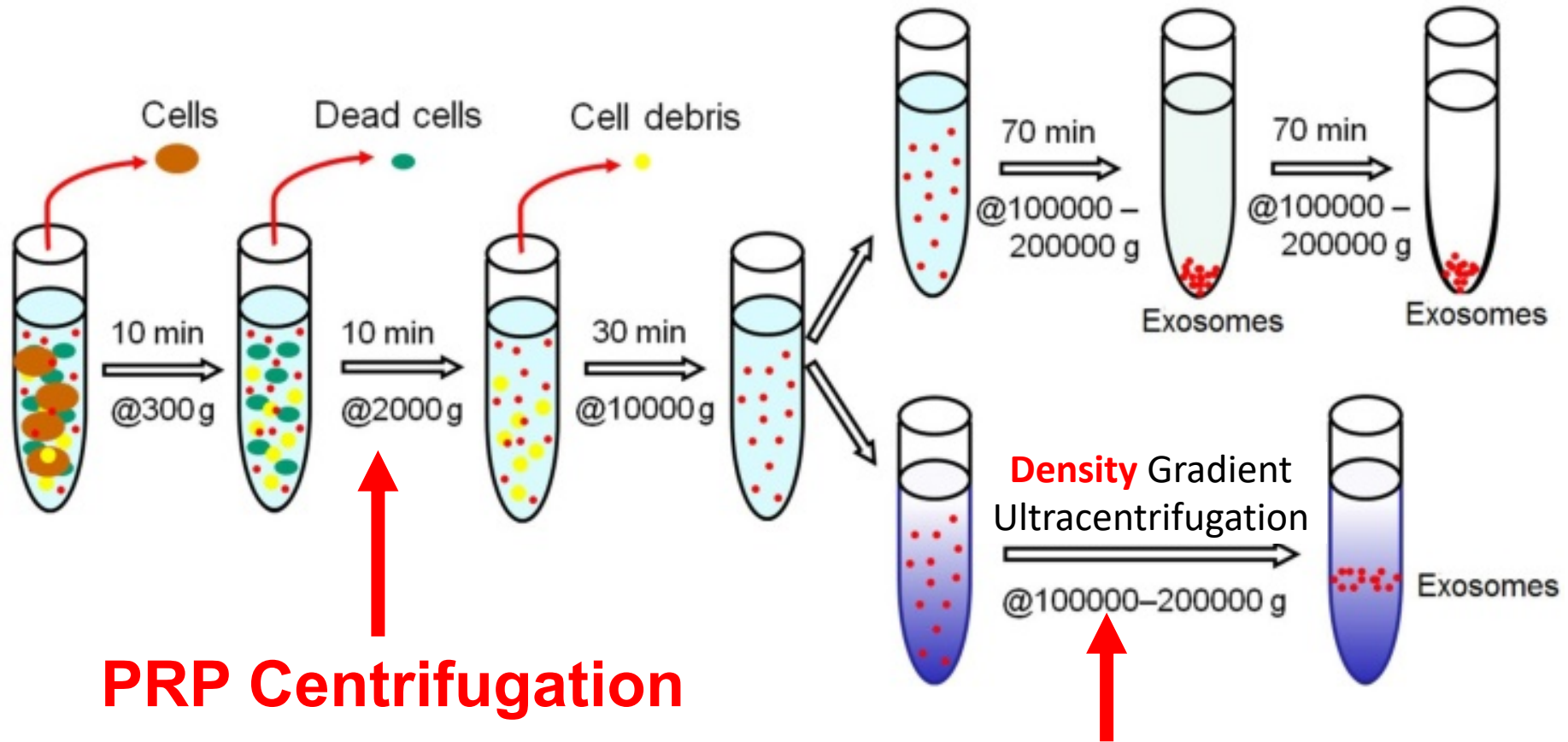
3D Gel



Col-T Gel



Ultra High Centrifuge



PRP Centrifugation

Ultra High Centrifugation



Methods

- Day 0
Cell Expansion

- Expanded hADSCs (Human Derived Adipose Stem Cells)

Day 0
Cell Seeding

- hADSCs (2million cells/ml) were encapsulated in Soft, Med and Stiff Col-Tgel, and 20ul of the mixture was seeded in 48-well suspension

Day 3
Treatment

- Treated with 3 types exosomes from part 1
 - Exosome 1 (Ex1): hADSCs in Soft gel
 - Exosome 2 (Ex2): hADSCs in Med gel
 - Exosome 3 (Ex3): hADSCs in Stiff gel
- Non-treated cells and cells treated with TGF-beta3 were included as the controls.

Day 5
Analysis (1st time point)

- Collected samples for analysis

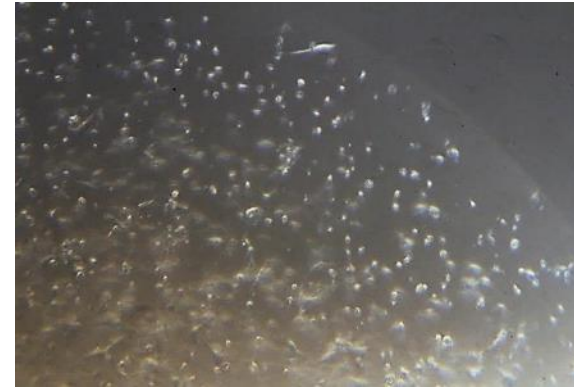


Results – Morphology of hADSCs (2D vs. 3D)

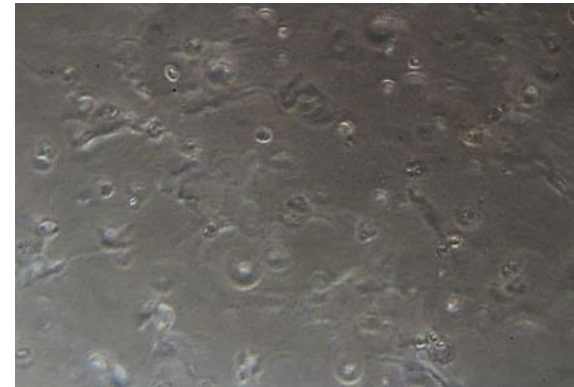
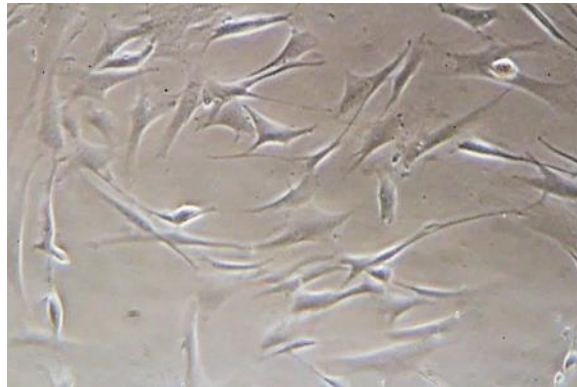
2D

