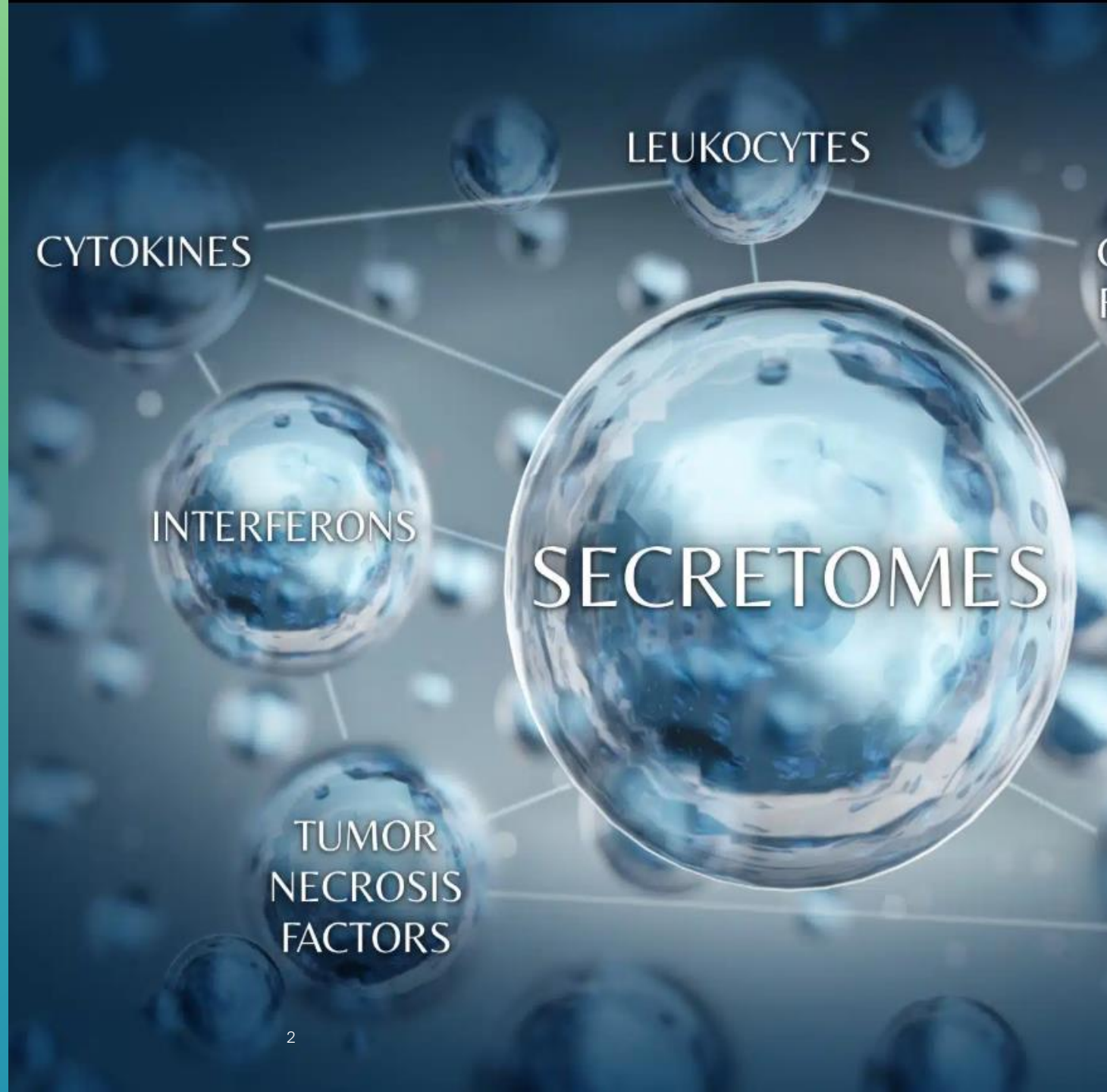
Welcome to the inaugural BEBPA Journal Club

BEBPA Journal Club:
Safety and Regenerative Properties of Immortalized Human Mesenchymal Stromal Cell Secretome

Karagyaur M, Primak A, Basalova N, Monakova A, Tolstoluzhinskaya A, Kulebyakina M, Chechekhina E, Skryabina M, Grigorieva O, Chechekhin V, Yakovleva T, Turilova V, Shagimardanova E, Gazizova G, Vigovskiy M, Kulebyakin K, Sysoeva V, Dyachkova U, Dzhauari S, Bozov K, Popov V, Akopyan Z, Efimenko A, Kalinina N, Tkachuk V.
Safety and Regenerative Properties of Immortalized Human Mesenchymal Stromal Cell Secretome. Int J Mol Sci. 2025 Sep 24;26(19):9322. doi: 10.3390/ijms26199322. PMID: 41096593; PMCID: PMC12525087.

Why Secretomes?



