Biology of Orthokine Therapy

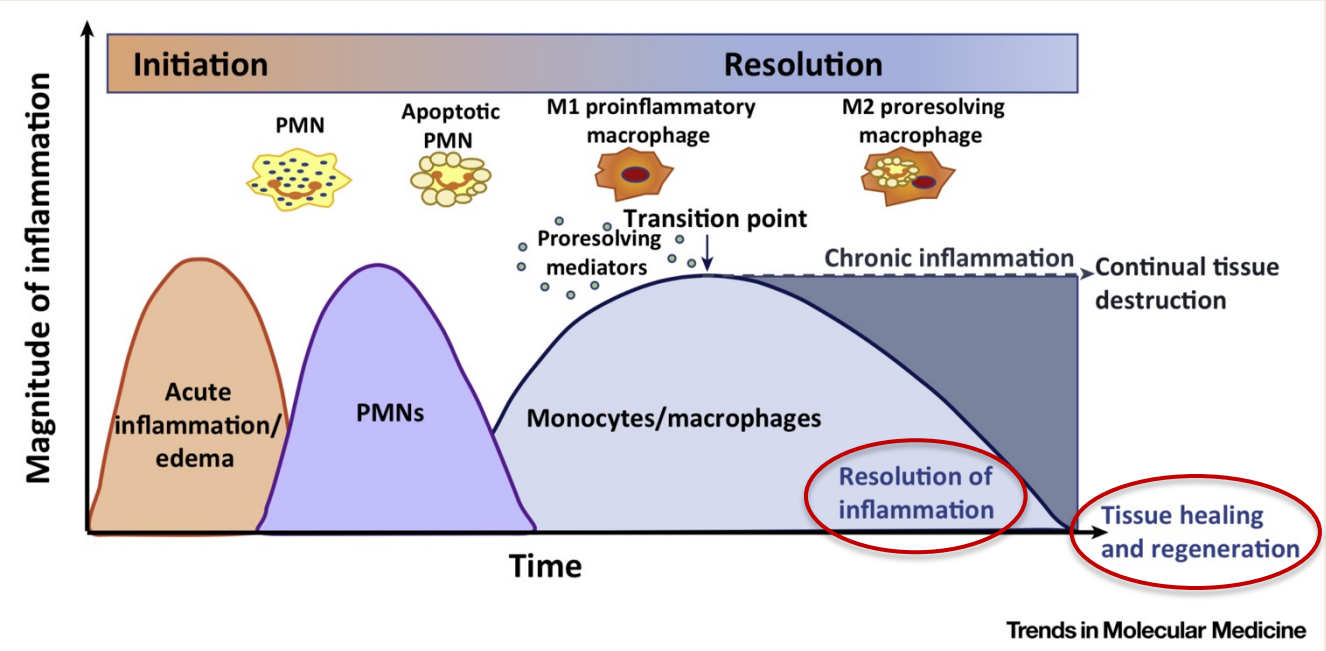
A “Symphony of Healing”:
1. stop bleeding,
2. block pathogens,
3. initiate healing
(restore dysfunctional biochemistry, cells, tissues and organs)

Aim of Regenerative Medicine:
Restoration of dysfunctional
cells, tissues and organs.

Chronic, non-communicable diseases affect patients and healthcare systems

Various organ systems are affected (classically defined as separate diseases).

Yet, chronic inflammation, stress and dysregulation of immune functions are common threads in such disease development and progression.



Standard model of injury/disease /inflammation and its resolution.

Orthokine and biologic therapies are **epigenetic interventions** that induce **cellular reprogramming** and **tissue restoration**

Here is **one** mechanism of Orthokine:

The repair of tissue is dependent on factors that initiate e.g. matrix synthesis and cell devision. Repair requires **massive energy supply**.

ATP is supplied by functional mitochondria.

Injured, chronic or senescent tissues suffer from too high radical loads which are released by too many dysfunctional mitochondria.

Excess radicals harm **all** biological molecules and suppress cell functionality.

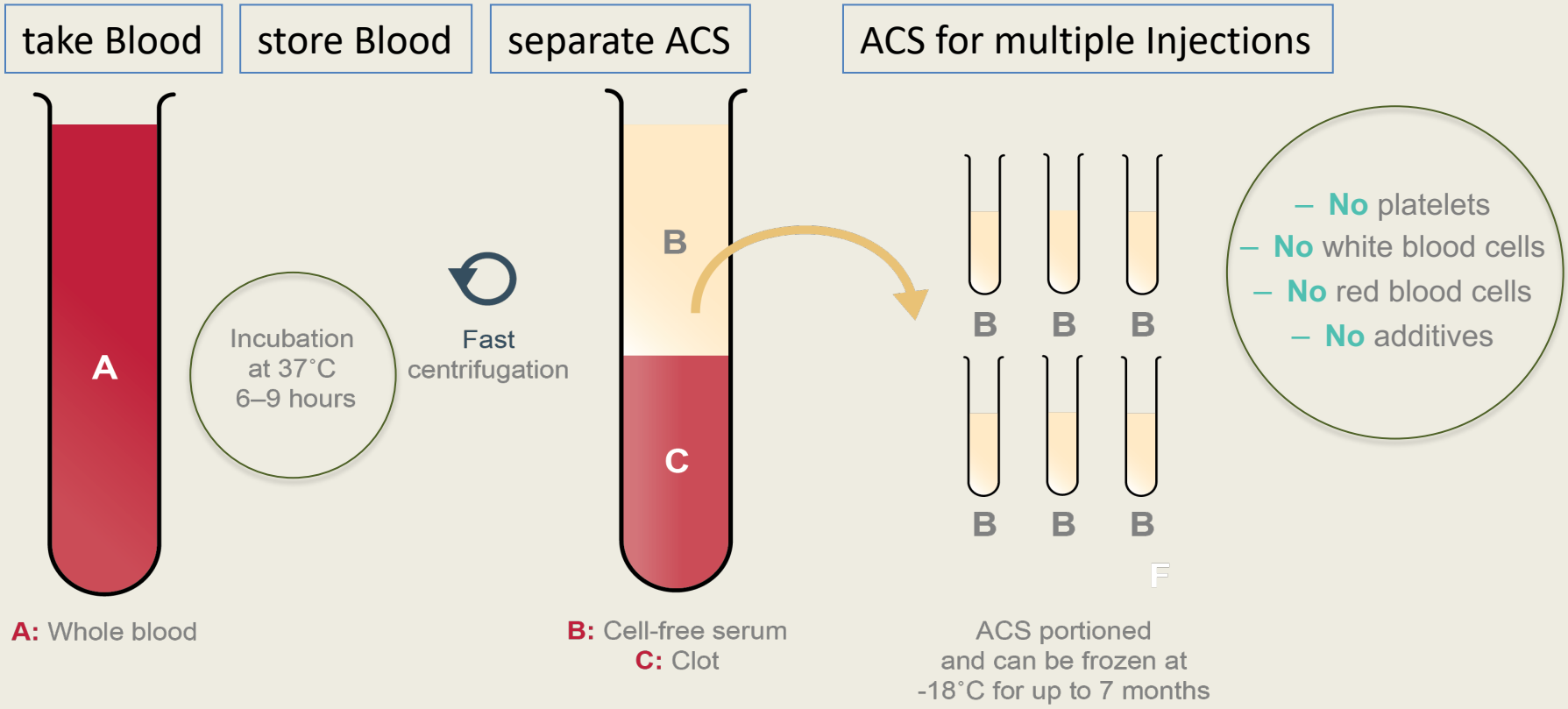
Locally injected ACS reduces radicals, improves mitochondria function and provides cytokines, growth factors, resolvins, exosomes and building blocks for tissue repair.

ACS enables the local requirements for healing.

ACS enables tissue to enter the resolution phase.

ACS for Orthokine Therapy is easy to process

Controlled processing under physiologic conditions triggers a standardized secretory response.



„I do PRP, that‘s the same“

NO!

Each PRP is different.

All therapeutic factors are in the blood cells and need to be released.

No guarantee that this happens consistently.

PRP coagulates and causes painful inflammation in joints.[e.g. 1]

In all studies ACS outperformed PRP.