3D

4x

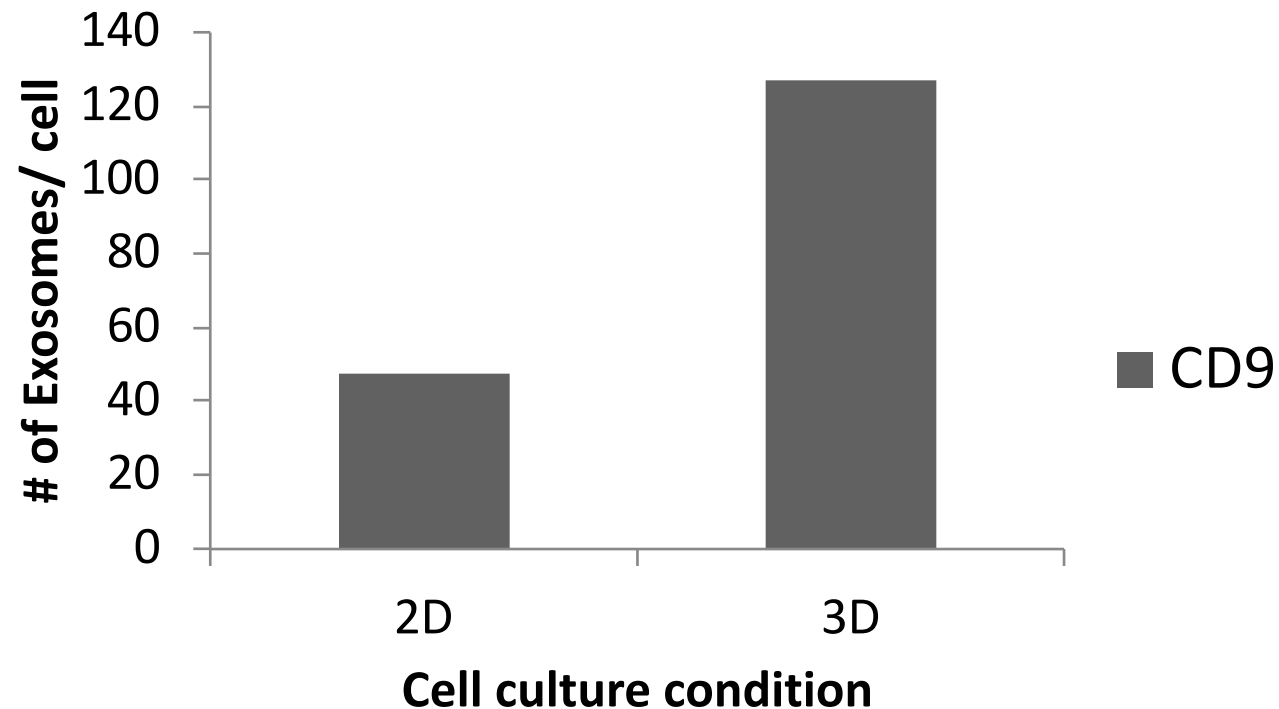


10x





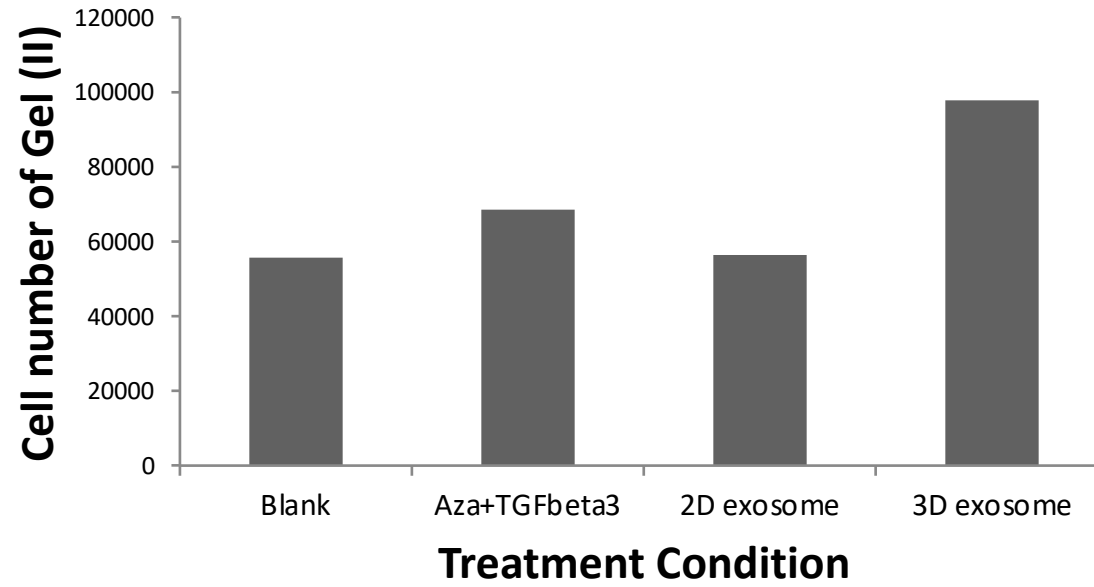
Results – Quantity of Exosome by CD9 surface marker



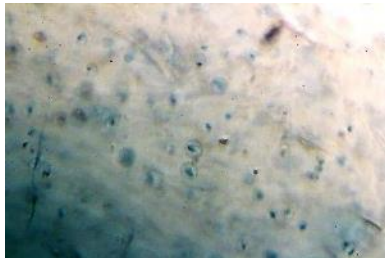
3D cell culture of hADSCs expressed more exosome marker (CD 9) compared to hADCs seeded on 2D surface.



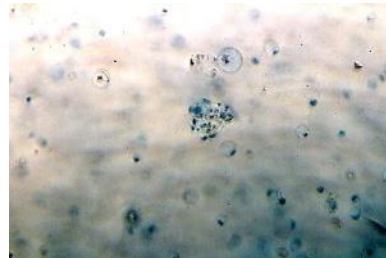
Results – The Effect of Exosome on Chondrogenic Expressions



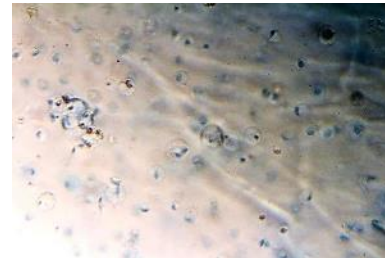
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TGFbeta3



2D Exosome



3D Exosome

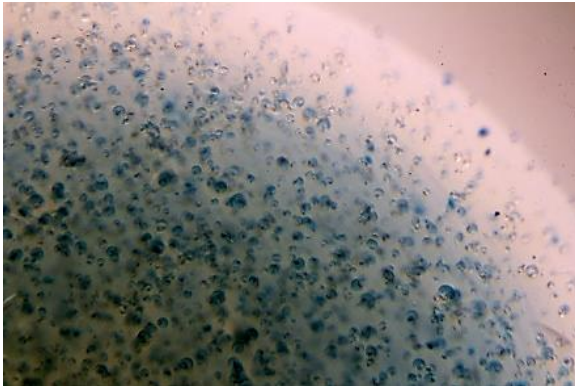




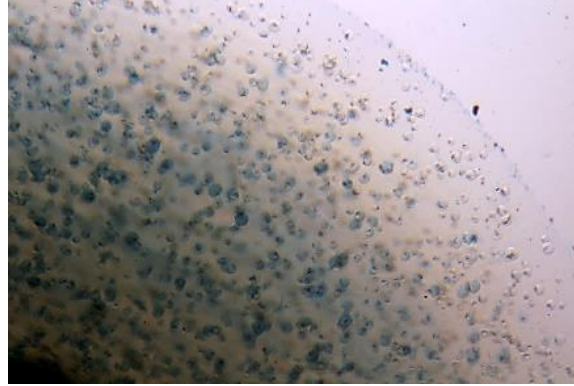
RESULTS - ALCIAN BLUE (GAG STAINING)

