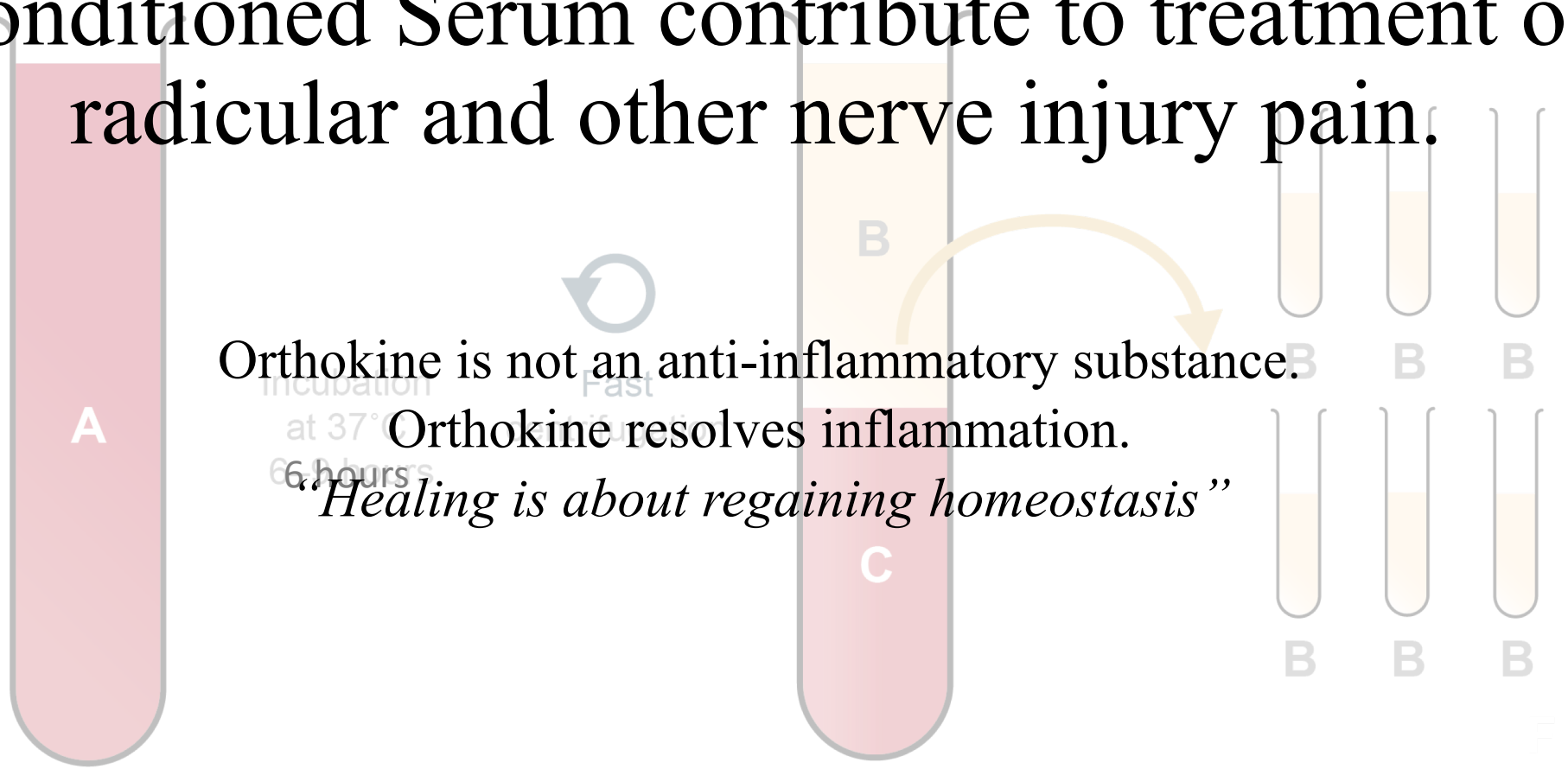
Extracellular Vesicles in Orthokine® Autologous Conditioned Serum contribute to treatment of radicular and other nerve injury pain.