1. Sung K, Zheng K, Williams C, Cunningham D, Sussman WI. Pseudoseptic reaction to an intra-articular platelet-rich plasma injection into the knee: a case report. *Regen Med*. Published online May 15, 2023. doi:10.2217/rme-2022-0216

ACS vs PRP/ACP

ACS	PRP/ACP
Processing	
Administered after **incubation and centrifugation**	Administered directly after centrifugation
Processed **only once** and divided in aliquots	Must be **processed for each single** injection
Cell-free serum administered with an antibacterial filter	Can not be administered with an antibacterial filter due to presence of cells
Content	
Cell-free serum contains resolving cytokines and growth factors, exosomes et al., no clotting factors.	**Plasma** contains platelets, white blood cells, fibrinogen, active complement and clotting factors.
Standardised processing	**Content differs** /depends on each manufacturer's IFU
No anticoagulants	Presence of anticoagulants
No additives	**Plasma activation** with 2nd additive Ca^{2+}
Storage	
Frozen storage possible	Frozen storage not possible

ACS vs PRP/ACP

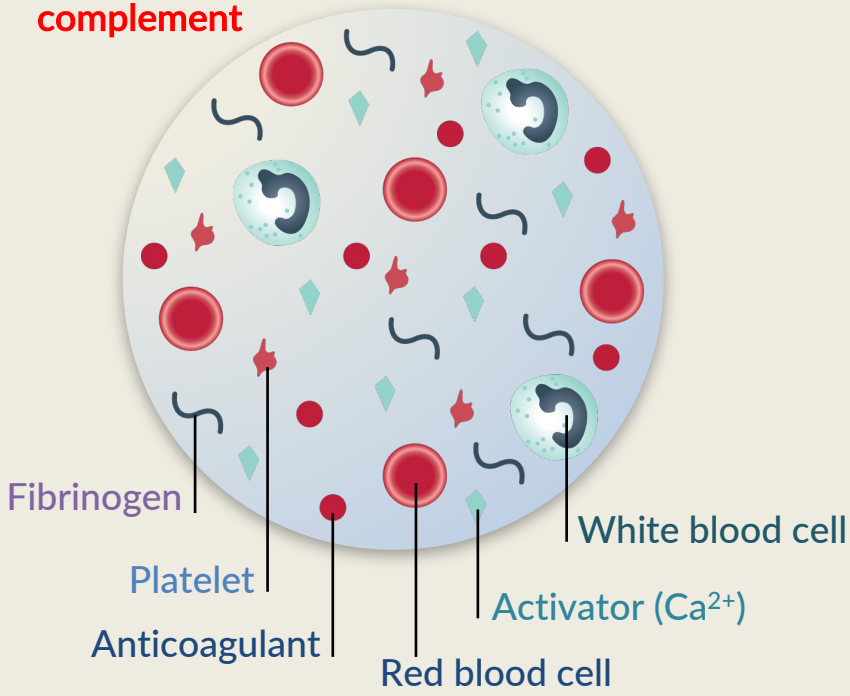
ACS

cell-free
no clotting factors
no additives

– No platelets
– No white blood cells
– No red blood cells
– No additives

PRP/ACP

cells
clotting factors
additives
complement



Mediators in ACS secretome (2007)

Table I. Content of cytokines and growth factors in human autologous conditioned serum (ACS) using 10mL whole blood in the Orthokine® system. The system is optimized to limit the accumulation of pro-inflammatory mediators. The list of cytokines present in the ACS is probably much more extensive than the number measured in this experiment. Note the high standard deviation (SD) of the individual mean concentrations detected indicating that cytokine concentrations vary greatly between individuals. Unless indicated, the values are from measurements of serum and/or unspecified plasma samples[a]

	Cytokine										
	IL-1ra	IL-1β	IL-6	TNFα	IL-10	FGFb	VEGF	HGF	IGF1	PDGF AB	TGFβ
No. of patients	224	224	200	92	92	92	92	92	92	92	80
Concentration (pg/mL) prior to incubation (0 hours)											
Basal[b]	236	<3.9	<12.5	<15.6	<7.8	14.6	61	431	86 000	205	1 165
Concentration (pg/mL) after incubation (6 hours)											
Mean	2 014.8	7.9	28.7	10.1	33.4	26.6	508.6	1 339.3	117 208.8	39 025.6	97 939.0
SD	4 381.1	8.7	48.1	9.6	18.9	20.8	307.7	928.7	51 644.4	10 515.8	113 418.3
Minimum	390.3	1.4	0.9	3.0	4.1	2.8	114.1	691.4	37 430.0	19 601.0	13 067.0
Maximum	31 057.0	48.9	250.2	69.7	105.0	104.5	1 694.0	6 473.0	440 000.0	66 208.0	823 000.0

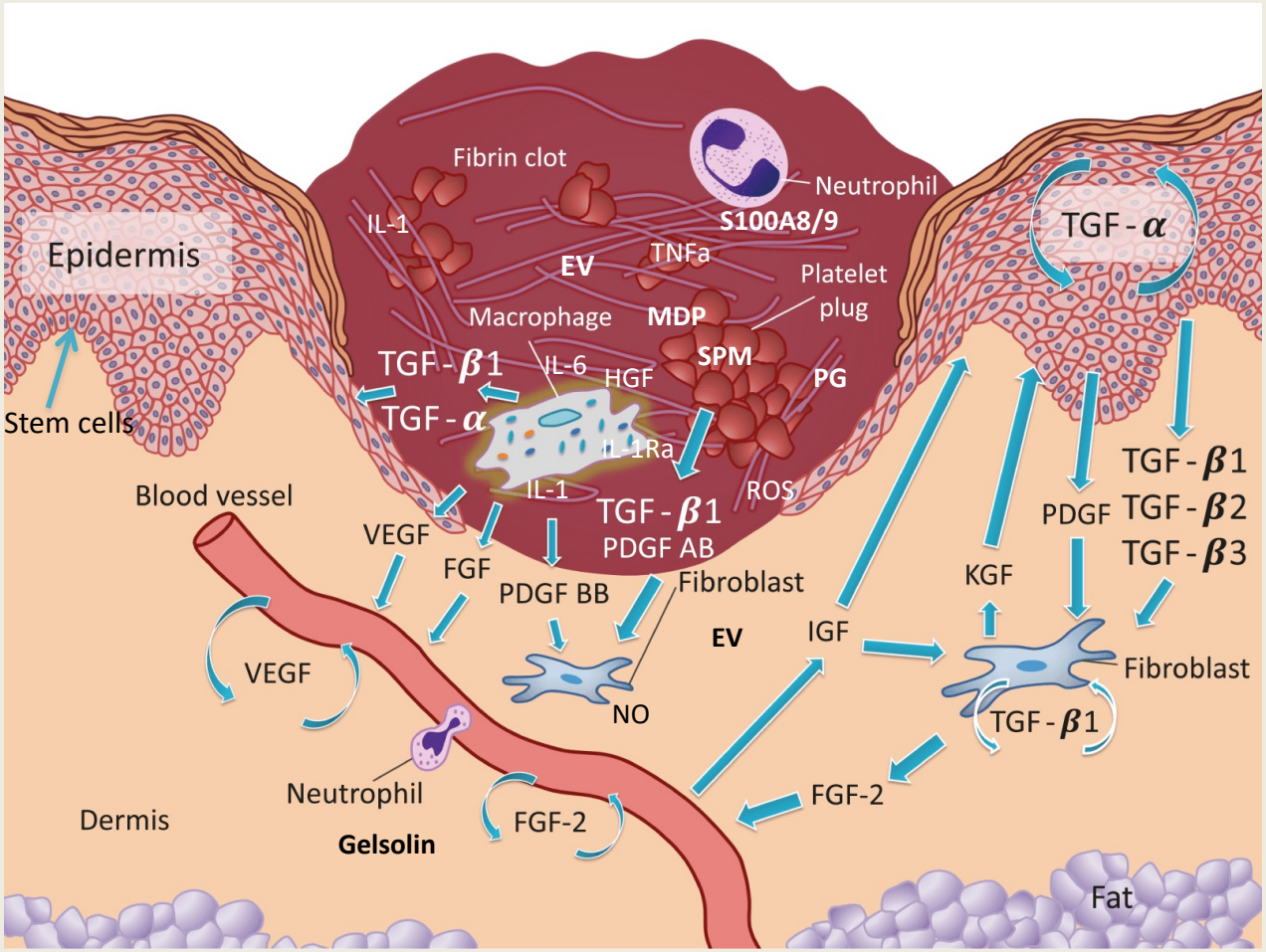
a All measurements were performed using enzyme-linked immunoabsorbant assay kits (ELISA; R&D Systems, Minneapolis, MN, USA). Basal values are normal values of healthy donor as measured by the kit manufacturer. Serum retrieved from 10mL of whole blood.

b All basal levels given with a '<' are lower limits of the kit's sensitivity. The accuracy of readings lower than these values are suboptimal.