In vitro human chondrocytes

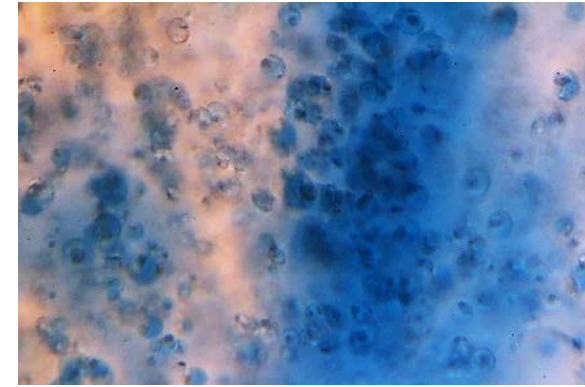
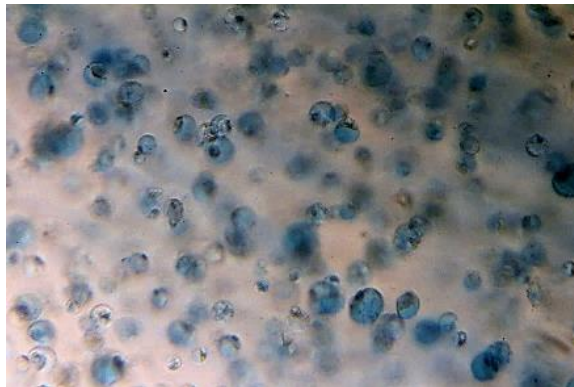
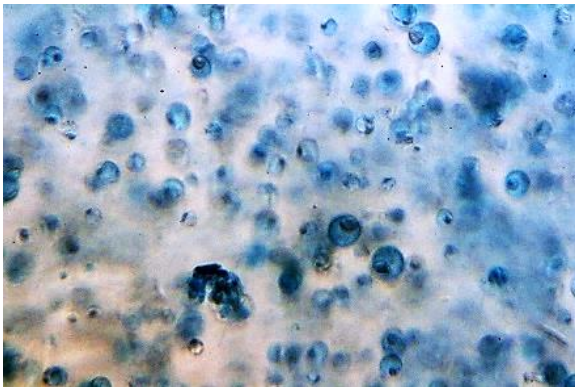
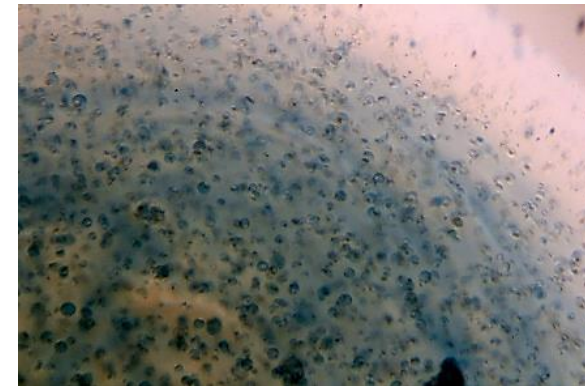
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2D Exosome



3D Exosome



CHONDROGENIC FUNCTIONAL STUDIES

Col(II)

Blank



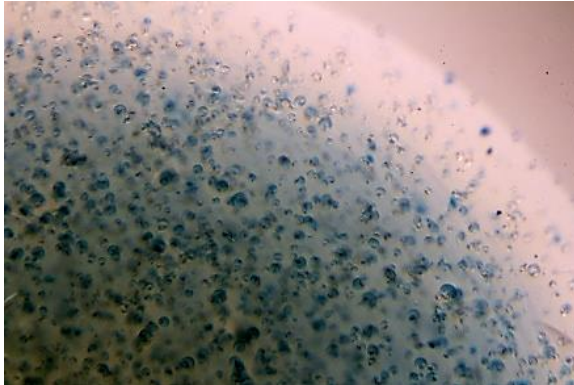
3D exosome



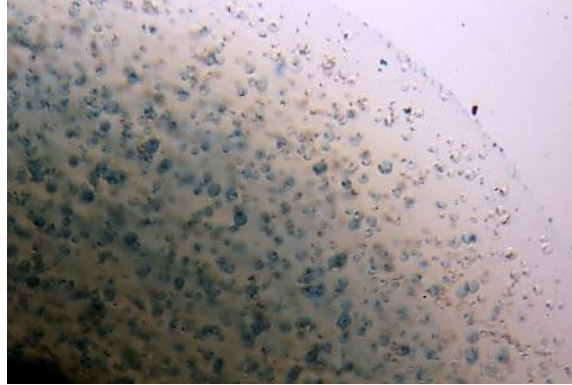
CHONDROGENIC FUNCTIONAL STUDIES

AB staining

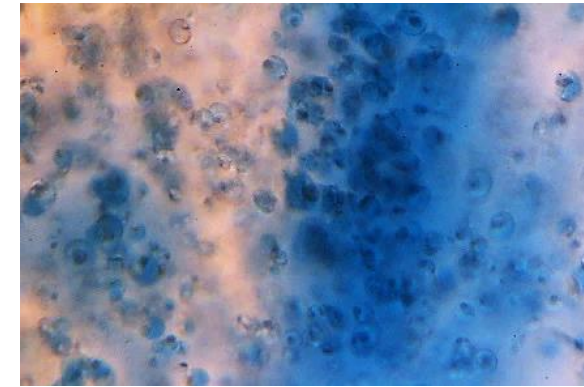
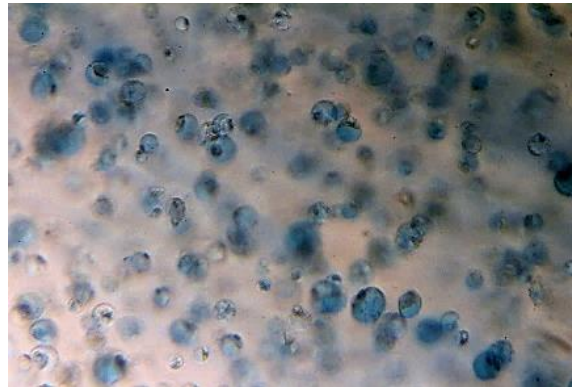
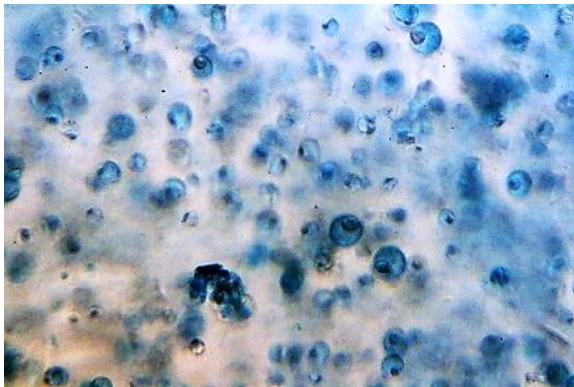
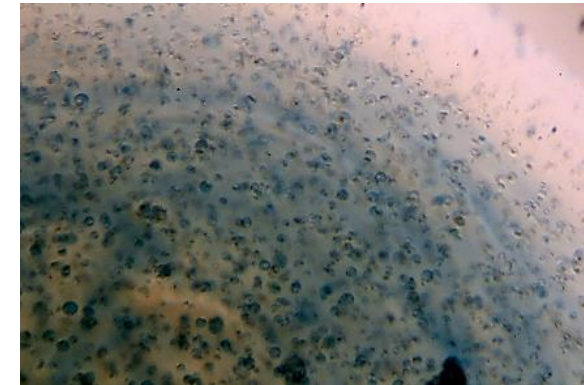
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2D Exosome