MSC vs. MSC……

	Mesenchymal Stem Cells (MSCs)	Mesenchymal Stromal Cells (MSCs)
Primary Function	Differentiation & Self-Renewal	Immunomodulation & Secretion
Commonly Found	Tissue progenitor populations	Bulk cultured populations
Role in Therapy	Regeneration via cell replacement	Repair via trophic/signaling factors

Why are we talking about this? Because I am *NOT* an expert….but I am fascinated with the complexity of cellular therapies and how we can provide appropriate potency data.

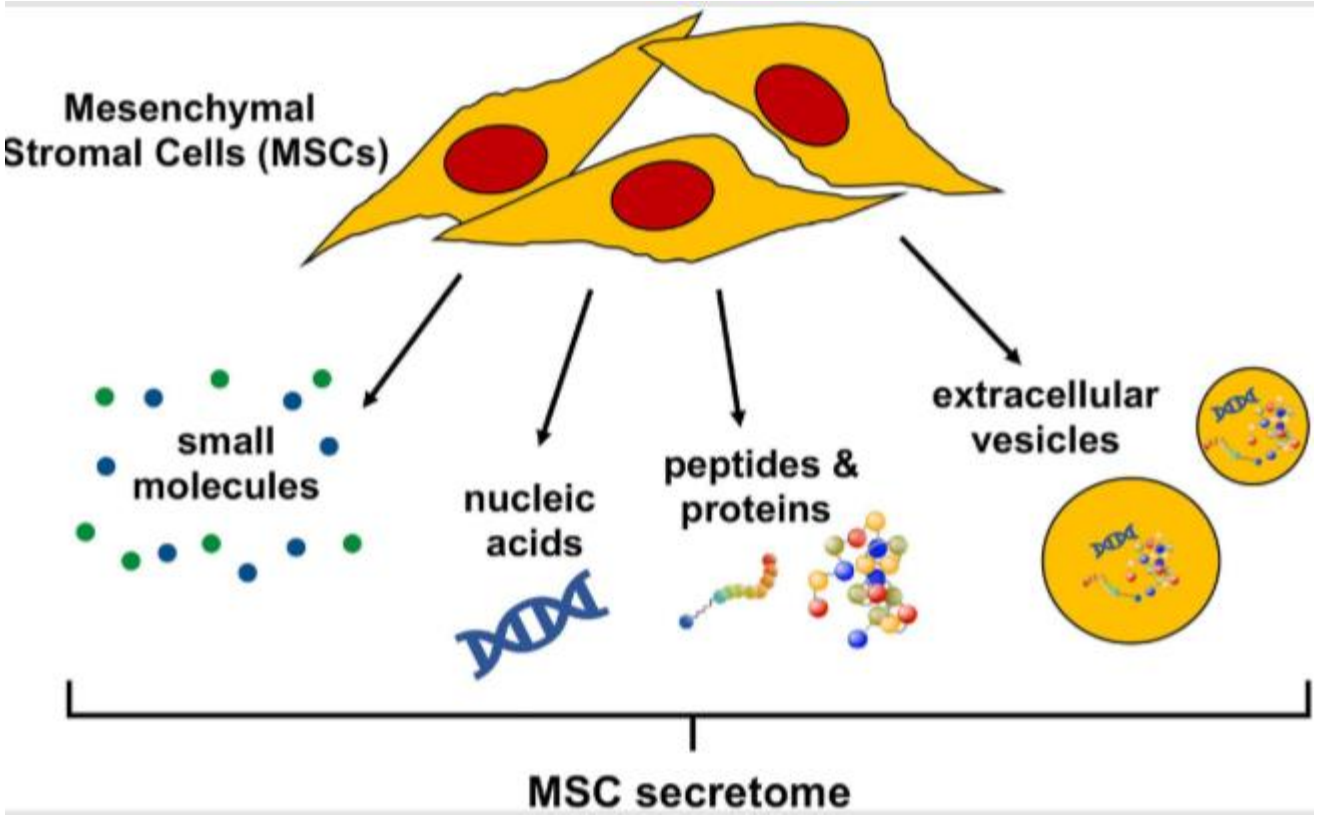
I have worked with early stage MSC products in research institutes and the “arrogant naiveté” was horrifying. These are not easy products to ensure batch-to-batch consistency and assuming that a simple viability assay is *NOT* going to be considered a CMC product potency release assay.

The Secret of MSC-based Therapies Lies in their Secretomes

It is widely recognized that the main mechanism for the regenerative potential of MSCs is not their differentiation.