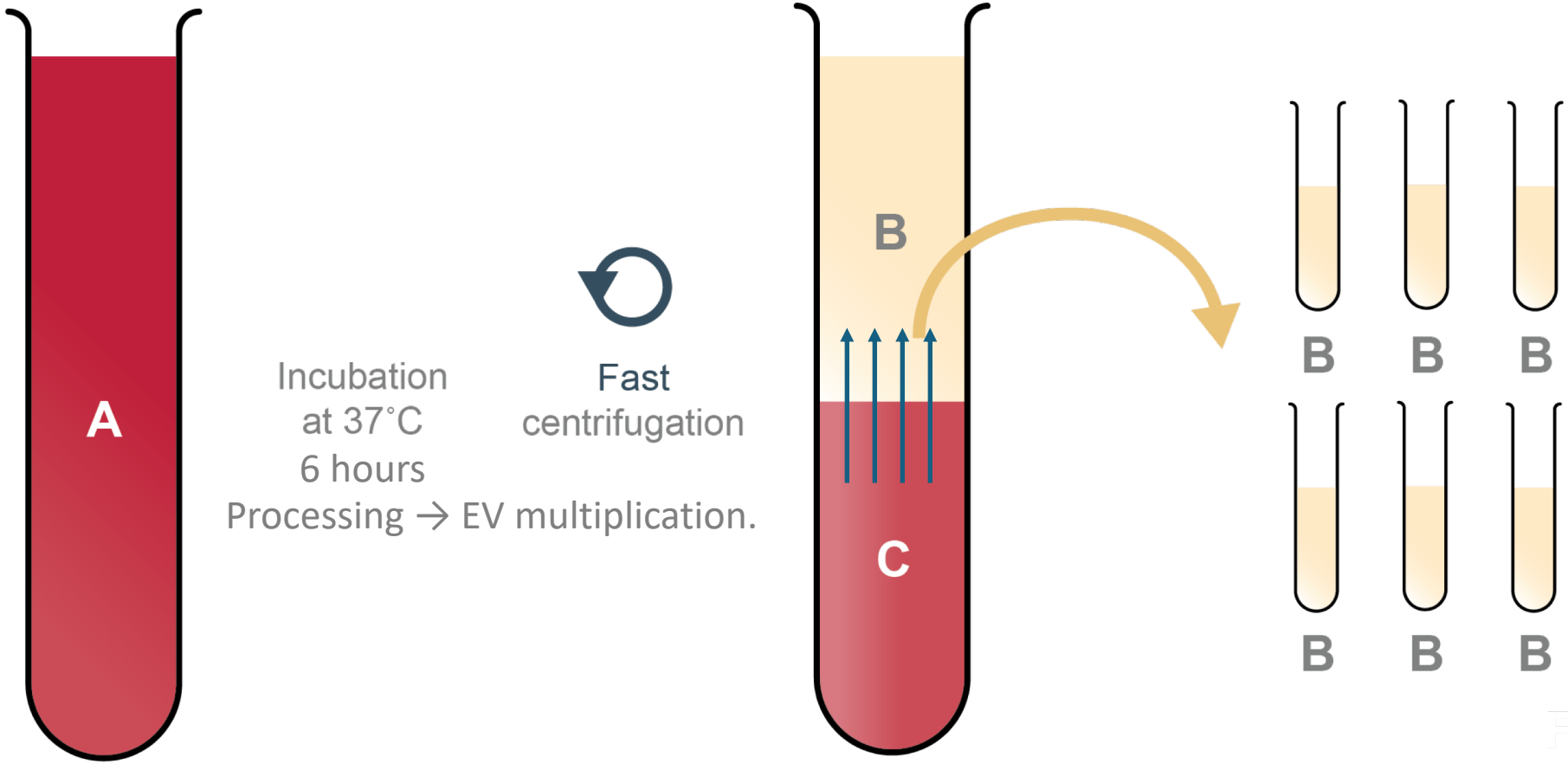


Orthokine is not an anti-inflammatory substance.
Orthokine resolves inflammation.
"Healing is about regaining homeostasis"

Dr. Julio Reinecke
Private Clinic Prof. Wehling, Düsseldorf
ORTHOGEN, Düsseldorf

When does an injury heal well?

Common medical wisdom says: when it bleeds and blood clots.



A: Whole blood

B: Cell-free serum
C: Clot

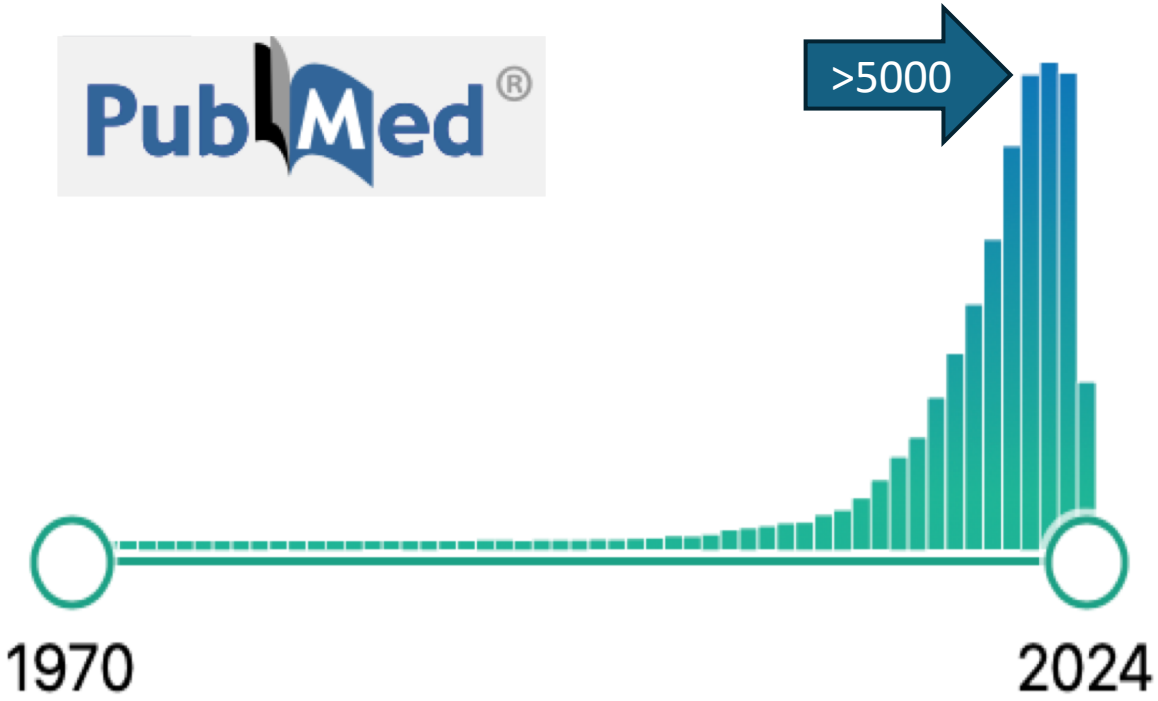
ACS portioned
and can be frozen at
-18°C or lower for up to 12 months