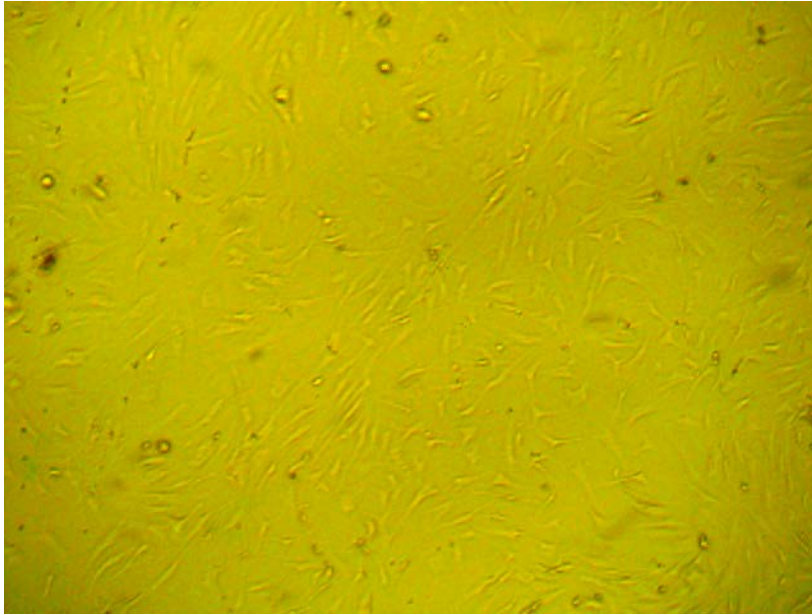
3D Exosome



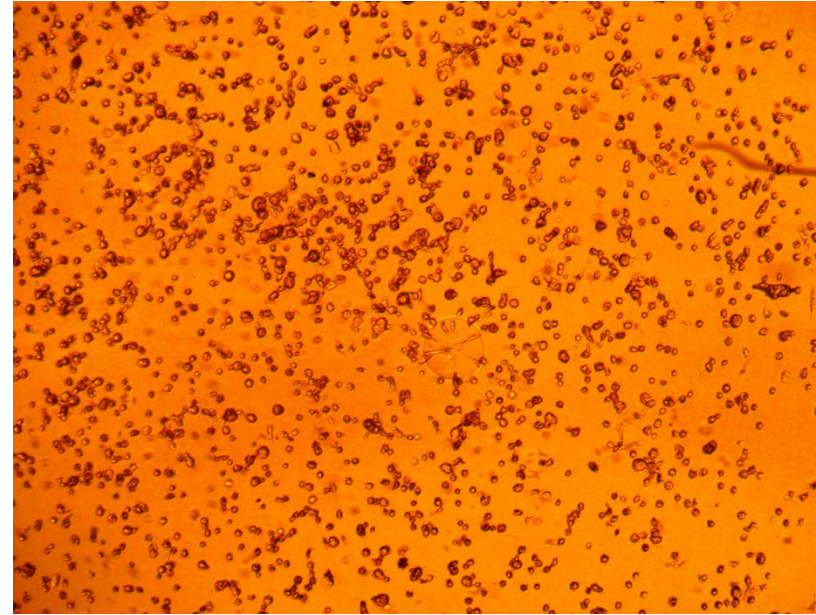
2D exosome has attenuated effect 3D induced chondrogenic differentiation

3D exosome promote chondrogenic differentiation

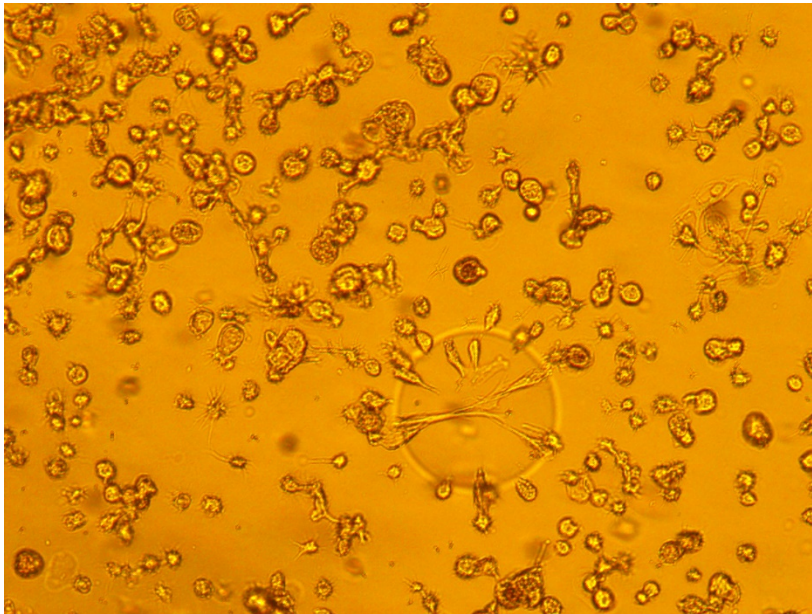
Synovial Fluid Derived Cells 2D/3D



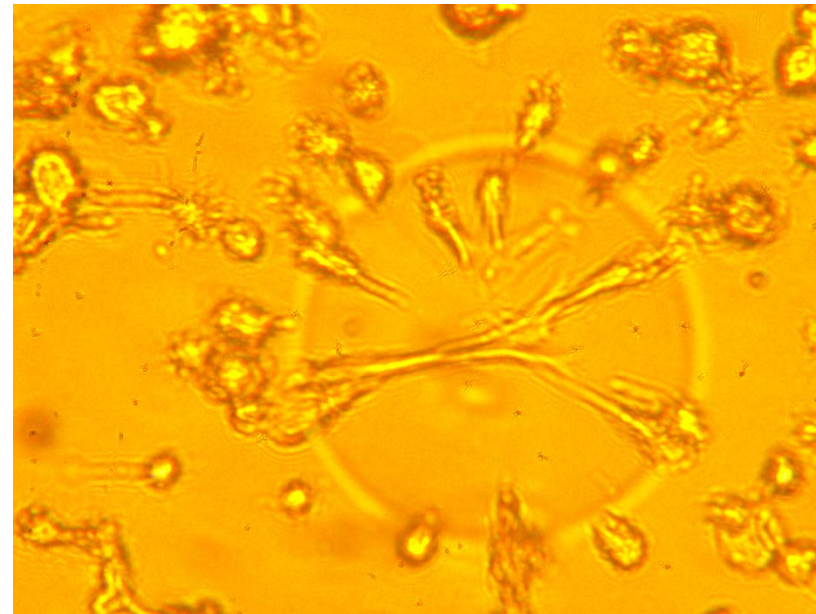
SF#6 2D cell culture 4x10



SF#6 3D cell culture 4x10



SF#6 3D cell culture 10x10



SF#6 3D cell culture 25x10

CULTURE CONDITIONS AND CELLULAR FUNCTION AND EXOSOME EXPRESSION

Human synovial fluids derived cells

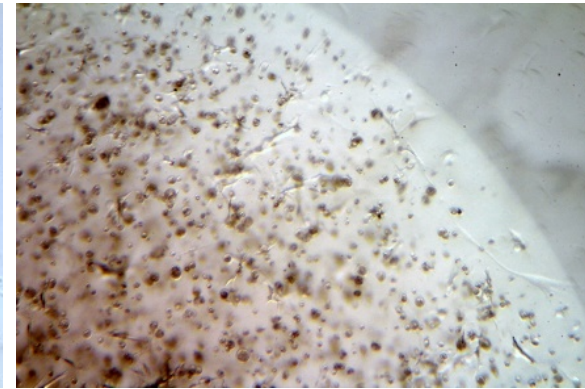
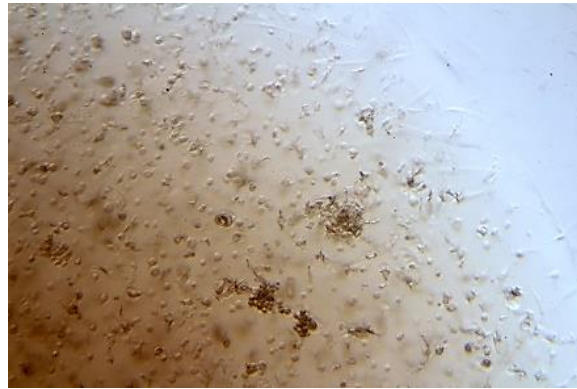
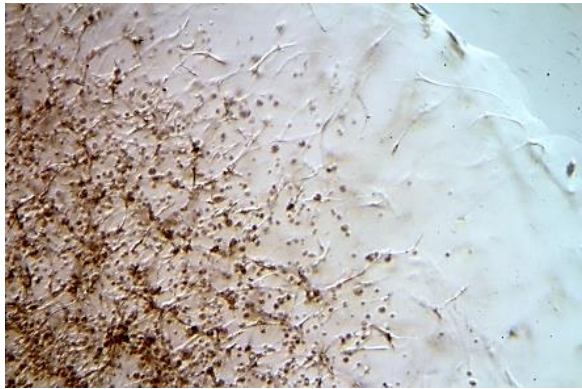
Day 7