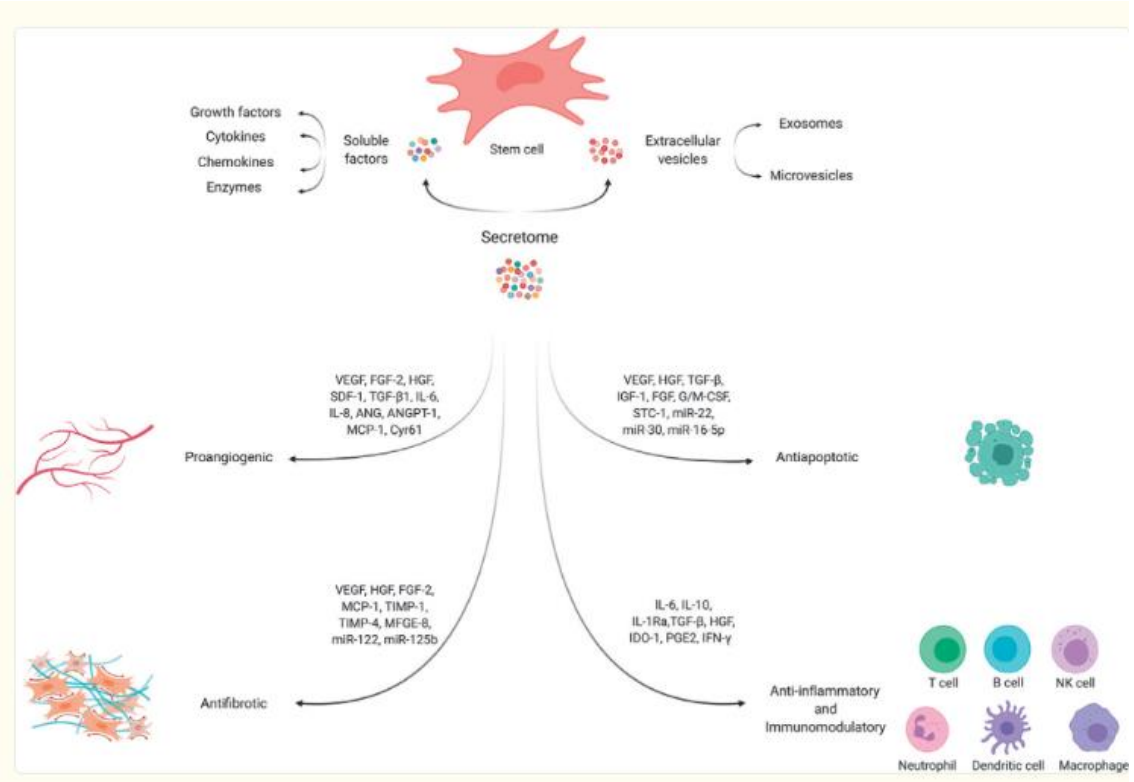
In the context of MSC transplantation *in vivo* data revealing transient and low engraftment rates.

MSCs therapeutic effects are mainly attributed to its secretome, i.e., paracrine factors secreted by these cells…



Secretomes have many Potential Active Ingredients which are important for a Variety of Different Therapeutic Targets

Recent Clinical Studies with Secretomes
Chronic Ulcer/Wound Healing
Cerebral Palsy
Ligament Rupture
Keloid
Osteoarthritis, Knee
Residual Burn Wound
Ischemic Stroke
Retinitis Pigmentosa
Alopecia, Androgenetic
Keloid
Skin Grafting (care of donor sites)
Hypertrophic Scar
Bone Loss
Lupus Erythematosus
Rheumatoid Arthritis
Polycystic Ovary Syndrome
Corneal Defect
Bone Loss
Skin Aging
Muscle Atrophy



This is Good News….Bad News Scenario

Good News: Opens the door for cell-free cellular therapy!

Bad News: Primary MSC Cells are the producers of the MSC Secrotome!