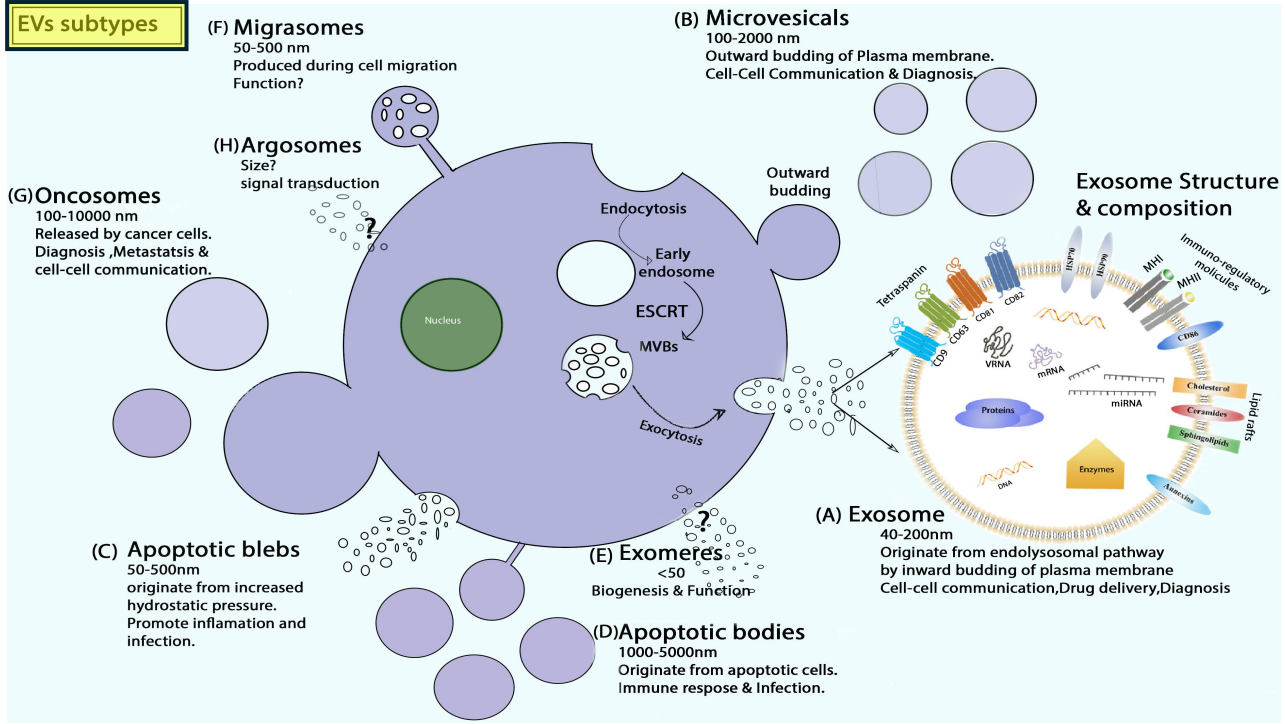
ACS mediator summary.

Ingredient	Detail/Effect
Cytokines	IL-1Ra, IL-10, IL-4, MCP-1, TNF, Rantes et al.
Growth Factors (PDGF, VEGF, IGF et al.)	Regenerative (cell division, vascularization et al.)
Lipid Mediators (omega3-derived SPM)	resolve inflammation, Macrophage shift M1→M2
Extracellular Vesicles (incl. Exosomes)	Immune-modulatory, Inflammation resolving
Mitochondrial derived peptides	modulate adaptative responses to metabolic stress
Neutrophil mediators	Alarmins
Others	Gelsolin, Thy-1, SDF-1a, SCGFb, NFkB et al.

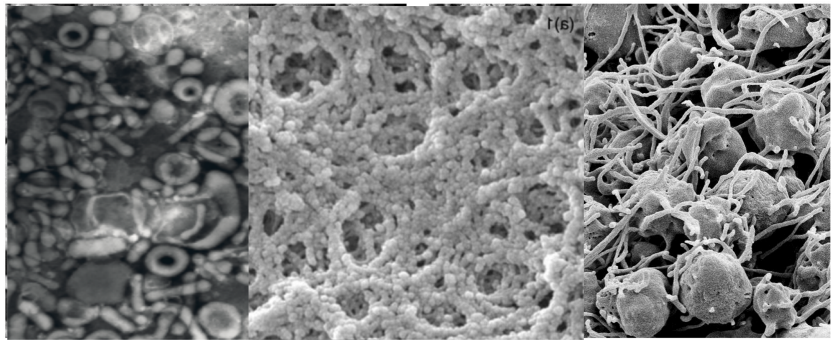
What are these fancy EVs?

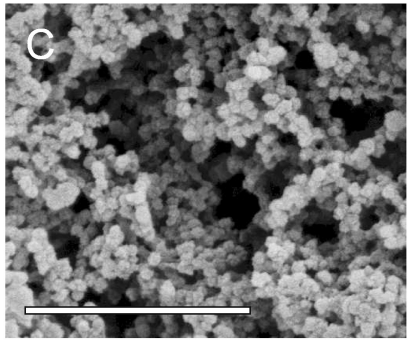
EVs have gained high popularity and show amazing effects.

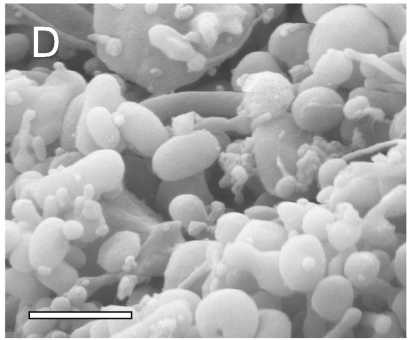




EVs should be cup-shaped and are multi-sized.







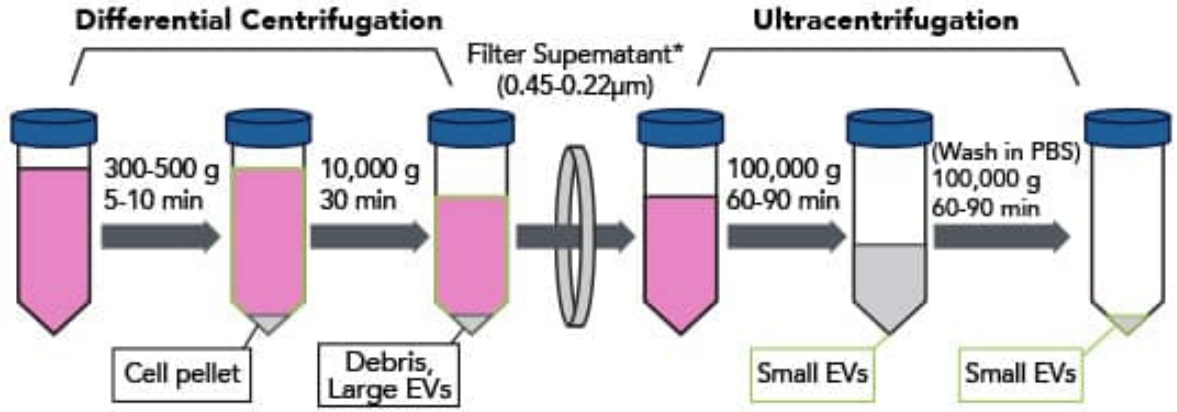
EVs are released by all cells and enter the body fluids.