Soft 3D

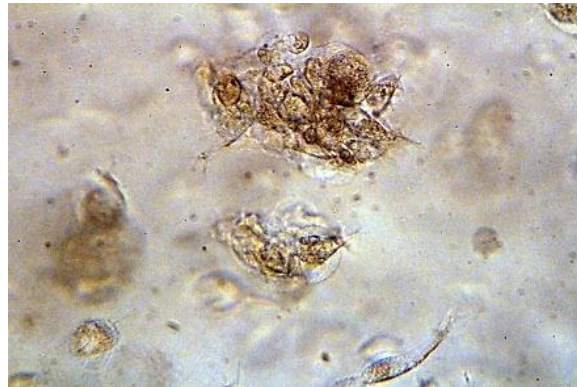
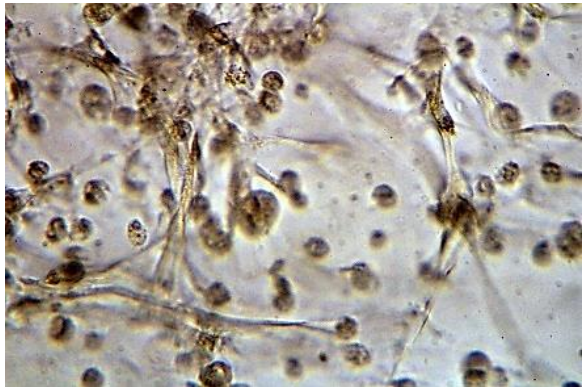
Med 3D

Stiff 3D

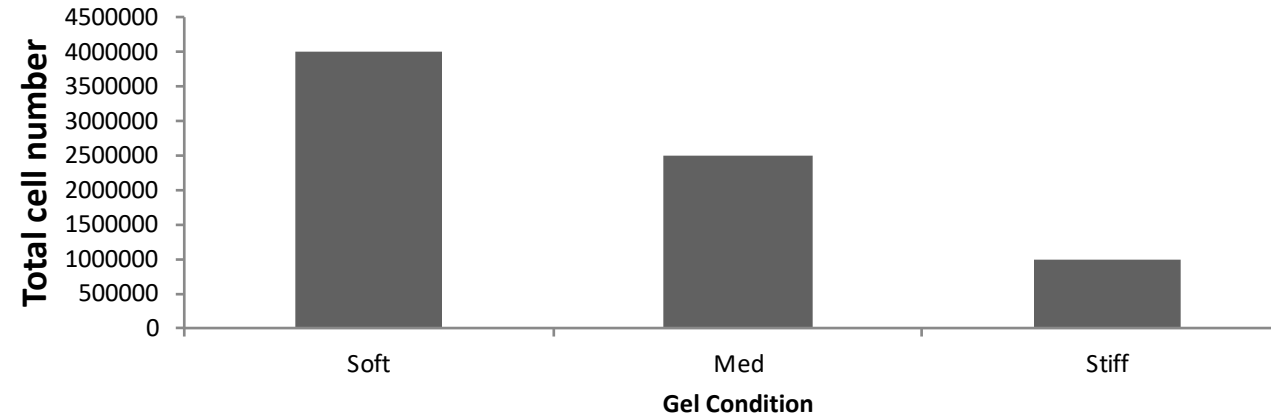
4x



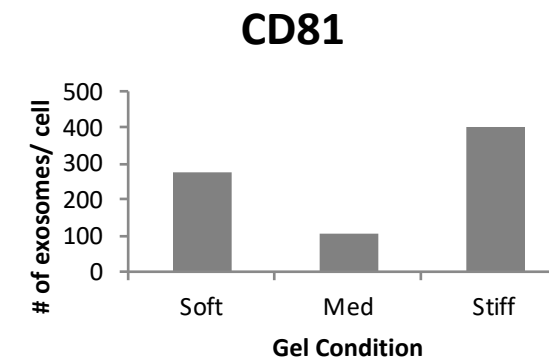
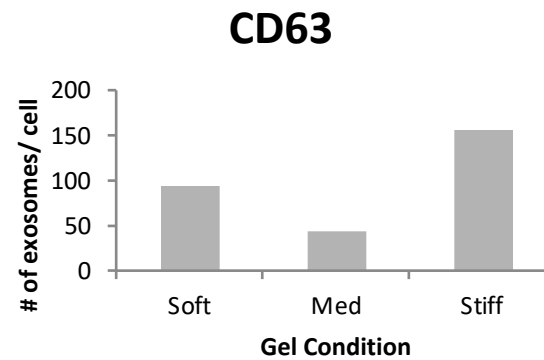
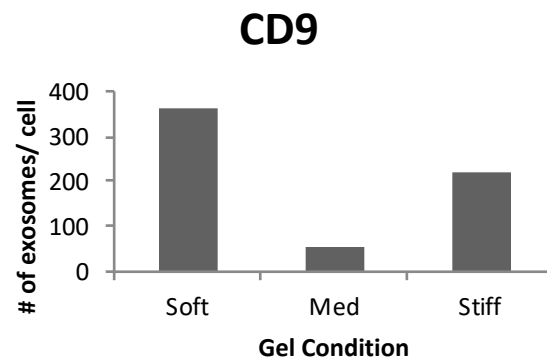
40x



CELL PROLIFERATION RATE CHANGES



Exosome expression (# of exosome /cell)





Discussion

- Pilot study
- Confirm our observation that cells in the optimal cultured condition favor their phenotypical homeostasis



Discussion

- Matrix is very important
 - Affected exosome quantity and quality
 - Determined the effect on cell homeostasis
 - E.g. exosome #2 + Med Col-Tgel on the expressions of sox9 and Col(II).
 - E.g. exosome #1 + Soft Col-Tgel on the expression of Col(I)
- Ongoing studies
 - **In vivo** animal study
 - In cartilage defect models, we will compare exosome isolated from 2D vs 3D cell culture on cartilage repair



Future

- A New Biology
- Biologic Injections / Clinics
- Improvement/Definitions with Stem Cells
- FDA
- Insurance Companies
- **Cash**
- Use Will Increase – Safety?
- Call for Level I Studies
- Measure your Results





Thank You



USC Keck School of Medicine
Orthopedic Surgery





Questions?



Where can we get MSCs?

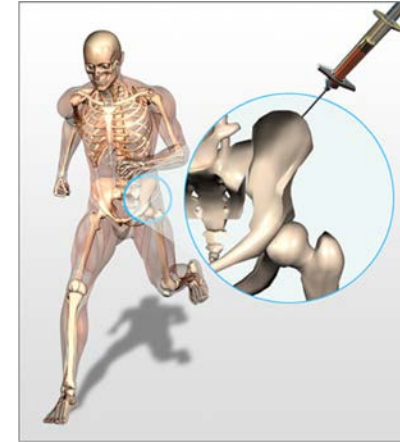
1. Platelet Rich Plasma

2. Bone marrow

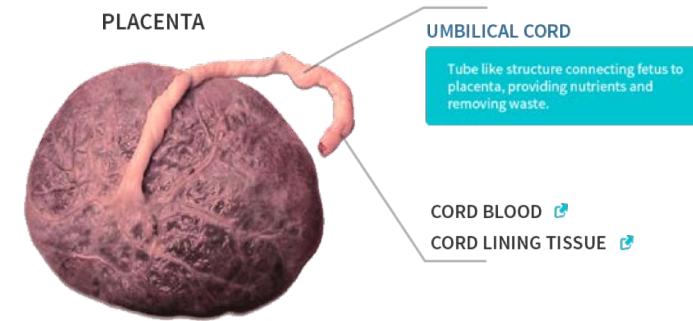
3. Fat

Largest microvascular organ in the body

4. Placental Tissues



Overview



No longer considered as "medical waste"



Today and Tomorrow Cell Therapies

There are two main principles by which cells are thought to facilitate therapeutic action:

1. Engraftment, differentiation, and long term replacement of damaged tissue
2. By releasing soluble factors such as cytokines, chemokines, and growth factors which act in a paracrine or endocrine manner.