Primary Cell Problems

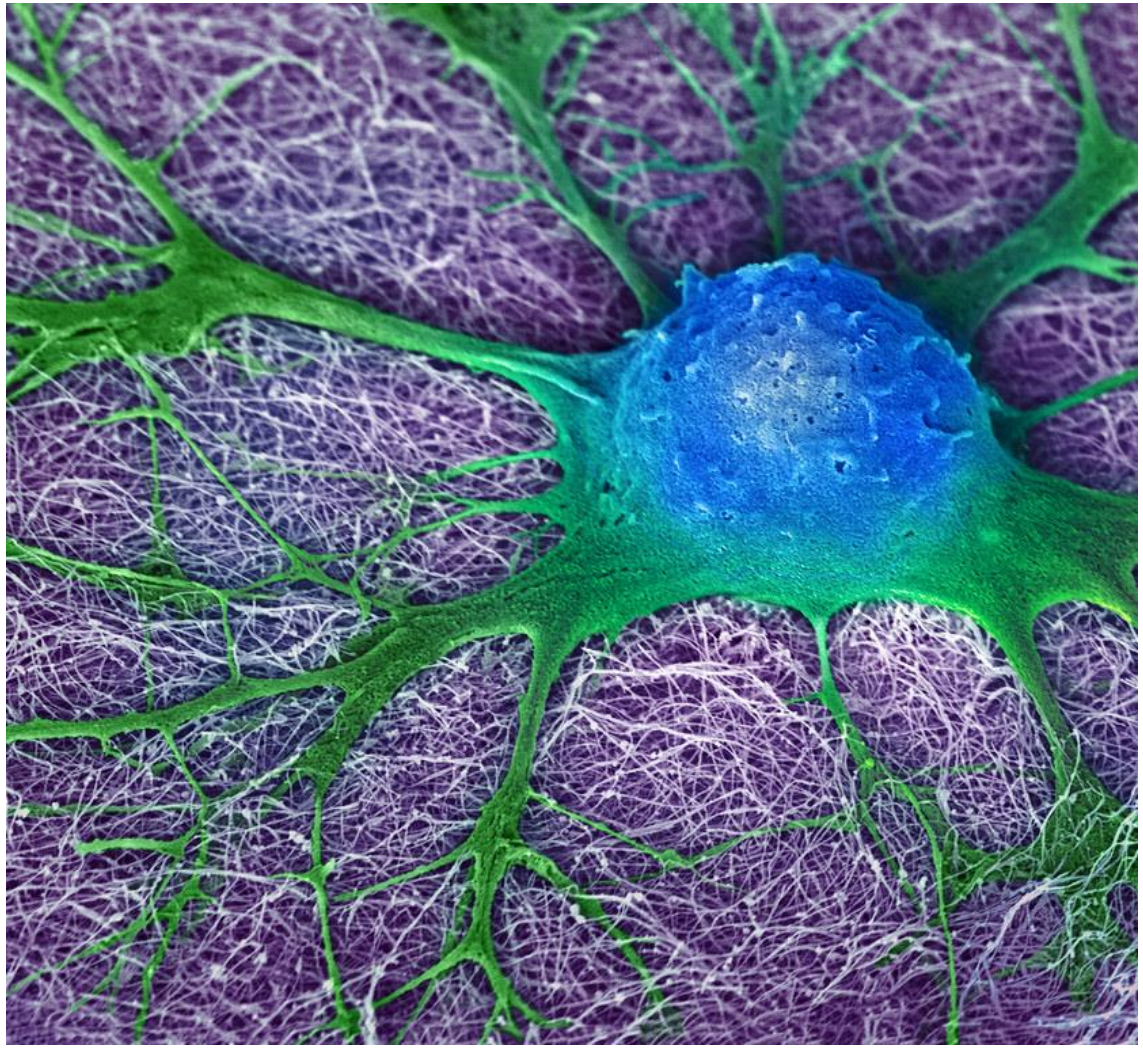
- Need a large number of donors
- Source- and donor-dependent variability of MSC and secretome properties
- Rapid aging of an MSC culture (up to 7–9 passages)
- Biological and microbiological characterization of each new MSC culture

Immortalized MSC Cultures

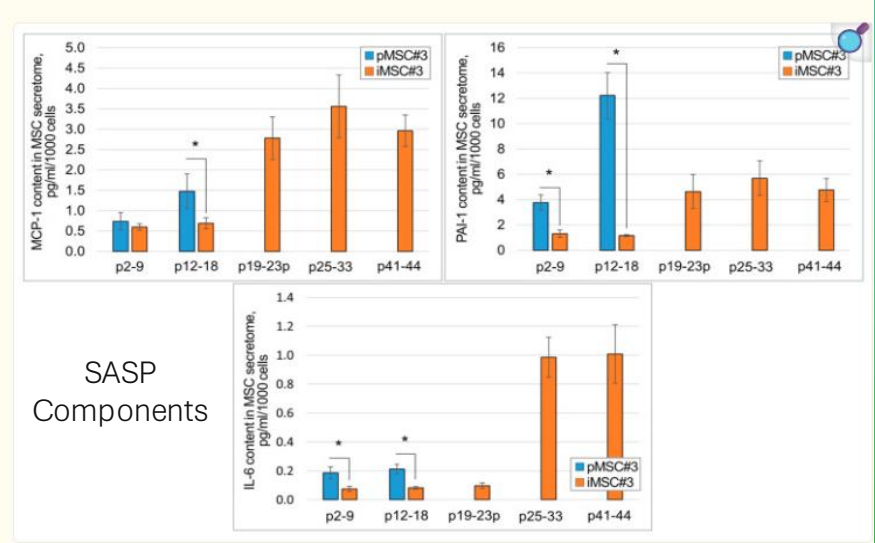
Cells are immortalized by overexpression of the telomerase reverse transcriptase catalytic subunit gene (TERT)

Two Main questions raised in the paper:

- What is the influence of the immortalization procedure on the stability of MSC secretome composition, its pro-regenerative properties?
- What is the potential tumorigenicity of the immortalization?