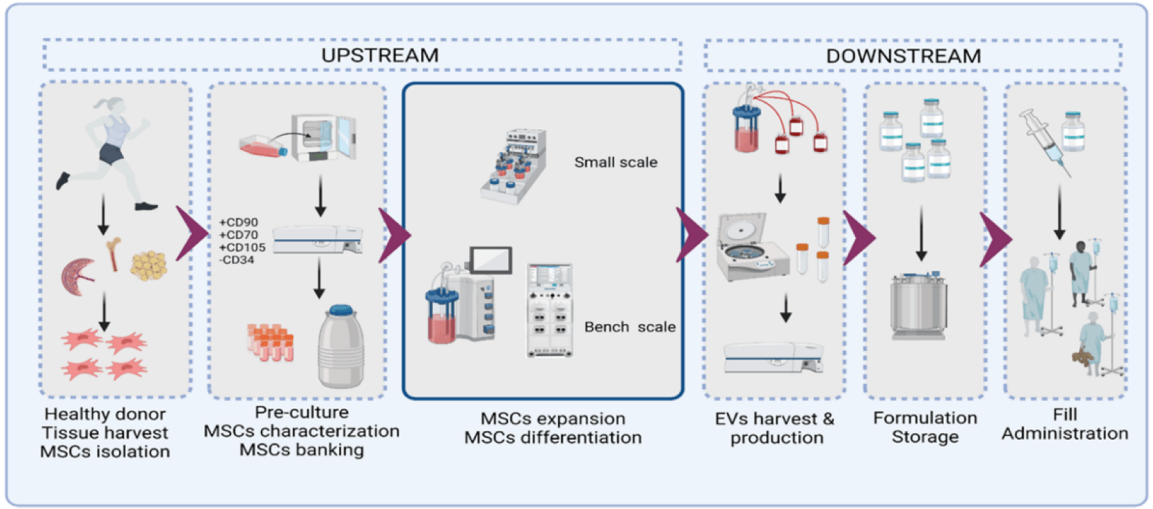
EVs orchestrate the communication between cells/tissues/organs.

You want to use EVs for therapy?

- Blood is the most reliable and sustainable source for autologous Exosomes.
- Blood-EVs are geared to tissue healing.
- EVs hone their efficacy during ACS-processing.
- The protocol is designed to be a self optimizing process.
- You only need the ACS, EVs come for free.
- No worries about "*how many platelets or WBC do I have in my PRP*".

You want to purify from cell culture?
Or rather take the culture supernantant directly?

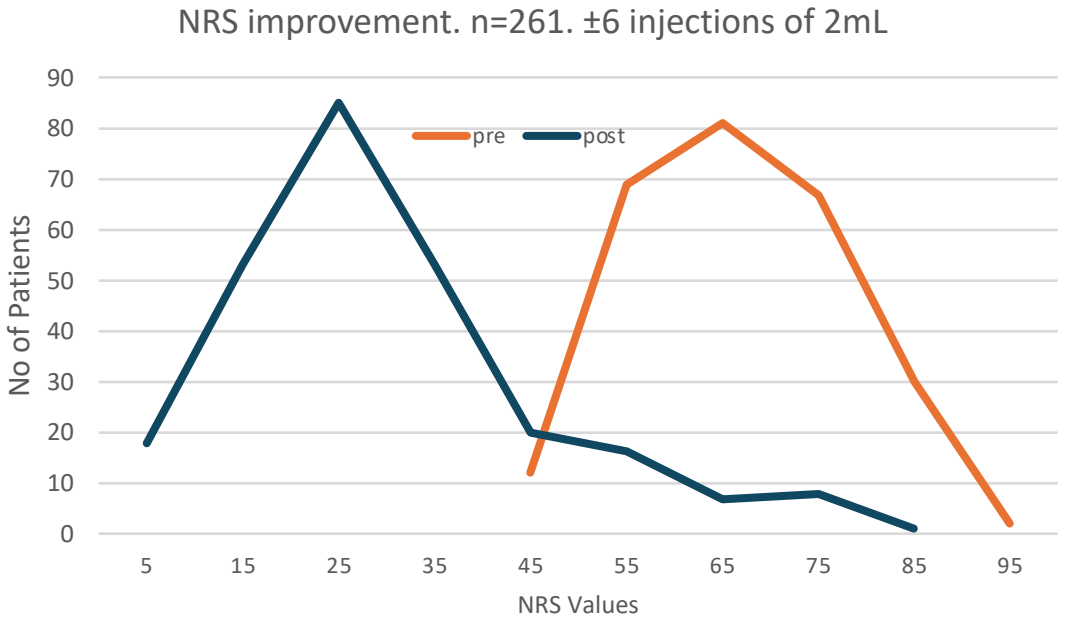




ACS contains ≥10^{10} EVs/mL, *de-chronifies* neural pain.

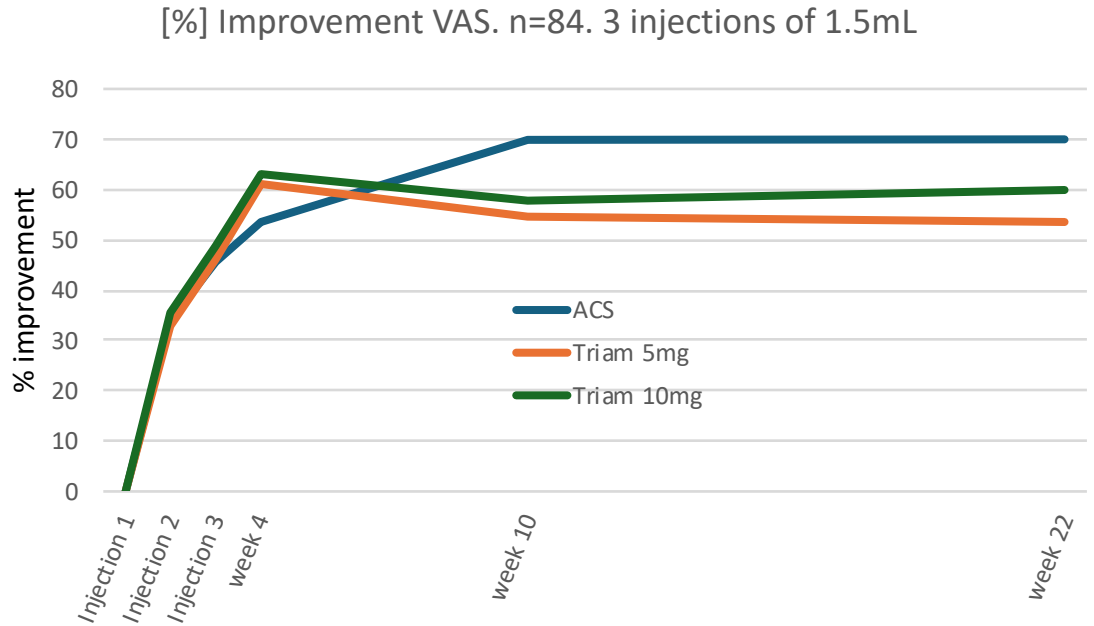
That's:10,000,000,000 per mL

Orthokine®-Therapy: Autologous Conditioned Serum for the Spine. Observational study. N= 261 Patients



Randomized Controlled Trial > Spine (Phila Pa 1976). 2007 Aug 1;32(17):1803-8.
doi: 10.1097/BRS.0b013e3181076514.