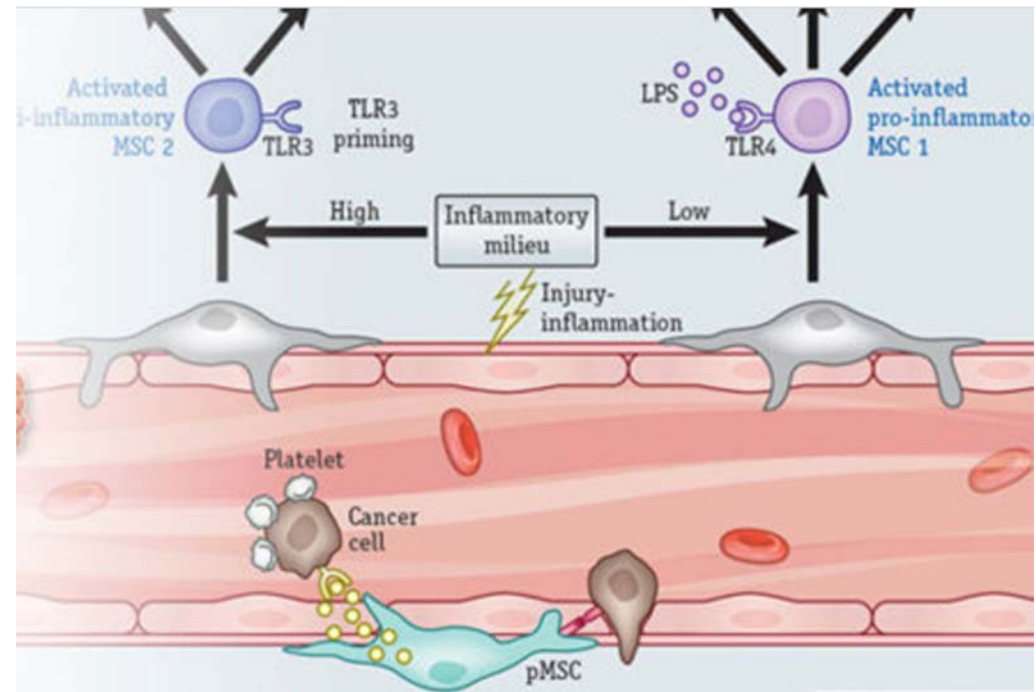




MSCs

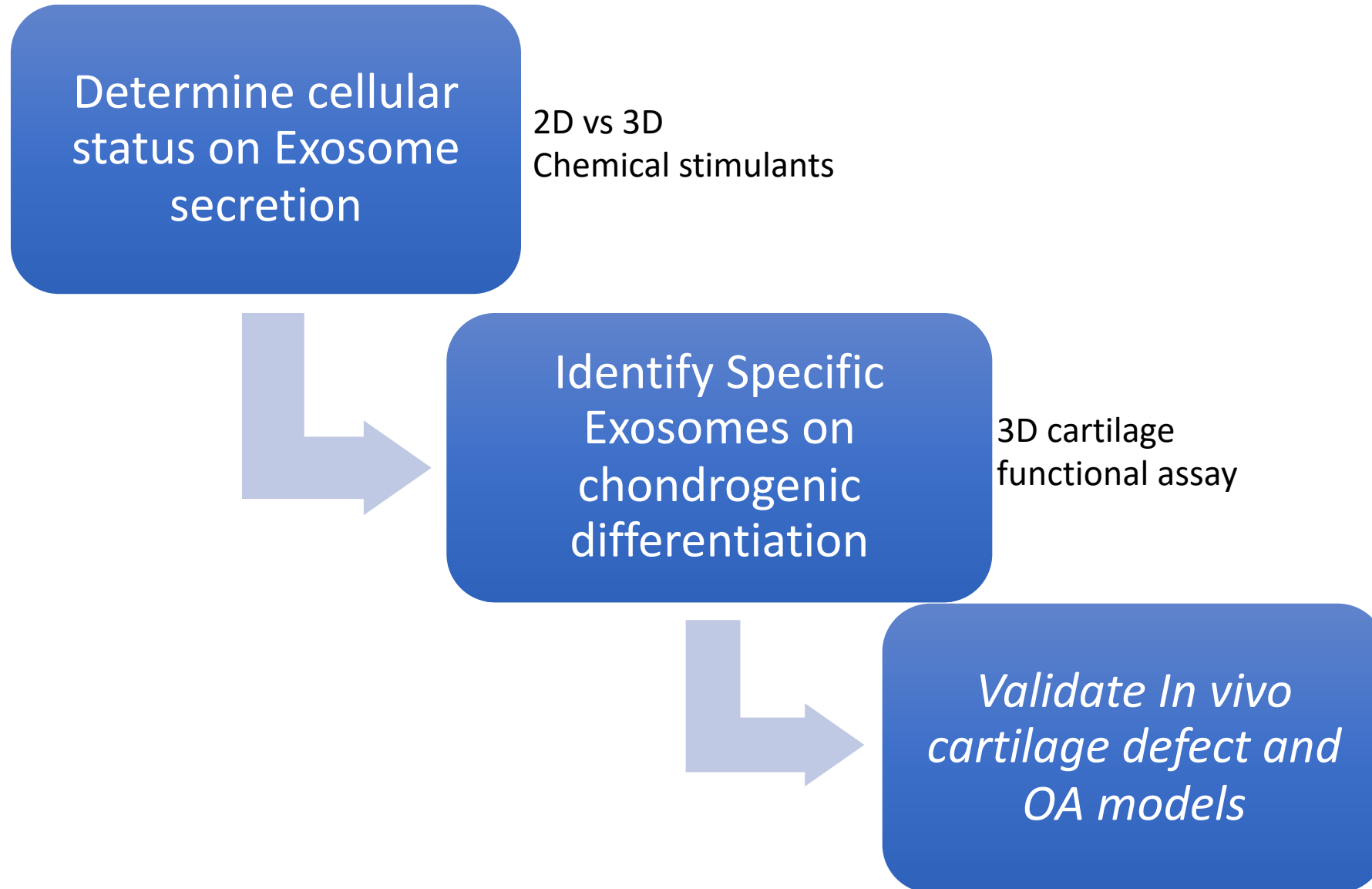
“Medicinal Signaling Cells”

1. Recruit other stem cells
2. Secrete bioactive factors
 - ✓ Angiogenesis
 - ✓ Mitosis
 - ✓ Anti-scarring
 - ✓ Anti-apoptotic
 - ✓ Anti-inflammatory
 - ✓ Immunomodulatory



Arnold Caplan Pericyte

WORK FLOW





Gel Stiffness Results – Col(II) (Day 5) (In Vitro human chondrocytes)

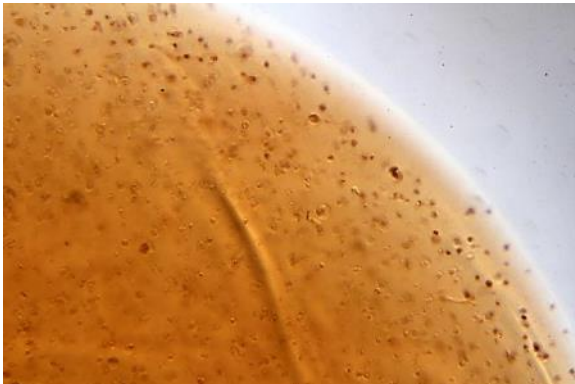
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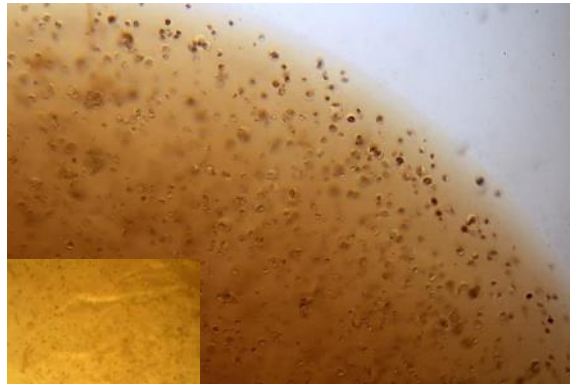
TGF-Beta3