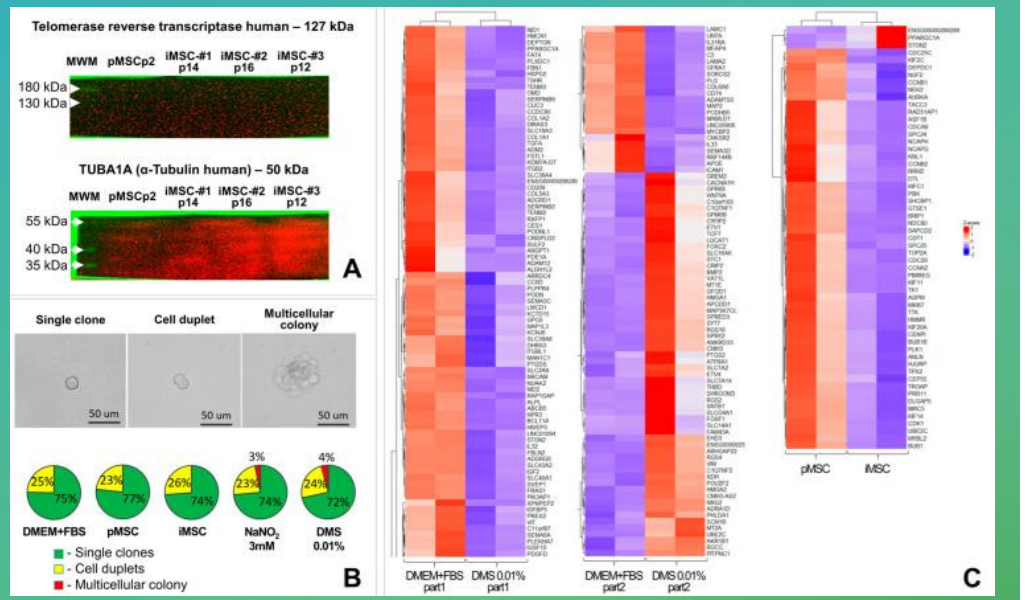
How did they study these Questions?



SASP Components

1. Lots of non-potency content assays including:
   - Proteomic analysis of secretory of primary MSC (pMSC) vs. TERT-immortalized MSC(iMSC)
     - Total of 1338 proteins identified: 1207 in pMSC and 1214 in iMSC. They were 94.5% identical
     - qPCR and ELISA tests of the pMSC and iMSC secretomes. for key neurotrophic and proangiogenic molecules (BDNF, VEGF, uPA and HGF) showed no differences
     - Expression of the secretome components associated with cell culture senescence (the so-called SASP components) were decreased with iMSC

Potential Tumorgenicity



Took 3 approaches:

1. Looked for detectable amounts of telomerase activity in iMSC.
2. Does iMSC transform primary human dermal fibroblasts into colonies in soft agar colony formation assay
3. Does iMSC Secretome alter the expression of Pro- and Anti-Oncogenes in the Culture of Primary Human Dermal Fibroblasts?

Three BioAssays used to Assess potency of the Secretome: Assay 1 Increased Testosterone Production

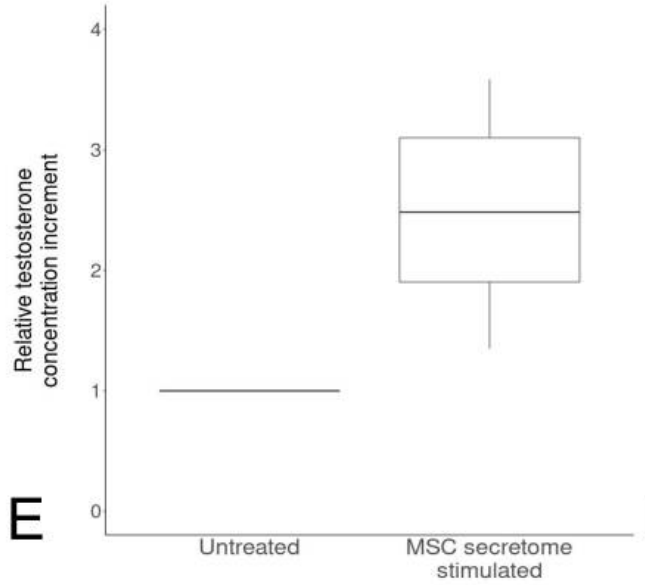
ASSAY #1Testosterone production by Leydig cells (Male infertility)

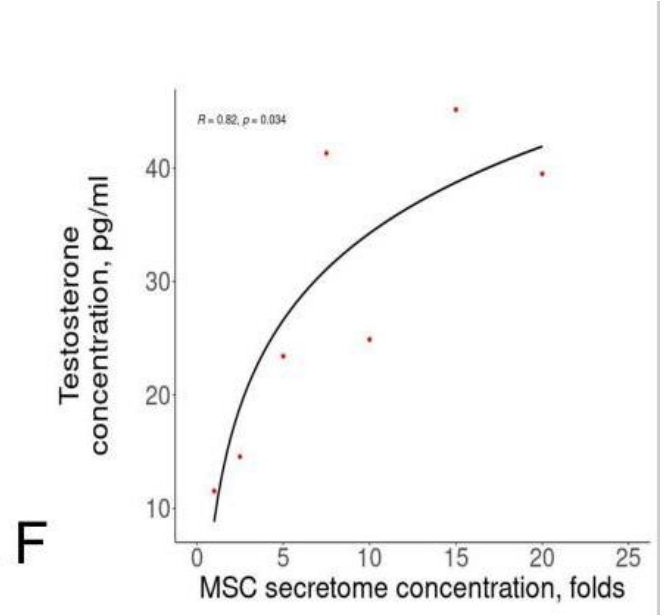
Monakova A., Sagaradze G., Basalova N., Popov V., Balabanyan V., Efimenko A. Novel Potency Assay for MSC Secretome-Based Treatment of Idiopathic Male Infertility Employed Leydig Cells and Revealed Vascular Endothelial Growth Factor as a Promising Potency Marker. Int. J. Mol. Sci. 2022;23:9414. doi: 10.3390/ijms23169414. [DOI] [PMC free article] [PubMed] [Google Scholar]

a) Leydig cells were seeded in DMEM:F12 with 2% FBS and supplements.