FGFb = fibroblast growth factor-b; **HGF** = hepatocyte growth factor; **IGF1** = insulin-like growth factor-1; **IL** = interleukin; **PDGF** = platelet-derived growth factor; **TGFβ** = transforming growth factor-β; **TNFα** = tumor necrosis factor-α; **VEGF** = vascular endothelial growth factor.

Biodrugs, 2007. doi: 10.2165/00063030-200721050-00004

Mediators in ACS secretome (2023)



cell-cell communication

Serum proteins scavenge Radicals

Alarmins (e.g. S100, cell debris)

Cytokines & Growth Factors

Gelsolin (cell-mobility)

Extracellular Vesicles (EV, Exosomes)

Lipid-mediator *class switch* (Prostaglandins->SPM)

Mitochondria Derived Peptides (Humanin, MOTS-c etc.)

modified: https://ticebacellnews.wordpress.com/tag/ticeba/

Release of ACS - Secretome

Phase I: (minutes)

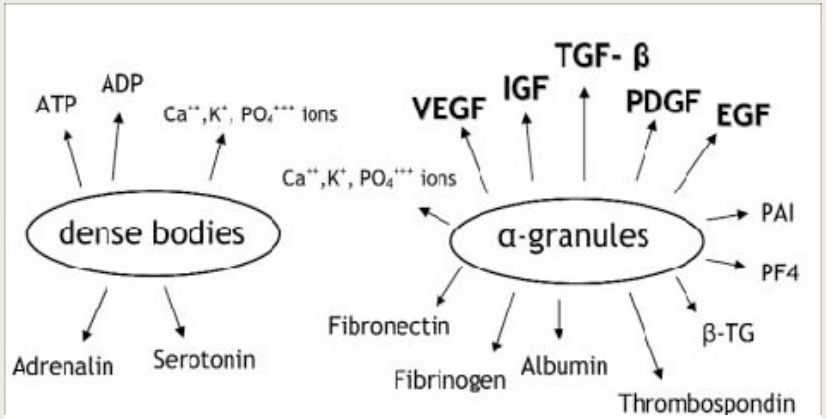
Acute-Mediators

- Coagulation
- Complement activation
- Alarmins: e.g. ATP, ADP, Histamin, Serotonin, Adrenalin, exRNA
- Growth factors
- Neutrophils -> S100A8/9-Proteins
- Lipid Mediators (SPM) -> *Mediator switch*

Phase II: (hours)

Regenerative Mediators

- Growth factors
- Gelsolin
- Extracellular Vesicles
- Cytokines
- Lipid Mediators (SPM)
- Mitochondria-derived peptides (MDP)
- S100A8/9-Proteins



Platelet activation: Devescovi et al. Growth factors in bone repair. *Chir Organi Mov*. 2008;92(3):161-168. doi: 10.1007/s12306-008-0064-1

You need incubation for effect

Growth Factors are produced during extended coagulation activated platelet concentrate

DOI: 10.1111/vox.12039

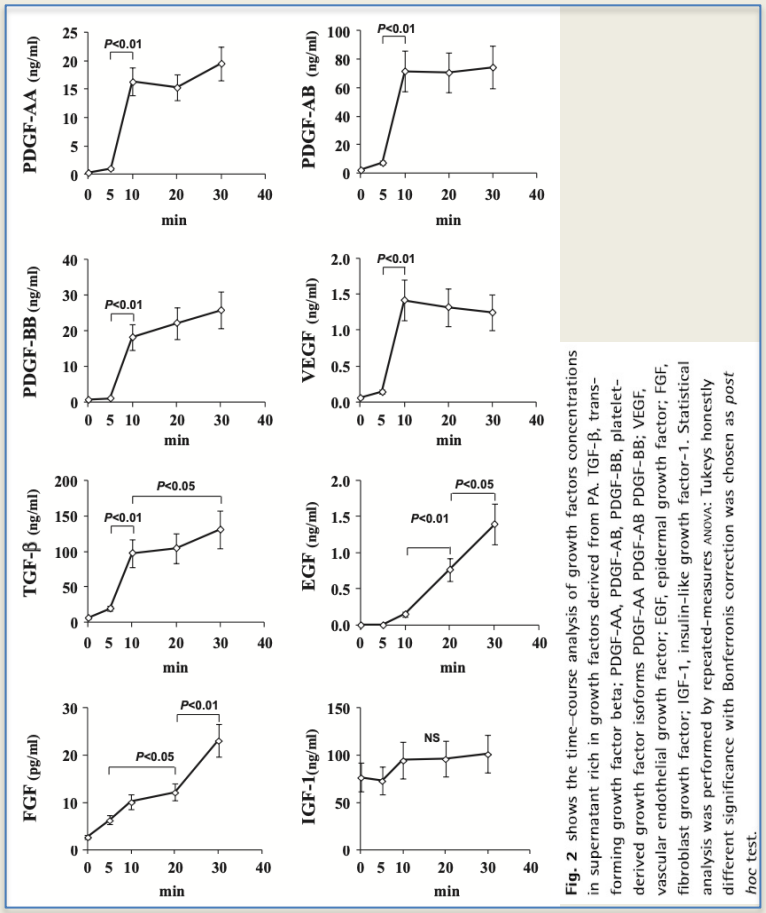
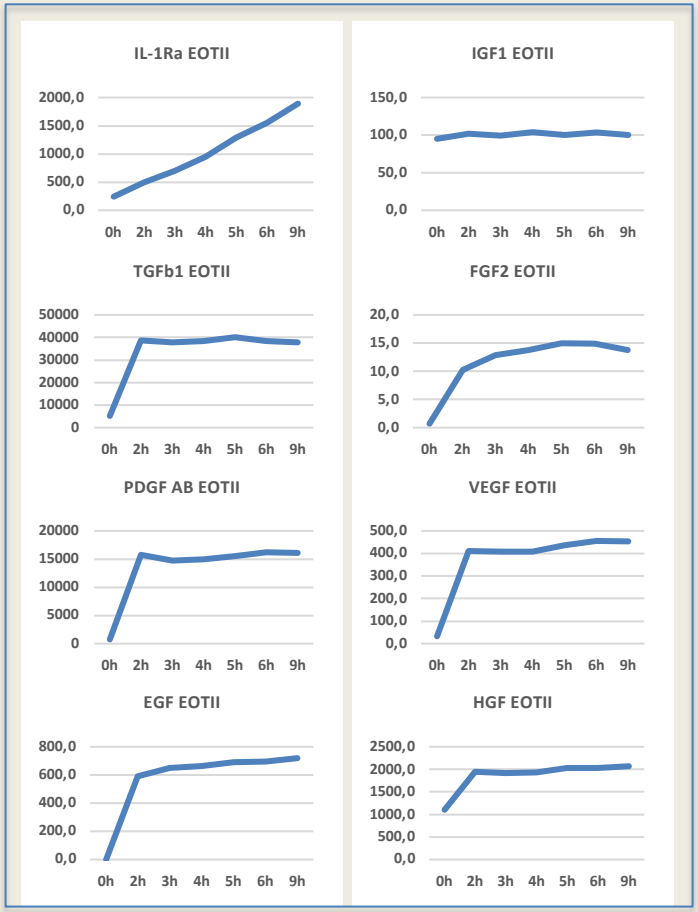


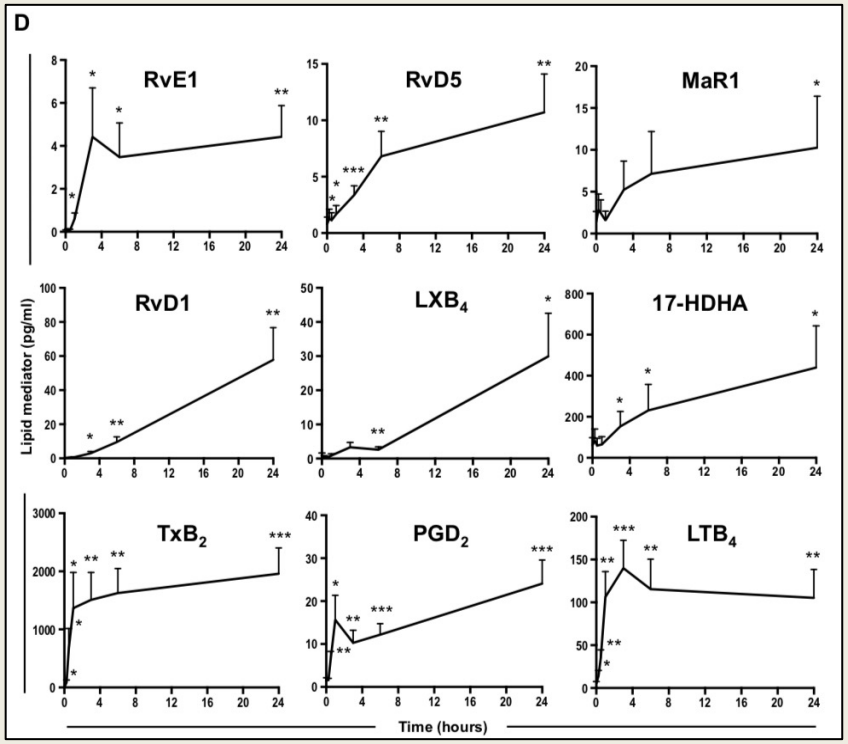
Fig. 2 shows the time–course analysis of growth factors concentrations in supernatant rich in growth factors derived from PA. TGF-β, transforming growth factor beta; PDGF-AA, PDGF-AB, PDGF-BB, platelet-derived growth factor isoforms PDGF-AA PDGF-AB PDGF-BB; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF-1, insulin-like growth factor-1. Statistical analysis was performed by repeated-measures ANOVA: Tukeys honestly different significance with Bonferronis correction was chosen as *post hoc* test.