Efficacy of epidural perineural injections with autologous conditioned serum for lumbar radicular compression: an investigator-initiated, prospective, double-blind, reference-controlled study



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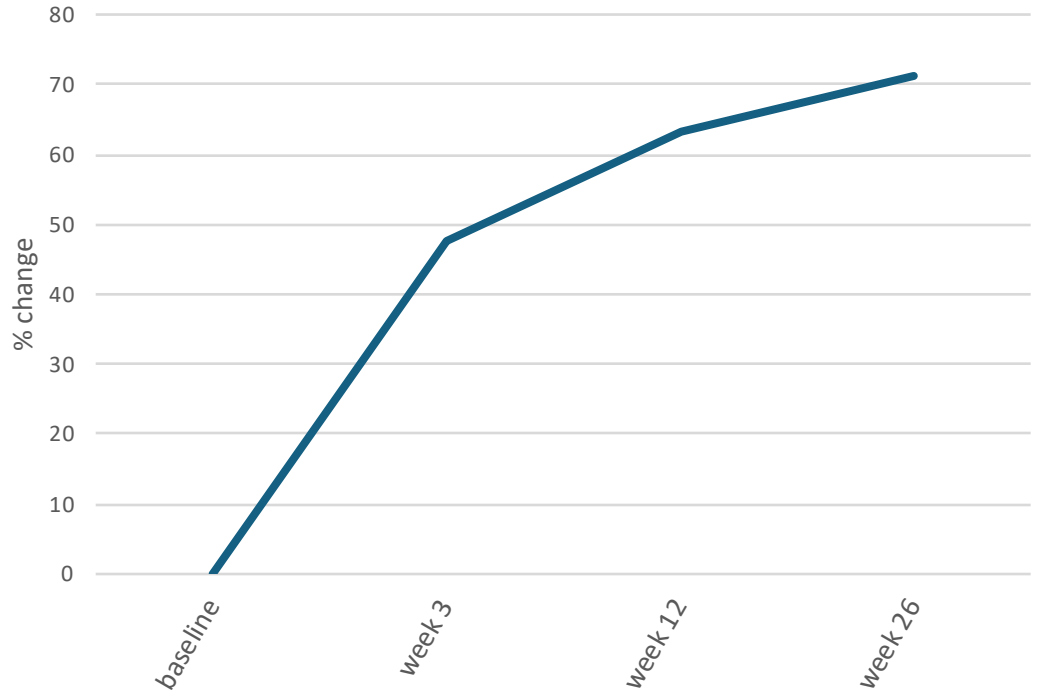
> Asian Spine J. 2015 Dec;9(6):916-22. doi: 10.4184/asj.2015.9.6.916. Epub 2015 Dec 8.

Autologous Conditioned Serum as a Novel Alternative Option in the Treatment of Unilateral Lumbar Radiculopathy: A Prospective Study

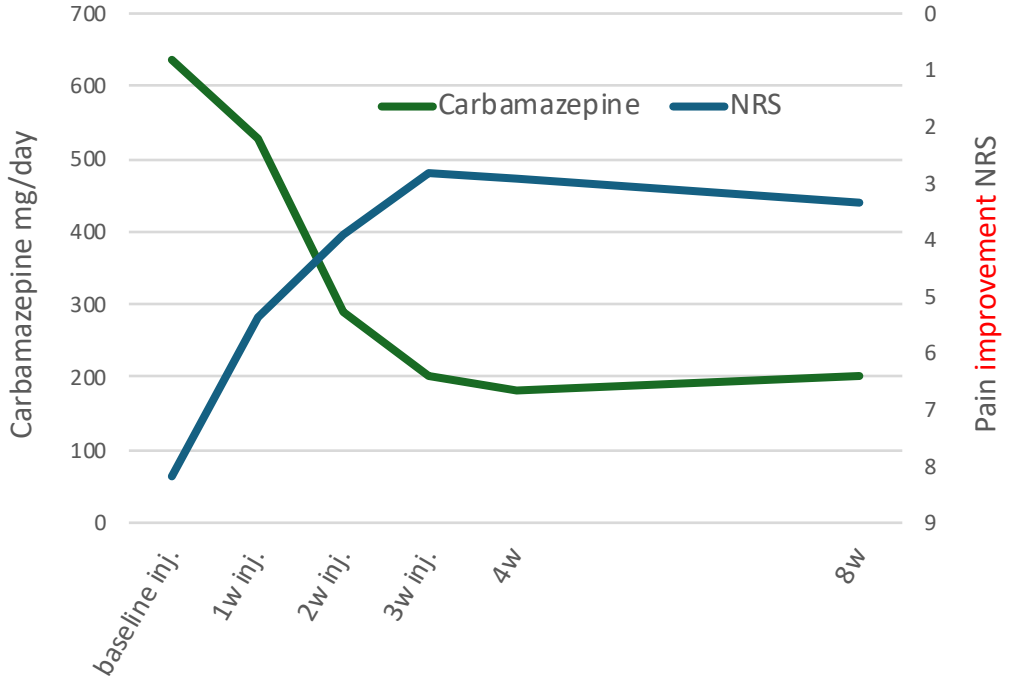
%VAS change vs baseline. n=20, "1-3" injections of 2mL



Case Reports > J Med Case Rep. 2022 May 8;16(1):183. doi: 10.1186/s13256-022-03393-9.

Autologous conditioned serum (Orthokine) injection for treatment of classical trigeminal neuralgia: results of a single-center case series

4x 2mL ACS per injection reduces pain and Carbamazepine consumption. n=11



Mouse model of “therapy-induced senescense“ allows a deeper look at ACS-EV’s action.