b) Two days after Leydig cell isolation, the medium was changed to either DMEM:F12 with supplements (control) or the MSC secretome.

c) Two days after the addition of the MSC secretome, testosterone concentrations were measured in secretome samples of Leydig cells treated with the control medium or the MSC secretome.





Three BioAssays used to Assess potency of the Secretome: Assay 2 (Prevent fibrosis)

<u>Assay 2: TGF-b induced fibroblast-to-myofibroblast differentiation (Cardiac Treatment)</u>

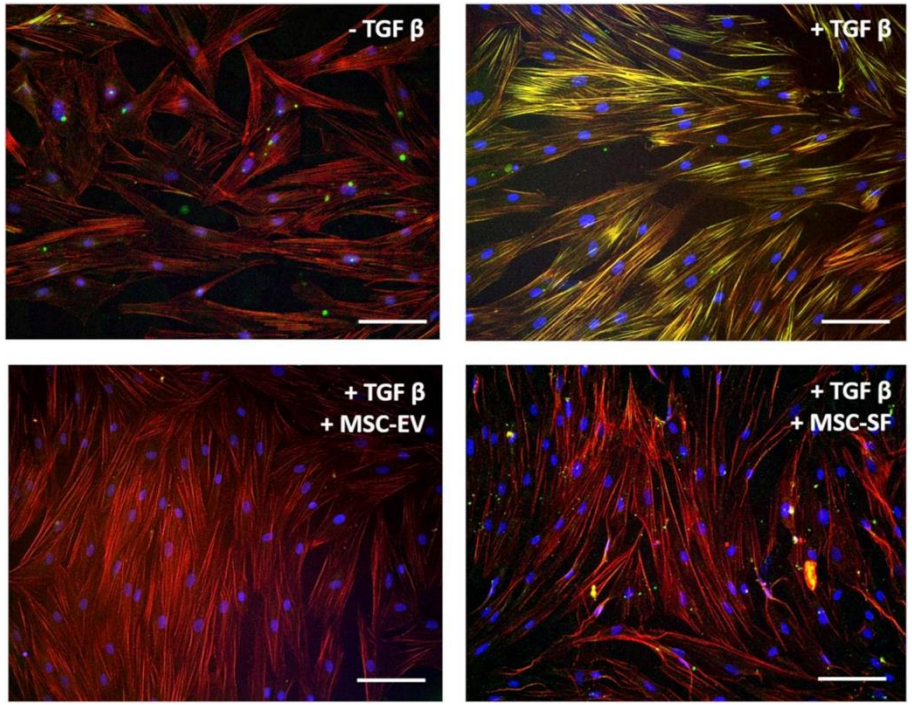
Basalova N, Sagaradze G, Arbatskiy M, Evtushenko E, Kulebyakin K, Grigorieva O, Akopyan Z, Kalinina N, Efimenko A. **Secretome of Mesenchymal Stromal Cells Prevents Myofibroblasts Differentiation by Transferring Fibrosis-Associated microRNAs within Extracellular Vesicles.** Cells. 2020 May 20;9(5):1272. doi: 10.3390/cells9051272. PMID: 32443855; PMCID: PMC7290371.

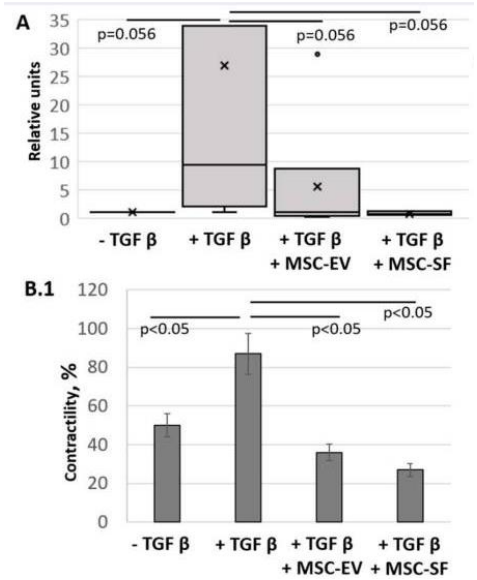
a) Culture Human dermal fibroblasts (HDF)

b) Induce differentiation of HDF to myofibroblasts by culturing in serum-free culture with 5 ng/mL TGFbeta ± Secretome