ACS



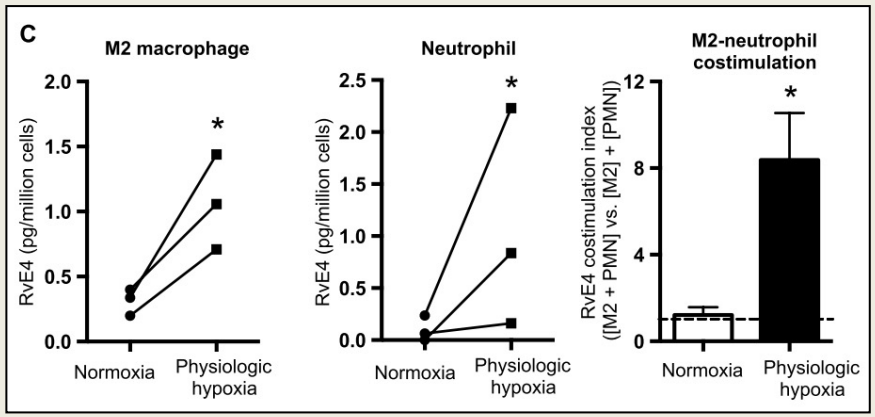
You need incubation for effect:

Lipid Mediators (SPM) are produced in case of extended coagulation



Whole blood: Concentration change of the indicated members of the SPM cluster and of eicosanoids in blood samples over time. (1)

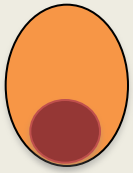
Treatment with MaR1 could attenuate mitochondrial dysfunction during sepsis through regulating ROS production. (3)



Increased production of RvE4 in human M2 (left) and PMN (middle) with physiologic hypoxia versus normoxia. Enhancement of RvE4 with coincubations of M2 and PMN versus summation of RvE4 from M2 and PMN incubated separately or co-incubated during hypoxia for 24h. (2)
At the same time PMN degranulation increases S100A8/9 protein concentration by 10fold.

Alterations in SPM production may affect joint lubrication, synovial inflammation, pannus formation, as well as cartilage and bone degradation and contribute to the pathogeneses of inflammatory joint diseases.(4)

1. Norris PC, Libreros S, Chiang N, Serhan CN. A cluster of immunoresolvents links coagulation to innate host defense in human blood. *Sci Signal*. 2017;10(490). doi: 10.1126/scisignal.aan1471
2. Norris PC, Libreros S, Serhan CN. Resolution metabolomes activated by hypoxic environment. *Sci Adv*. 2019;5(10):eaax4895. doi: 10.1126/sciadv.aax4895
3. Gu J, Luo L, Wang Q, et al. Maresin 1 attenuates mitochondrial dysfunction through the ALX/cAMP/ROS pathway in the cecal ligation and puncture mouse model and sepsis patients. *Lab Investig*. 2018;98(6):715-733. doi:10.1038/s41374-018-0031-x
4. Mustonen A, Nieminen P. Fatty Acids and Oxylipins in Osteoarthritis and Rheumatoid Arthritis—a Complex Field with Significant Potential for Future Treatments. *Curr Rheumatol Rep*. 2021;23(6):41. doi:10.1007/s11926-021-01007-9



You need incubation for effect

Non-cytokine factors in ACS produced during extended coagulation

	Baseline vs endpoint		Increase
Exosomes	±4,3x10^{10}/mL vs 4,7x10^{11}/mL	Immunemodulators, stop CINP	Up to 100fold
Bilirubin	similar	Radical scavenger	
Gelsolin	167 vs -> 300 ng/mL	cell mobility	2fold
Humanin (MDP)	15 vs -> 4360 pg/mL	Neuro-protective mt-peptide	Up to 200fold
THY-1 (CD90)	3000 vs -> 5000 pg/mL	MSC differentiation	2fold
Resolvin D1	30 vs -> 600 pg/mL	Resolving SPM formation is altered by COX Inhibitors, downregulate NFkB, upregulate efferocytosis	20fold
Protectin D1	2.3 -> 3.7 ng/mL		2fold
Resolvin D2	600 -> 1000 pg/ mL		2fold
S100A8/9[1]	338 -> 4871 ng/mL	Alarmin, prevents pain chronification	15fold

1. Parisien M, Lima L V, Dagostino C, et al. Acute inflammatory response via neutrophil activation protects against the development of chronic pain. *Sci Transl Med*. 2022;14(644):2-5. doi:10.1126/scitranslmed.abj9954

ACS **not** an anti-inflammatory

ACS restores Homeostasis in injured/chronified organs:

> Rejuvenation Res. 2020 Oct;23(5):401-410. doi: 10.1089/rej.2019.2263. Epub 2020 Feb 10.

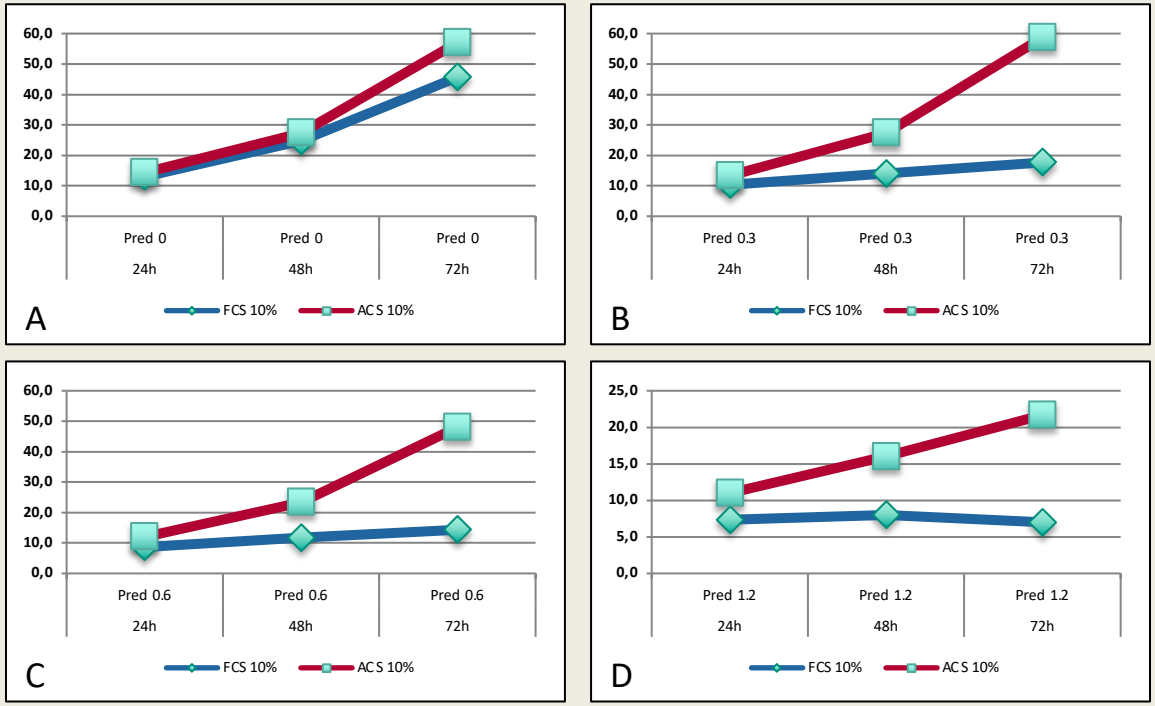
Intra-Articular Injections of a Whole Blood Clot Secretome, Autologous Conditioned Serum, Have Superior Clinical and Biochemical Efficacy Over Platelet-Rich Plasma and Induce Rejuvenation-Associated Changes of Joint Metabolism: A Prospective, Controlled Open-Label Clinical Study in Chronic Knee Osteoarthritis