Brain Behav Immun. 2023 Jul:111:298-311. doi: 10.1016/j.bbi.2023.04.013. Epub 2023 May 5.

Intrathecal administration of conditioned serum from different species resolves Chemotherapy–Induced neuropathic pain in mice via secretory exosomes

Conditioned Serum = CS

Paclitaxel (PTX) induces Neuropathic Pain[1]

PTX → disrupts axonal transport → ATP deficiency, oxidative stress, and mitochondrial damage.[2]

PTX → expression of cytokines such as IL-1b, IL-8, and TNFa, leading to glial activation and pain.

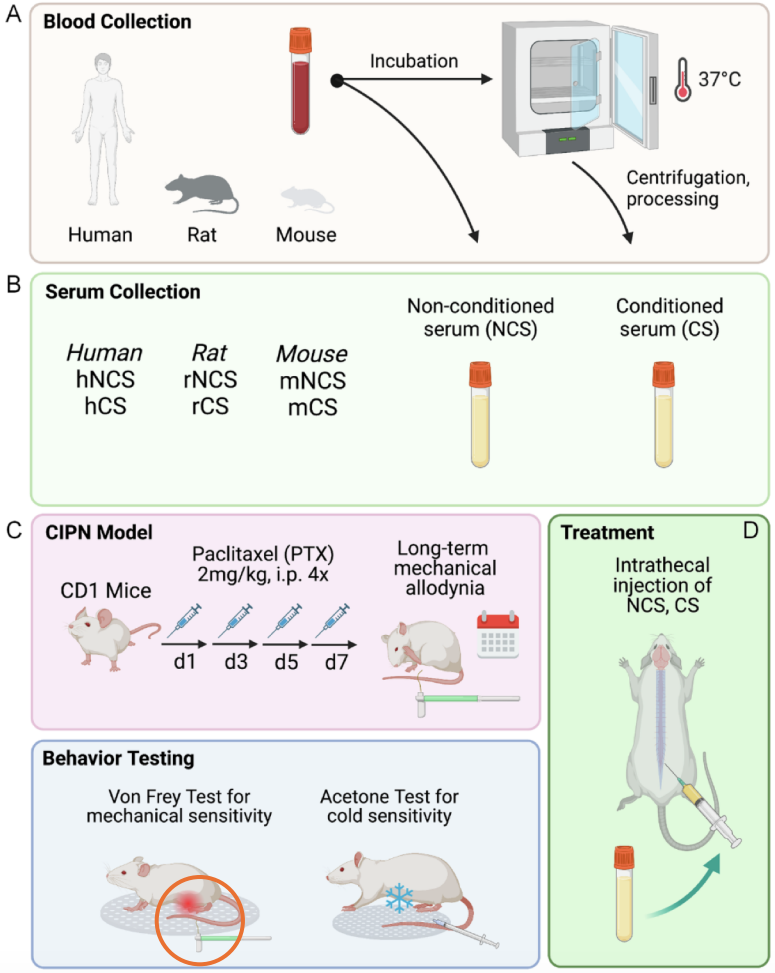
PTX → inflammation and axonal damage in spinal cord and dorsal root ganglia.

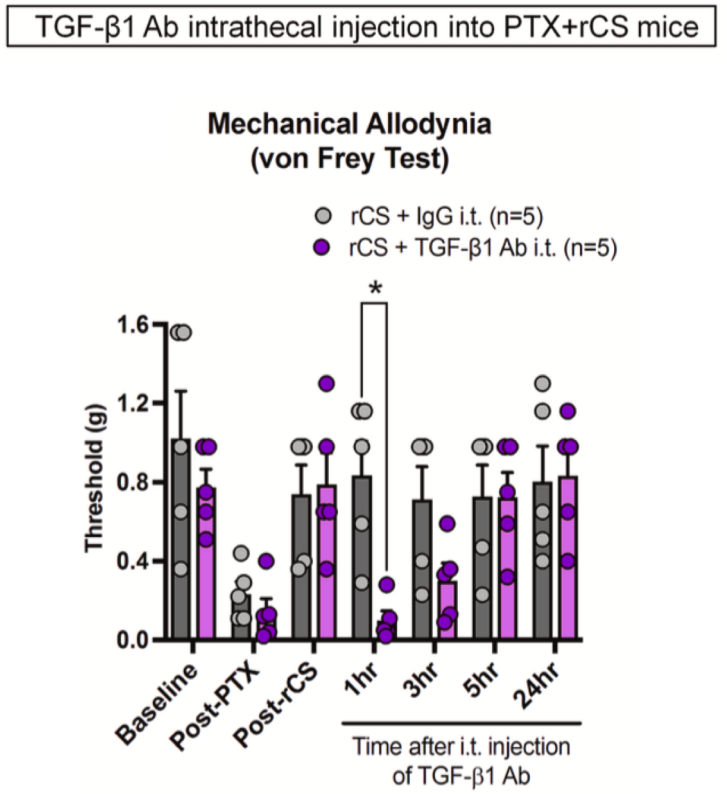
PTX → ROS (oxygen radicals from dysfunct mitochondria) and MMP13 production.[3]