c) Culture for 4 days

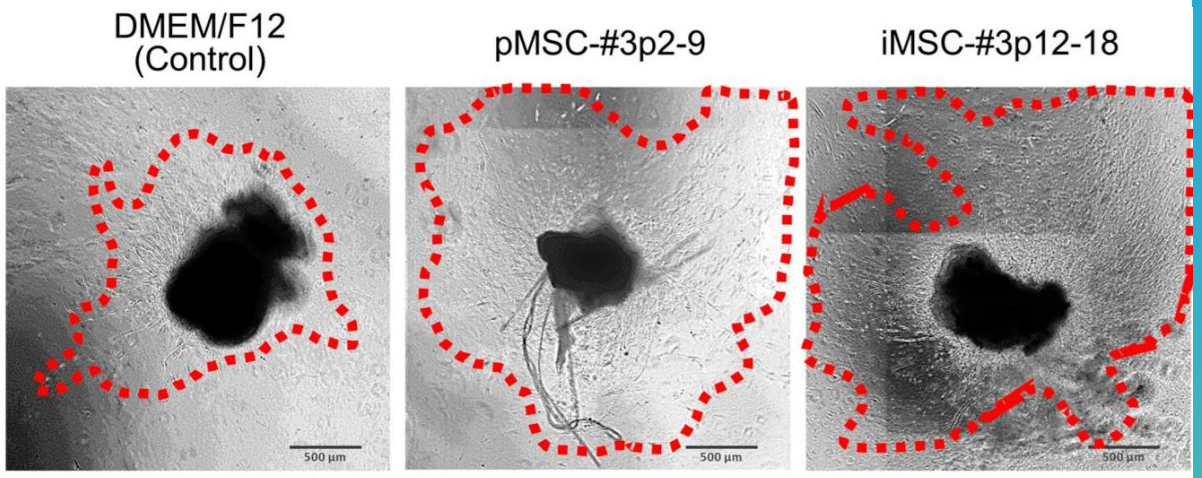
MSC secretome on the **expression of type I collagen** by myofibroblasts.

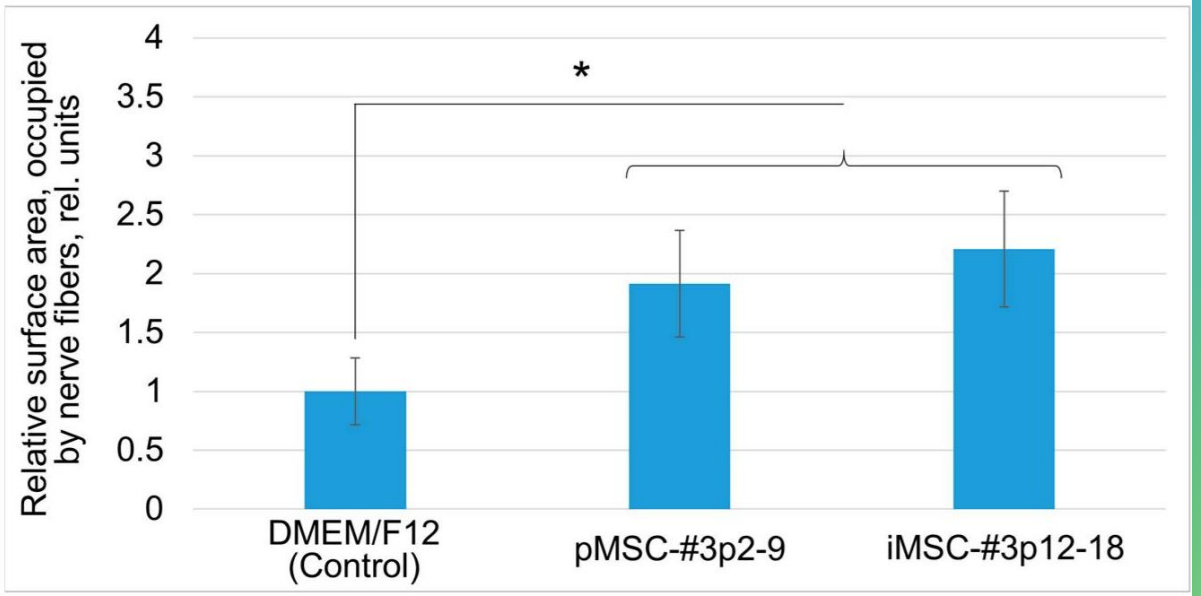




Three BioAssays used to Assess potency of the Secretome: Assay 3 (Regeneration of neuronal cells)