Larisa Shirokova [1], Sergey Noskov [1], Victoria Gorokhova [1], Julio Reinecke [2], Ksenia Shirokova [1]

Affiliations + expand

PMID: 31847701 DOI: 10.1089/rej.2019.2263

ACS **protects** Chondrocytes from GC

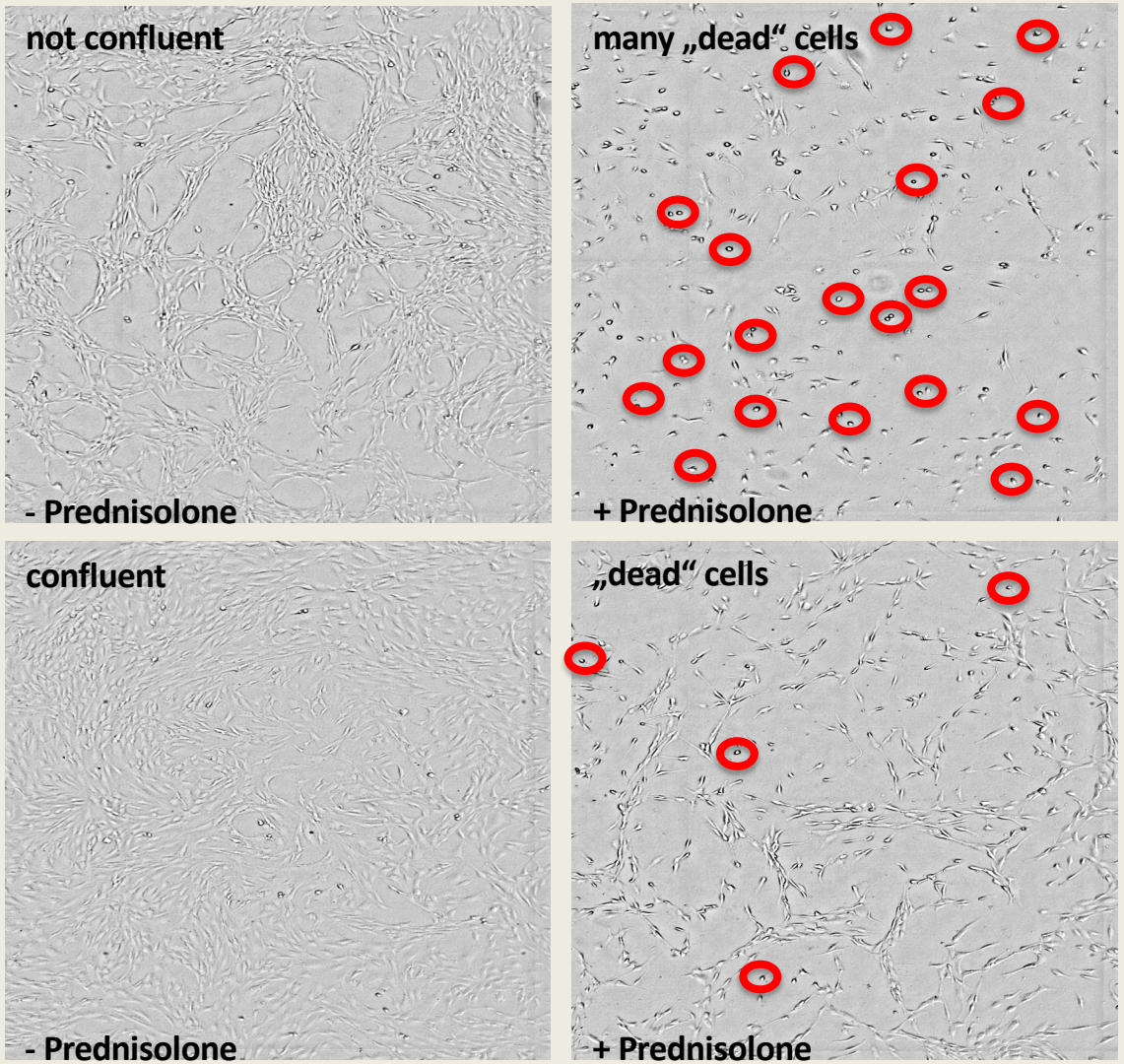


Vertical axis: % confluence of human primary chondrocytes. ACS supports cell viability better than FCS [A] and counteracts Prednisolone cell depression at 0.3, 0.6 and 1.2 mg/mL [B, C, D]. *All data points are means of triplicates*. Prednisolone succinate: 0.3mg/mL ≈ 0.63mM; 0.6mg/mL ≈ 1.2mM; 1.2mg/mL ≈ 2.5mM; 2.5mg/mL ≈ 5mM; 5mg/mL ≈ 10mM

In vitro primary human chondrocyte cell division kinetics as a function of ACS / FCS supplement and Prednisolone concentration.

ACS efficiently protects chondrocyte viability against Prednisolone cyto-depression *in vitro.*
Confluence determination at 24h, 48h, 72h in 96well plates.

Primary human chondrocytes grown for 72hrs.



10% FCS supplement (control)

10% ACS supplement protects

ACS exploits tissue (stem)cells

ACS enhances *in vitro* ***stemness*** of human fat derived stem cells ($CD90^{up}$).

ACS enhances *in vitro* ***immunmodulatory*** influence of human fat derived stem cells (CD4+ and CD8+ $Lymphocytes^{down}$ and CD45RA+ $CD62L+^{up}$).

ACS enhances *in vitro* ***cell division*** of human fat derived stem cells. (1)

ACS enhances *in vivo* ***emergence*** *of muscle* ***stem cells*** in mouse-contusion-model. (2)

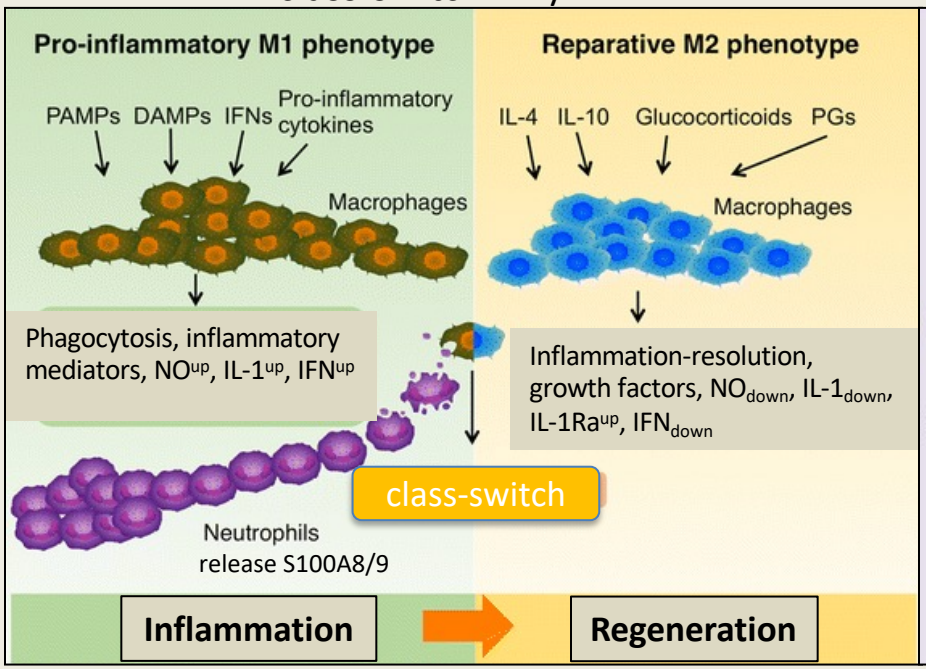
ACS supports ***growth*** *in vitro and in vivo* of cartilage, tendon-, bone- und skin cells.

ACS *regenerates/protects damaged* ***nerve*** *cells in vivo*.