Exosome #1



Exosome #2



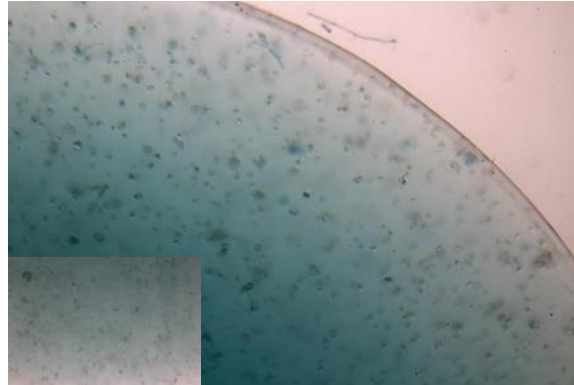
Exosome #3



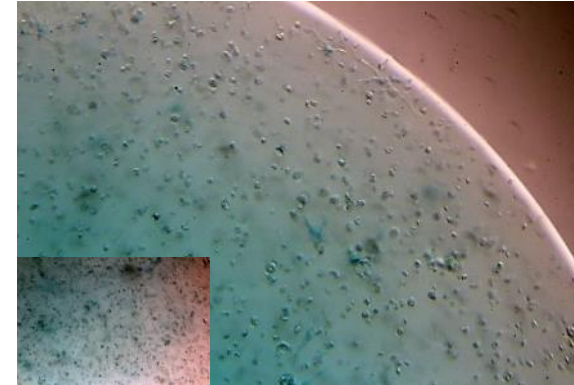


Gel Stiffness Results – Alcian Blue (Day 5) (In vitro human chondrocytes)

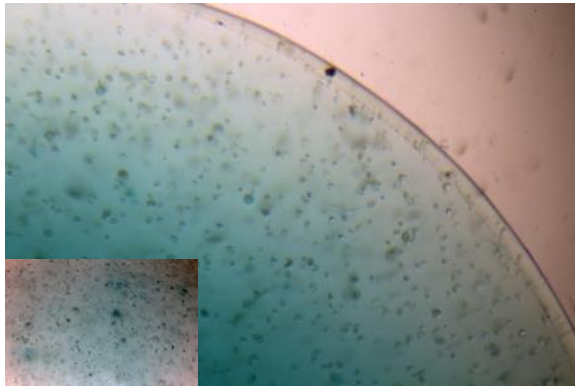
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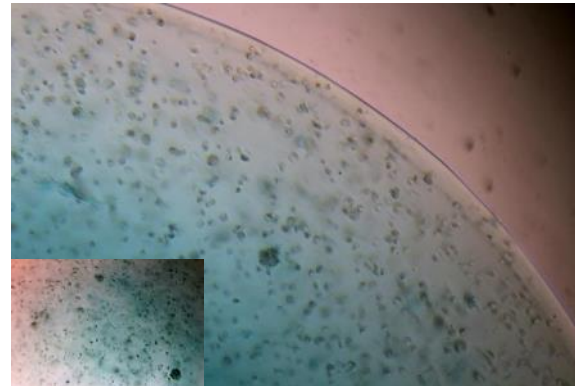
TGF-Beta3



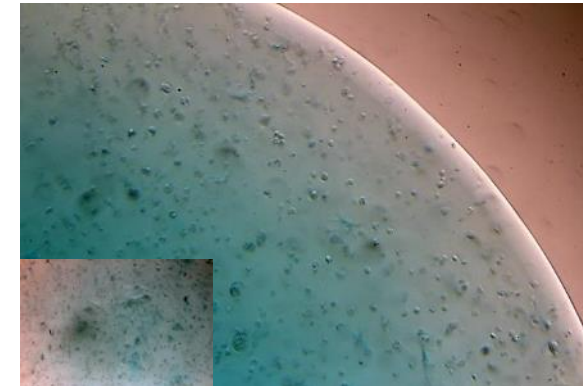
Exosome #1



Exosome #2



Exosome #3





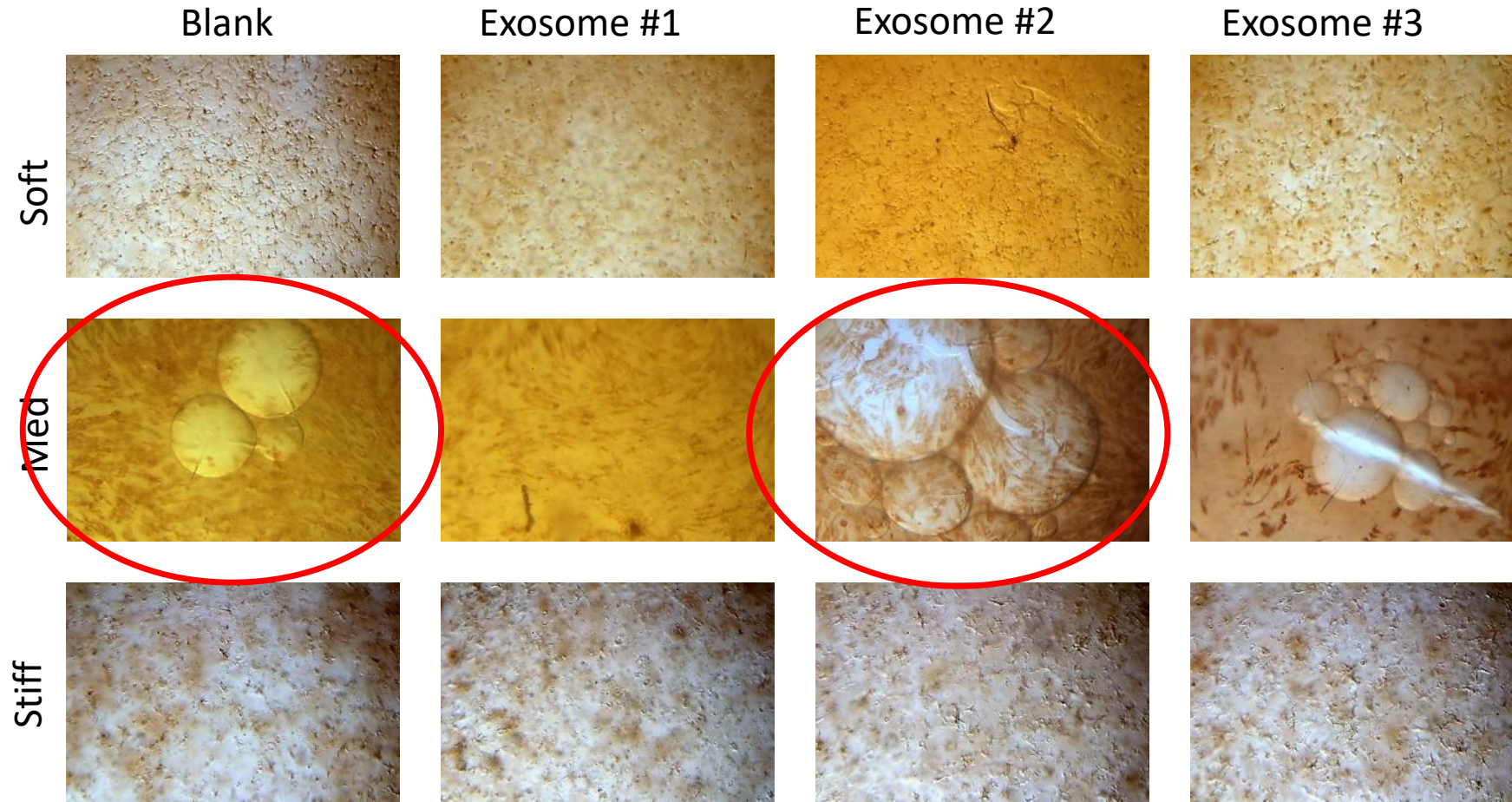
Exosomes: *Not Mentioned on FDA website* *Probable 351*