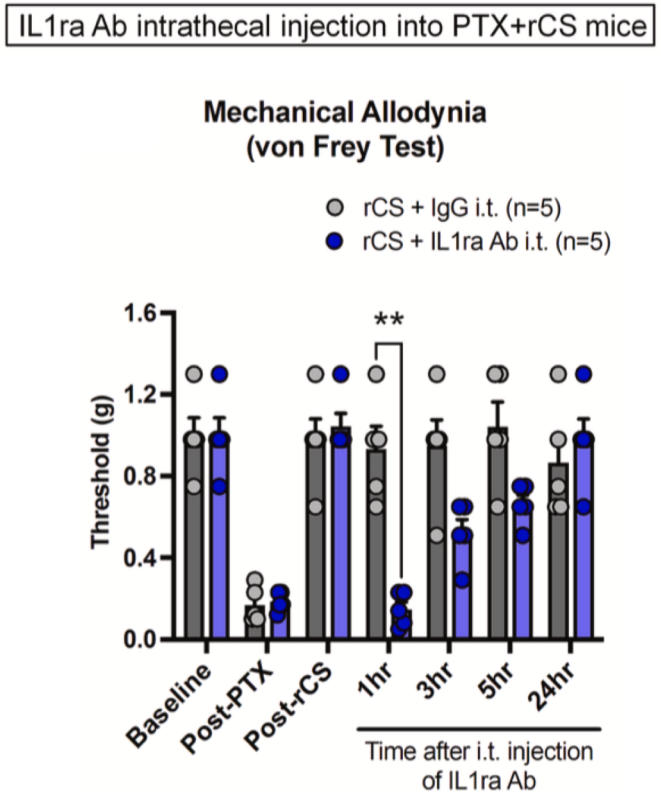
1. Buchheit T, Huh Y, Breglio A, et al. Intrathecal administration of conditioned serum from different species resolves Chemotherapy-Induced neuropathic pain in mice via secretory exosomes. *Brain Behav Immun*. 2023;111(April):298-311. doi:10.1016/j.bbi.2023.04.013
2. Klein I, Lehmann H. Pathomechanisms of paclitaxel-induced peripheral neuropathy. *Toxics*. 2021;9(10):229. doi:10.3390/toxics9100229
3. Cirrincione AM, Pellegrini AD, Dominy JR et al. Paclitaxel-induced peripheral neuropathy is caused by epidermal ROS and mitochondrial damage through conserved MMP-13 activation. *Sci Rep*. 2020;10(1):1-12. doi:10.1038/s41598-020-60990-8.

Mouse model of Paclitaxel induced peripheral neuropathy (CIPN).

ACS: The role of cytokines?
AB-inhibition IL-1Ra and TGFb1 reverses CS-caused analgesia temporally.



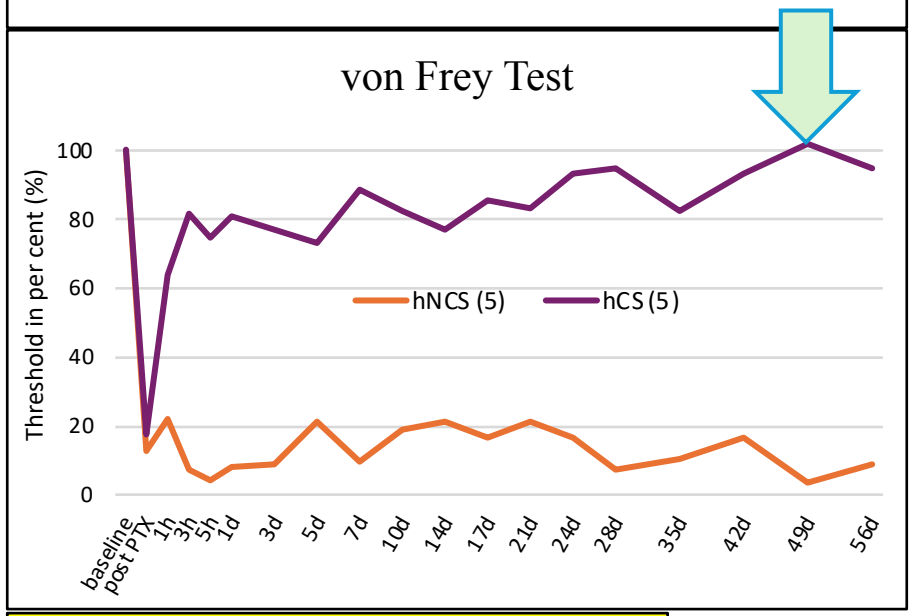




Buchheit T, Huh Y, Breglio A, et al. Intrathecal administration of conditioned serum from different species resolves Chemotherapy-Induced neuropathic pain in mice via secretory exosomes. *Brain Behav Immun*. 2023;111(April):298-311. doi: 10.1016/j.bbi.2023.04.013

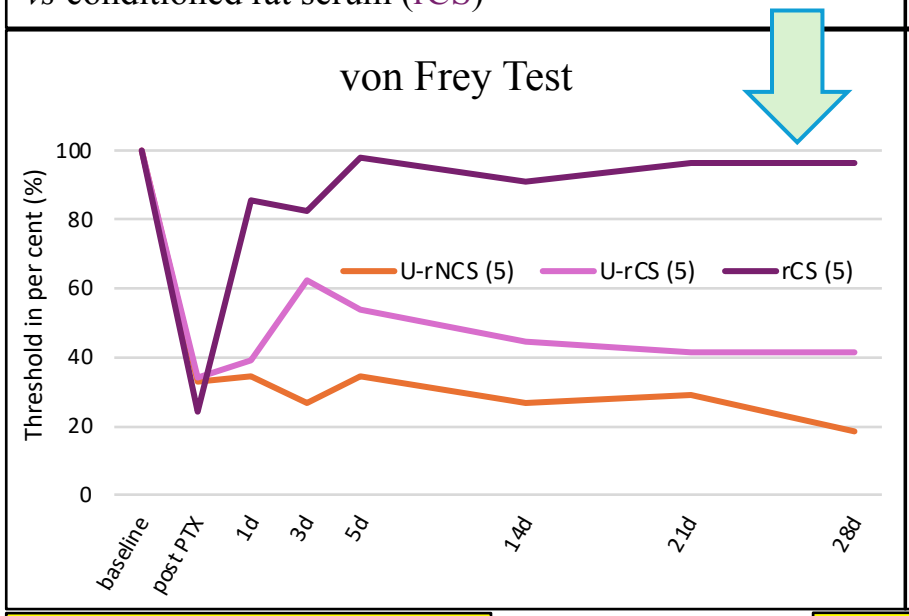
CS "wins" von Frey test over non-conditioned serum until 56 days. The role of EVs: CS without EVs is near impotent.