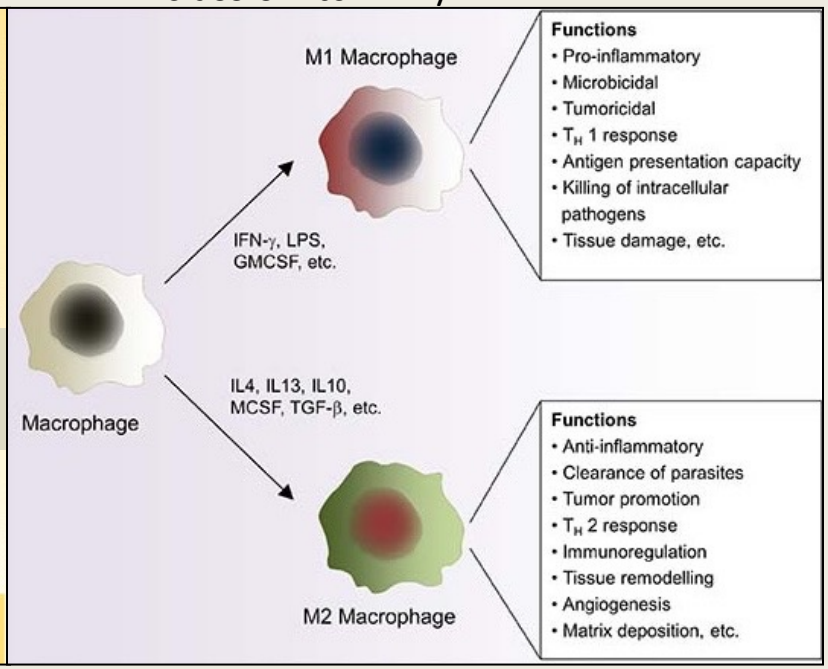
1. Blázquez et al. Conditioned Serum Enhances the Chondrogenic and Immunomodulatory Behavior of Mesenchymal Stem Cells. *Front Pharmacol*. 2019;10(June):699. doi:10.3389/fphar.2019.00699
2. Wright-Carpenter et al. Treatment of muscle injuries by local administration of autologous conditioned serum: animal experiments using a muscle contusion model. *Int J Sports Med*. 2004;25(8):582-587. doi:10.1055/s-2004-821303

ACS exploits tissue macrophages

class-switch M1/M2



class-switch M1/M2



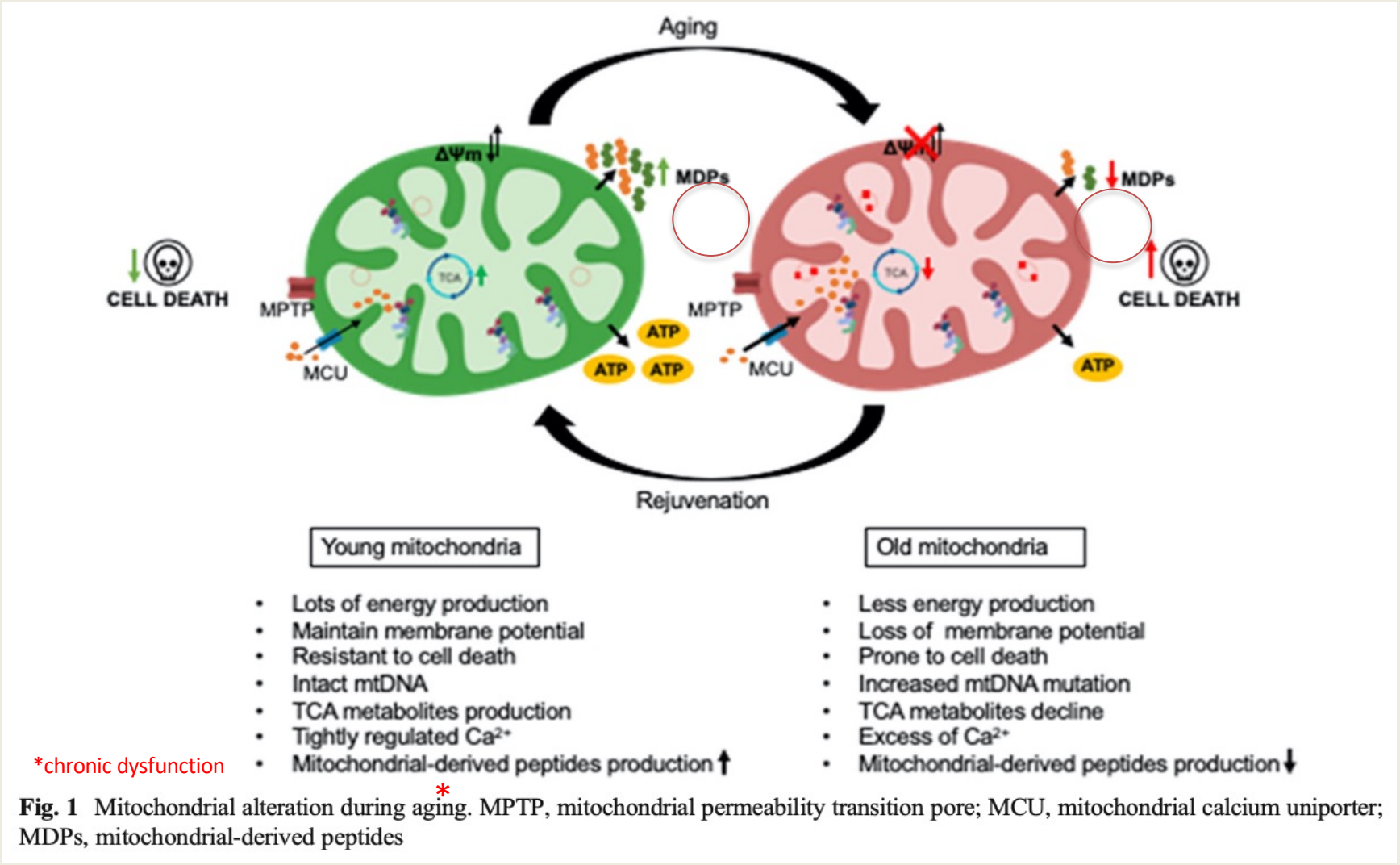
Macrophages are indispensible Custodians of tissue homöostasis. ACS supports M1->M2 class-switch

Osteoarthritis Synovia analysis correlates with clinic. (1)

ROS_{down} NO_{down} $IL\text{-}1_{down}$ $IL\text{-}1Ra^{up}$ HA^{up} VAS_{down} $WOMAC_{down}$

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

ACS exploits Mitochondria



*chronic dysfunction

Fig. 1 Mitochondrial alteration during aging. MPTP, mitochondrial permeability transition pore; MCU, mitochondrial calcium uniporter; MDPs, mitochondrial-derived peptides

Kim et al. Mitochondrial-derived peptides in aging and age-related diseases. *GeroScience*. 2021;43(3):1113-1121. doi:10.1007/s11357-020-00262-5

ACS exploits autophagy

(intra-cellular waste disposal)