Dozens of Exosome companies
Over 3,000 patents



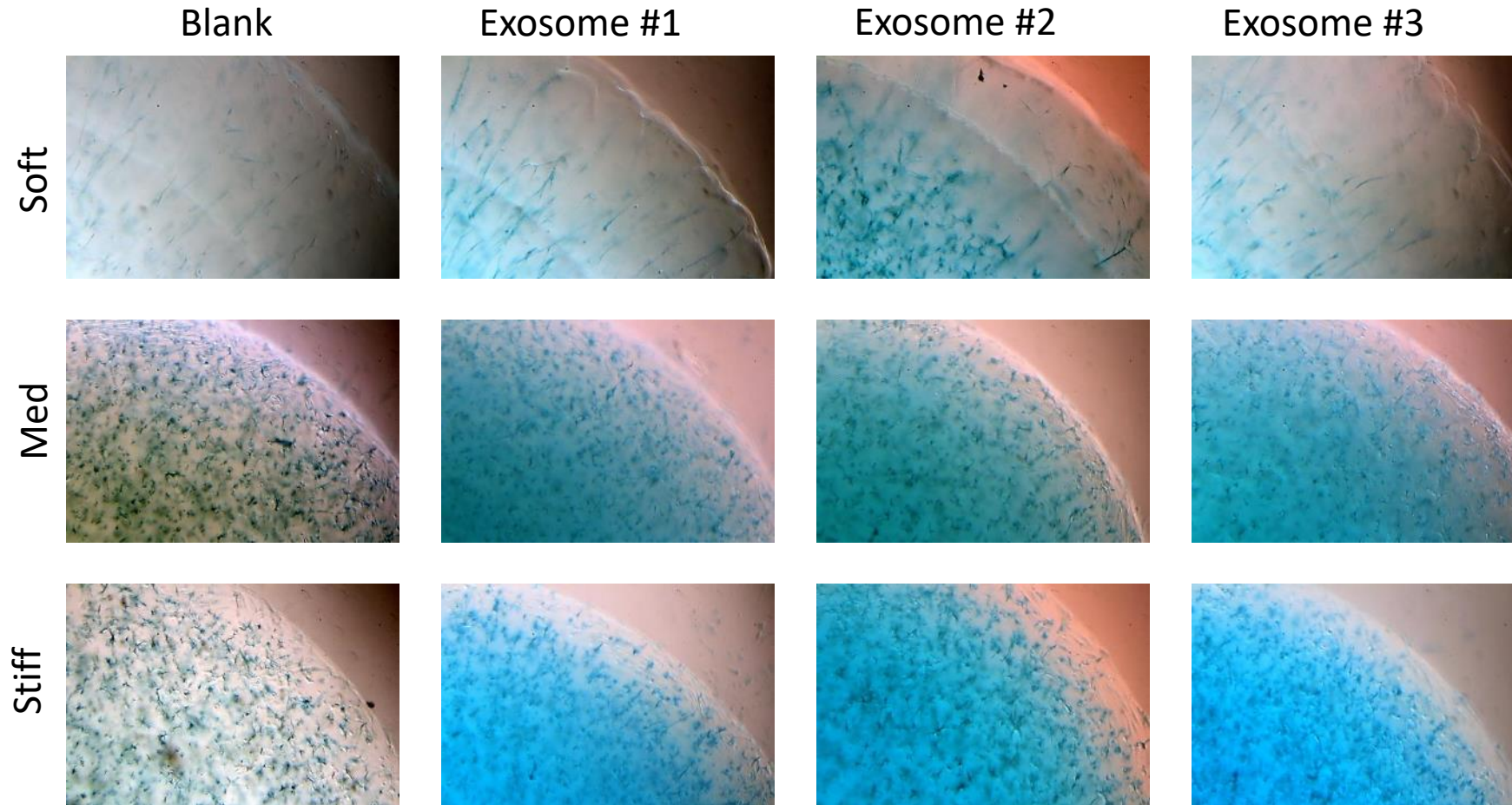


Gel Stiffness Results – Col(II) (Day 7) (In vitro human chondrocytes)



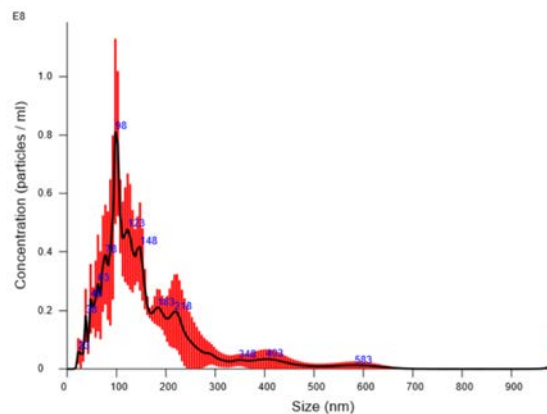


Gel Stiffness Results – Alcian Blue (Day 7) (In vitro human chondrocytes)

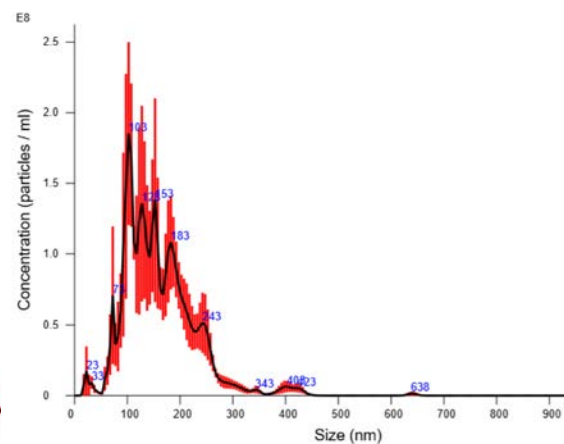


Compare quantity and quality of OA patient derived exosomes

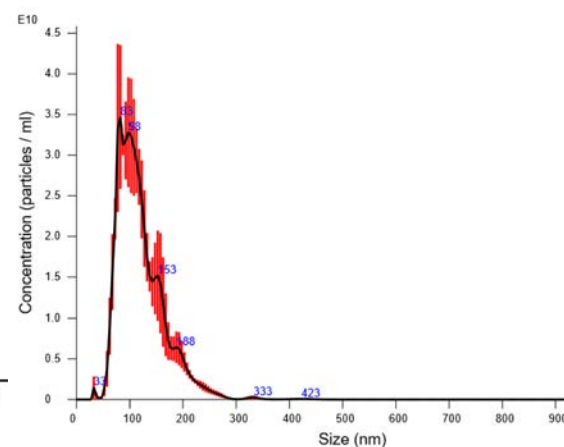
EXOSOME QUANTITATE ASSAY BY NANOSIGHT



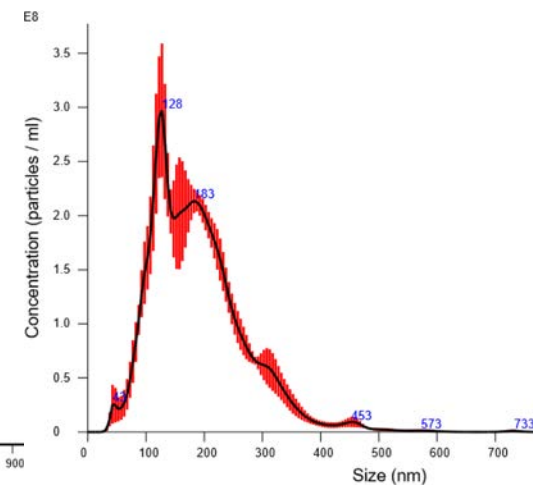
Averaged FTLA Concentration / Size for Experiment:
101_1 10x 2018-11-02 10-09-07
Error bars indicate + / -1 standard error of the mean



Averaged FTLA Concentration / Size for Experiment:
PEG + D + qEV 10x 2018-11-02 11-06-02
Error bars indicate + / -1 standard error of the mean



Averaged FTLA Concentration / Size for Experiment:
PEG + D 1000x 2018-11-02 11-18-37
Error bars indicate + / -1 standard error of the mean



Averaged FTLA Concentration / Size for Experiment:
PEG + qEV 10x 2018-11-02 10-54-44
Error bars indicate + / -1 standard error of the mean