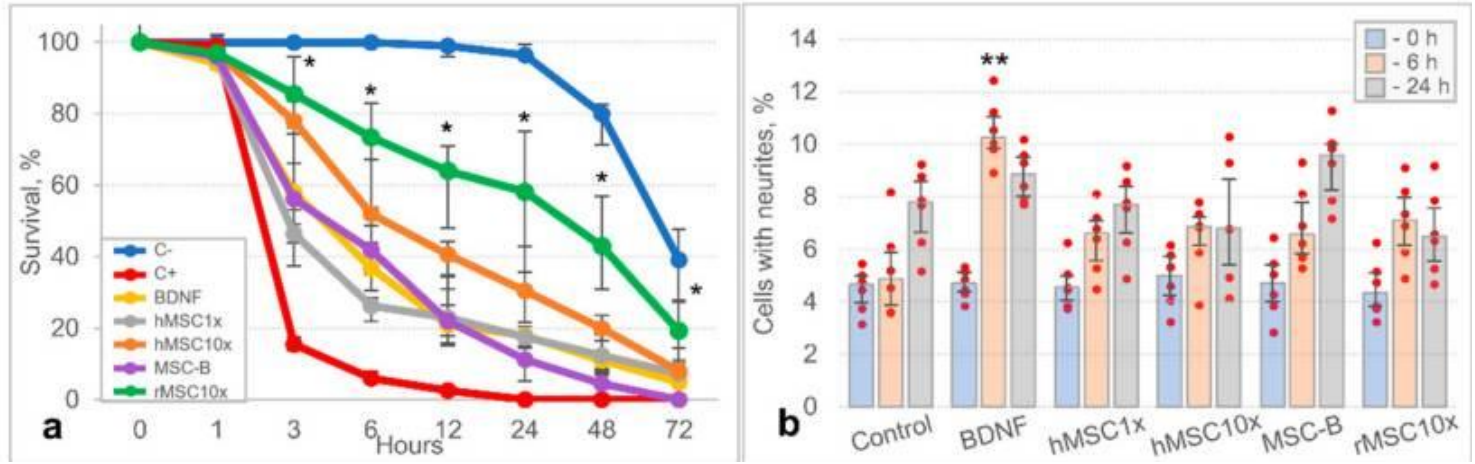
- Stimulation of Neurite Growth of Murine Sensory Ganglions (In Vitro Model of Neuritogenesis)
  - in vitro murine DRG explant model
  - Dorsal root ganglia (DRG) is isolated from adult Schwann cells (ScC). These are embedded into type I collagen gels and cultured for 3 days in serum-free medium.
  - The dynamics of neurite growth in the obtained ganglion cultures were recorded every 3 days (up to 20 days) by taking pictures using an inverted wide-field microscope, the Nikon Eclipse Ti2, equipped with a Kinetix digital monochrome camera (Teledyne Photometrics, Seattle, WA, USA) and a Ri2 color camera (Nikon, Tokyo, Japan). The images obtained were analyzed using NIS Elements AR 5.40.02 and FIJI ImageJ 1.54 p software (GitHub Inc.).





Looking thru the references the Following Assay was found for Neuroprotective Activity

Karagyaur M, Dzhauari S, Basalova N, Aleksandrushkina N, Sagaradze G, Danilova N, Malkov P, Popov V, Skryabina M, Efimenko A, Tkachuk V. MSC ***Secretome as a Promising Tool for Neuroprotection and Neuroregeneration in a Model of Intracerebral Hemorrhage***. Pharmaceutics. 2021 Nov 29;13(12):2031. doi: 10.3390/pharmaceutics13122031. PMID: 34959314; PMCID: PMC8707464.



- SH-SY5Y neuroblastoma cells were seeded in 48-well plates in complete growth medium at 40,000 cells/well in quadruplicates.
- The medium was removed the next day, and samples of serum-free medium (C+), serum-free medium with 3.5 ng/µL human BDNF (BDNF) or MSC secretome (hMSC1x, hMSC10x, MSC-B, or rMSC10x), were added, each supplemented with 100 mM L-glutamate [26] and 5 µM IncuCyte® Caspase-3/7 Apoptosis Reagent (Essen Bioscience, #4440, Ann Arbor, MI, USA).
- In the control group C-, no L-glutamate, BDNF, or MSC secretome were added to monitor the spontaneous cell death in serum-free medium. Glutamate causes a rapid increase in the cytosolic concentration of Ca^{2+} in SH-SY5Y cells (similar to what happens in neurons), with subsequent caspase activation.
- The IncuCyte® Caspase-3/7 Apoptosis Reagent, cleaved with activated caspase-3/7, stains nuclear DNA (green fluorescence). To monitor the death of SH-SY5Y cells, the plate was placed in the Incucyte® ZOOM Live Cell Analysis System (Essen Bioscience, Ann Arbor, MI, USA), located inside a carbon dioxide (CO_2) incubator. The time-lapse imaging of nine fields of vision (phase and green channel) of each well was performed 1, 3, 6, 12, 24, 48, and 72 h after medium replacement.

And that is all Folks…..

Seville, Spain Site of our Next EU BEBPA BioAssay Conference (23-25 September 2026)

Our next Journal Club is scheduled for 11 June 2026. We will meet 4x per year and we need:

- You….to present. Email us at contactus@bebpa.org
- These slides will be available on the BEBPA website https://bebpa.org/journal-club/
- At the link above you will also find potency assay literature

Thank you for attending the inaugural BEBPA Journal Club