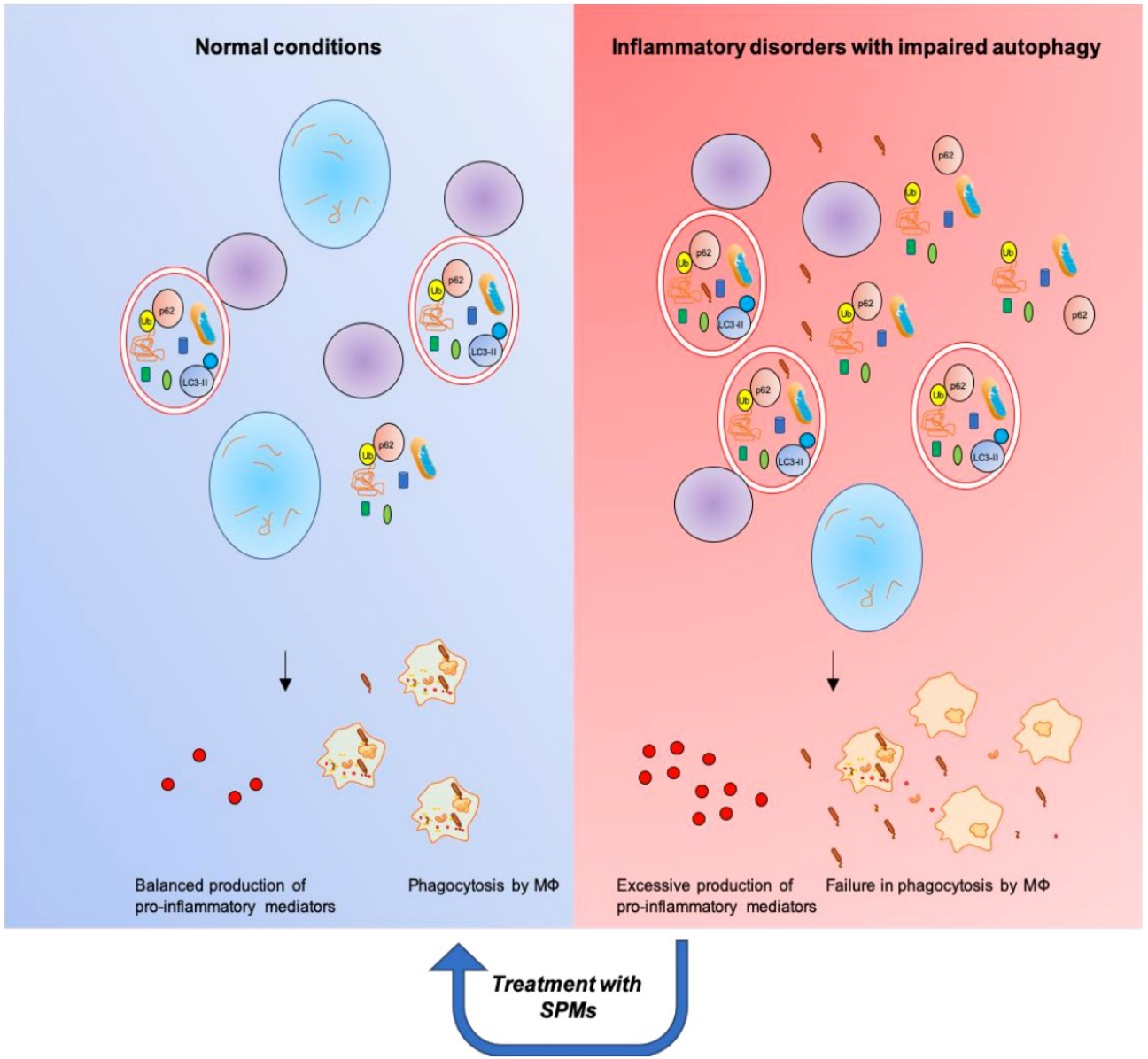


Figure 4. SPMs restore functional autophagic flux and resolution of inflammation in inflammatory disorders characterized by impaired autophagy.

Recchiuti et al. Roles of specialized pro-resolving lipid mediators in autophagy and inflammation. *Int J Mol Sci*. 2020;21(18):1-23. doi: 10.3390/ijms21186637

ACS exploits fundamental Healing-Mechanisms.

Cytokines are but one facet in this symphony.
ACS sets in motion a polymodal machinery to support tissue-homeostasis.
Relief from oxidative stress enables healing of various tissues and injuries, transcending clinical disease definitions.

Clinical Signs	Effectors
Pain relief	Cytokines
Cell division	Growth Factors
Matrix synthesis	Growth Factors
Cell protection	MDP
Resolution of inflammation	SPM
and pain	S100A8/9
	Extracellular Vesicles
Immune modulation	Extracellular Vesicles
Radical-modulation	MDP
Cell Senescence	Autophagy
Mitochondria-revitalisation	MDP

> Brain Behav Immun. 2023 May 5;111:298-311. doi: 10.1016/j.bbi.2023.04.013.
Online ahead of print.

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

Thomas Buchheit [1], Yul Huh [2], Andrew Breglio [3], Sangsu Bang [3], Jing Xu [3], Yutaka Matsuoka [3], Ran Guo [3], Andrey Bortsov [3], Julio Reinecke [4], Peter Wehling [5], Tony Jun Huang [6], Ru-Rong Ji [7]

Affiliations + expand

PMID: 37150265 DOI: 10.1016/j.bbi.2023.04.013

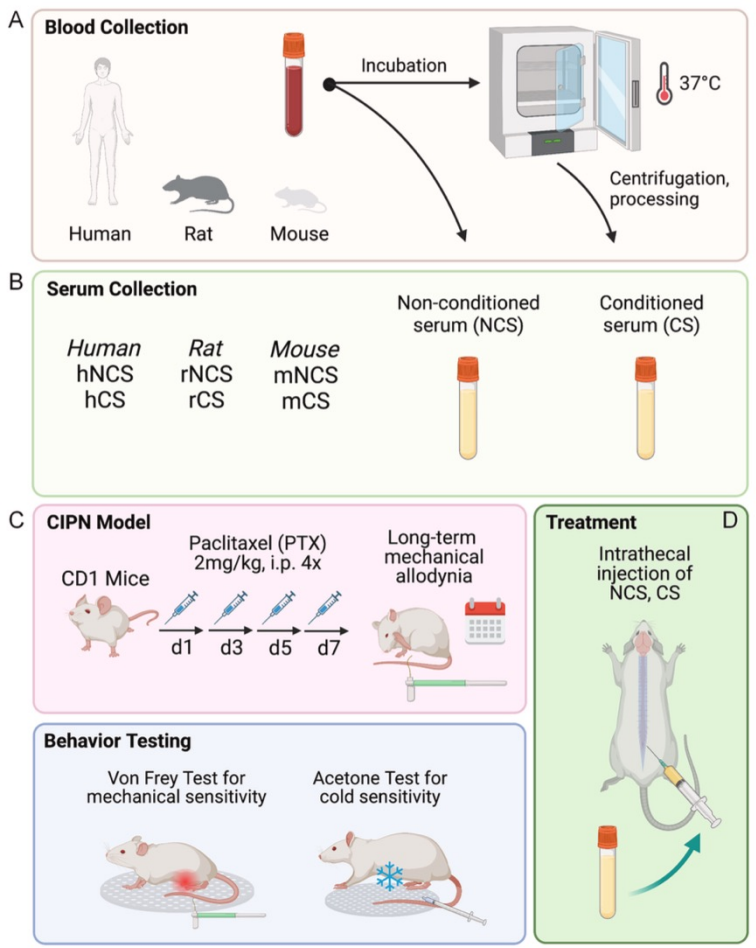
A single intrathecal injection of human conditioned serum (hCS) produced long-lasting inhibition of paclitaxel chemotherapy-induced neuropathic pain (mechanical allodynia) in mice, without causing motor impairment. The effect of hCS was maintained even 8 weeks after the treatment, compared with non-conditioned human serum (hNCS). The hCS transfer-induced pain relief in mice was fully recapitulated by rat or mouse CS transfer to mice of both sexes, indicating cross-species and cross-sex effectiveness.

CS treatment blocked the chemotherapy-induced glial reaction in the spinal cord and improved nerve conduction. CS contained significantly higher concentrations of anti-inflammatory and pro-resolving mediators, including IL-1Ra, TIMP-1, TGF-β1, and resolvins D1/D2 than NCS. *Intrathecal injection of anti-TGF-b1 and anti-IL-1Ra antibody transiently reversed the analgesic action of CS.*

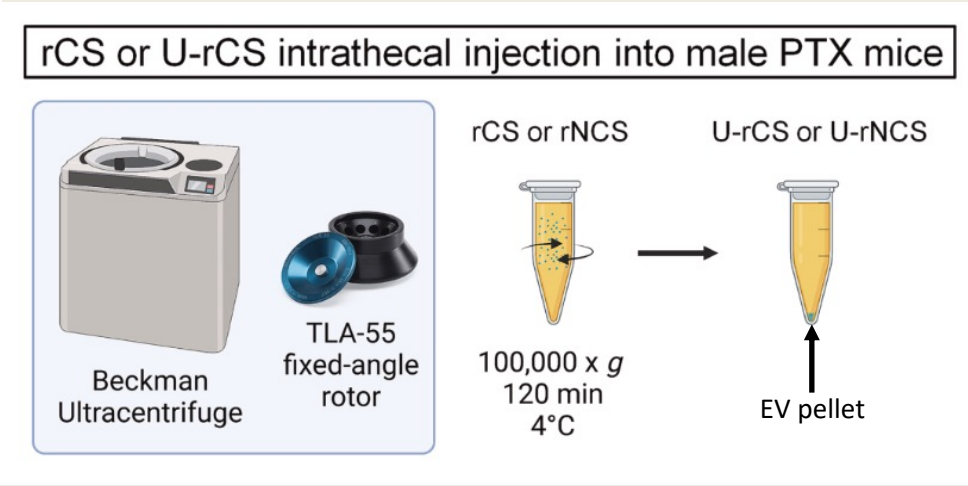
rCS contained a significantly greater number of exosomes than rNCS. Removal of exosomes by ultra-centrifugation largely diminished the CS-produced pain relief, suggesting critical involvement of small vesicles (exosomes) in the beneficial effects of CS.

Intrathecal CS produces a remarkable resolution of neuropathic pain mediated through a combination of small vesicles/exosomes and neuroimmune/neuroglial modulation.