Non-conditioned human serum (hNCS)
vs conditioned human serum (hCS)



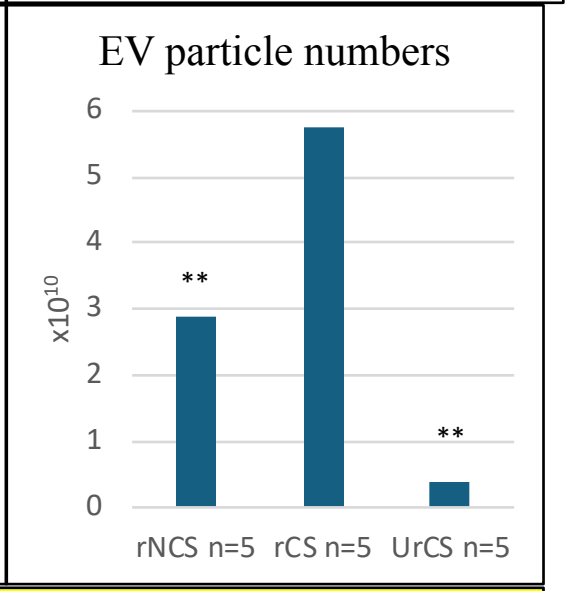
You need conditioning for effect

Non-conditioned EV-depleted rat serum (U-rNCS)
vs conditioned EV-depleted rat serum (U-rCS)
vs conditioned rat serum (rCS)



You need EVs for effect

Centrifugation at $1.2x10^5$g removes most EVs from serum.
rNCS: **rat** non-conditioned serum
rCS: **rat** conditioned serum
U-rCS: ultracentrifuged **rat** conditioned serum



You need conditioning for EVs

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Conclusions from PTX-CIPN mouse model.

Intra-thecal injection of 10µl of human or rat CS resolves PTX-CIPN for at least 15% of mouse life expectancy.

i.t. injection of 10µl of human or rat NCS does not resolve PTX-CIPN.

Human, rat, blood duplicates EV counts during conditioning process.

Removal of EVs from CS impairs the resolution effect.

- ACS-EV are main contributors to CIPN resolution.
- ACS-EV can act across species boundaries.
- ACS-EV have more profound effect than cytokines/growth factors.

ACS (EVs) employ tissue stem cells.

ACS fortifies *i.v.* ”stemness“ of human adMSC (CD90 ↑).[1]

ACS fortifies *i.v.* immunmodulatory effect of human adMSC ($CD4^+$ und $CD8^+$ Lymphocytes ↓ and $CD45RA^+$ $CD62L^+$ ↑ $=T_{cm}$).[1]

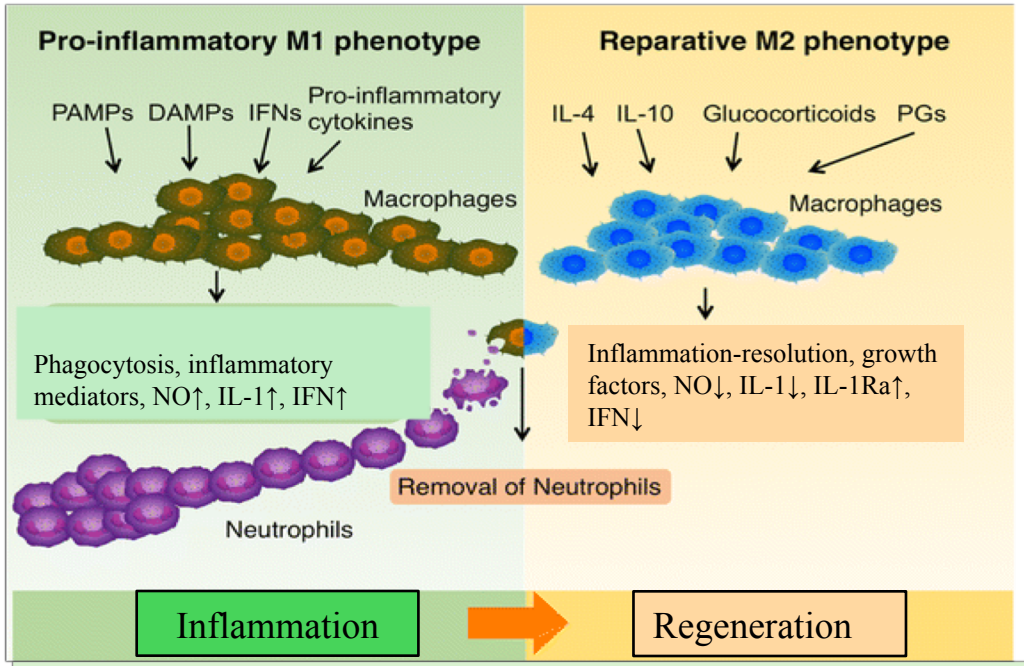
ACS fortifies *i.v.* cell division ↑ of human adMSC.[1]

ACS activates muscle stem cells *i.v.* ↑ in Mouse-Contusion-Model.[2]

ACS is clinically superior ↑ in muscle injuries vs standard of care.[3]

1: Blazquez et al. DOI 10.3389/fphar.2019.00699
2: Wright-Carpenter et al. DOI 10.1055/s-2004-821303
3: Wright-Carpenter et al. DOI 10.1055/s-2004-821304

ACS (EVs) employ tissue macrophages.



Macrophages are indispensable sentinels of tissue homeostasis.

Biomarkers show:
ACS favours M1→M2 polarisation[1]

b.t.w. → **Glia are improved by ACS**[2]

Shirokova et al.: Osteoarthritis synovia analytics correlates with improved clinics.
ROS↓ NO↓ IL-1↓ IL-1Ra↑ HA↑ VAS↑ WOMAC↑

1. Shirokova et al. Intra-articular injections of a whole blood clot secretome, autologous conditioned serum, have superior clinical and biochemical efficacy over platelet-rich plasma and induce rejuvenation-associated changes of joint metabolism. A prospective, controlled, open label clinical study in knee osteoarthritis. *Rejuvenation Res*. 2020;23(5):401-410. doi: 10.1089/rej.2019.2263
2. Buchheit T, Huh Y, Breglio A, et al. Intrathecal administration of conditioned serum from different species resolves Chemotherapy-Induced neuropathic pain in mice via secretory exosomes. *Brain Behav Immun*. 2023;111(April):298-311. doi: 10.1016/j.bbi.2023.04.013

Orthokine-ACS:
Exosome Therapy for epigenetic re-programming → tissue homeostasis, resolves cellular stress.

Orthokine-ACS is *resolving*, not *anti-inflammatory*

ACS self optimizing process provides EVs et al. for tissue repair.

Restoration of tissue requires massive ATP supply, requires functional mitochondria.

Injured/chronic/senescent tissues suffer excess ROS loads from dysfunctional mitochondria

ACS reduces ROS, restores mitochondrial function[1], enables resolution and healing

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