ACS preparation and ultra-centrifugation

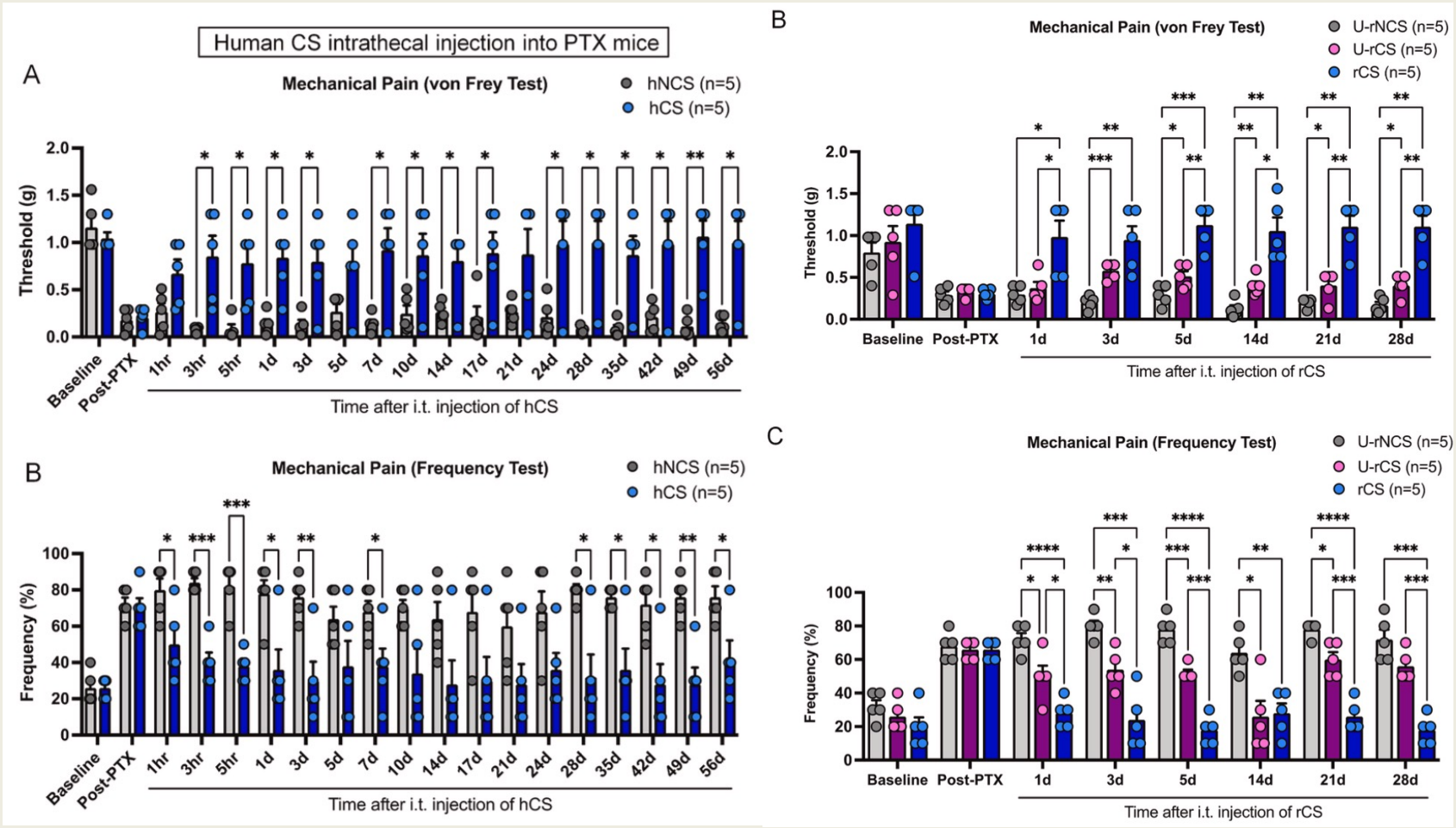


EV depletion by ultra-centrifugation



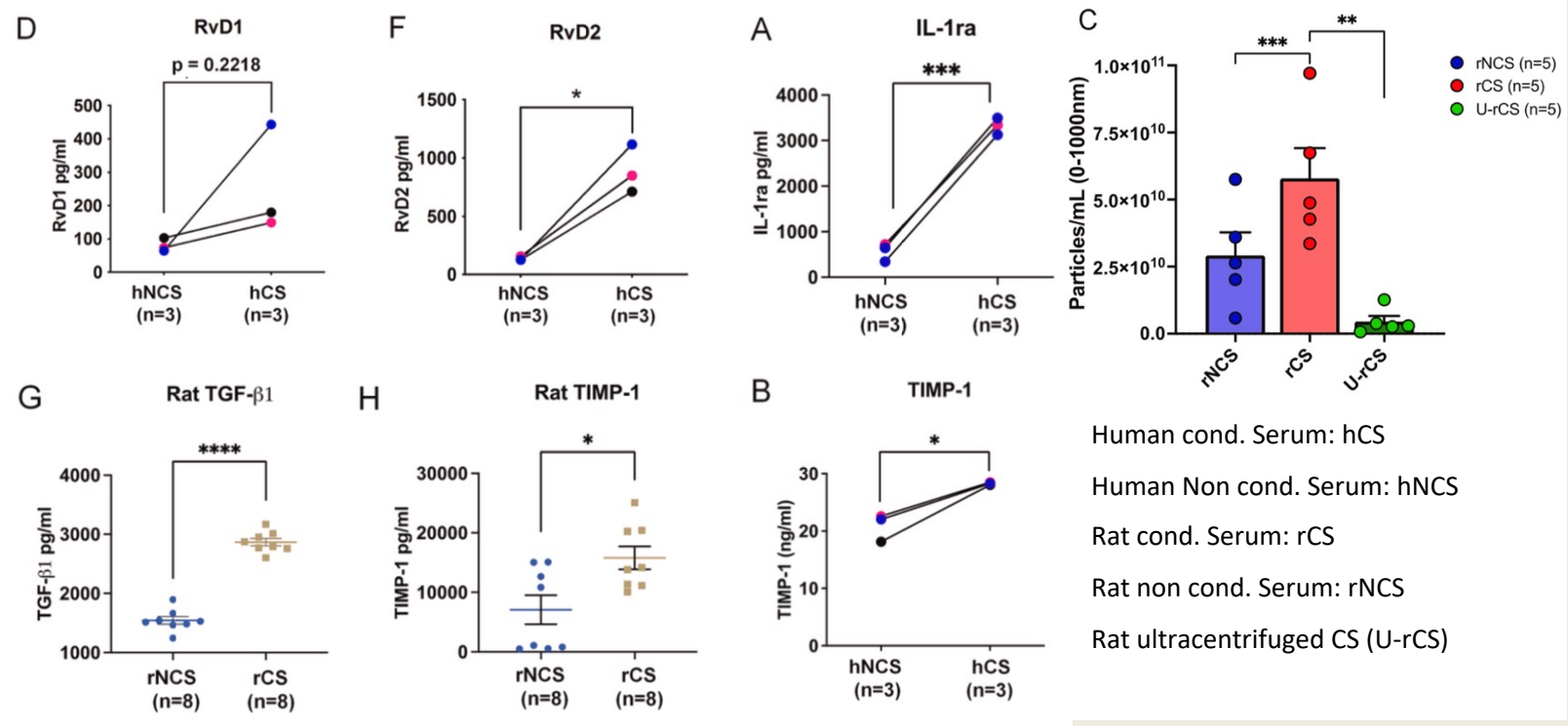
Comment: Paclitaxel leads to ATP undersupply and *oxidative stress*.
Mitochondria morphology is altered during paclitaxel treatment.

ACS stops paclitaxel chemotherapy-induced neuropathic pain. EV are key players.



Comment: Paclitaxel leads to ATP undersupply and *oxidative stress*.
Mitochondria morphology is altered during paclitaxel treatment.

Summary: ACS mediator content



Human cond. Serum: hCS

Human Non cond. Serum: hNCS

Rat cond. Serum: rCS

Rat non cond. Serum: rNCS

Rat ultracentrifuged CS (U-rCS)

Efficacy of ACS in radiculopathy and other neuro-indications (e.g. trigeminus, carpal-tunnel, endometriosis) explained:

Exosomes cross the BBB

„Insights into Exosome Transport through the Blood-Brain Barrier and the Potential Therapeutical Applications in Brain Diseases.“ https://pubmed.ncbi.nlm.nih.gov/37111328/

Perineural injection is as effective as epidural

„Comparison of Analgesic Efficacy between Epidural and Perineural Administration of Autologous Conditioned Serum in the Conservative Treatment of Low Back Pain Due to Lumbar Degenerative Disc Disease: A Randomized, Open-Label, Controlled Clinical Trial.“
https://doi.org/10.3390/brainsci13050749/

Intrathecal injection may not be necessary but may require less ACS.