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10ο Συνέδριο Βιοχημείας & Φυσιολογίας της Άσκησης με Διεθνή Συμμετοχή

21-23 Οκτωβρίου 2022
Αμφιθέατρο Σ.Ε.Φ.Α.Α. - Ε.Κ.Π.Α.
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Στρογγυλή τράπεζα
Φυσιολογικές και βιοχημικές αρχές της μυϊκής λειτουργίας

The Role of Platelet Secretome in Skeletal Muscle Biology and Satellite Cell Function

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Outline

- Broad aspects of platelet biology
- Platelet-based applications in regenerative medicine
- Composition of platelet secretome
- The role of platelet secretome in skeletal myogenesis
- The role of platelet secretome in reparative skeletal muscle regeneration
- Mechanistic insights
- The role of platelet secretome in skeletal muscle stem cell function
- Conclusions

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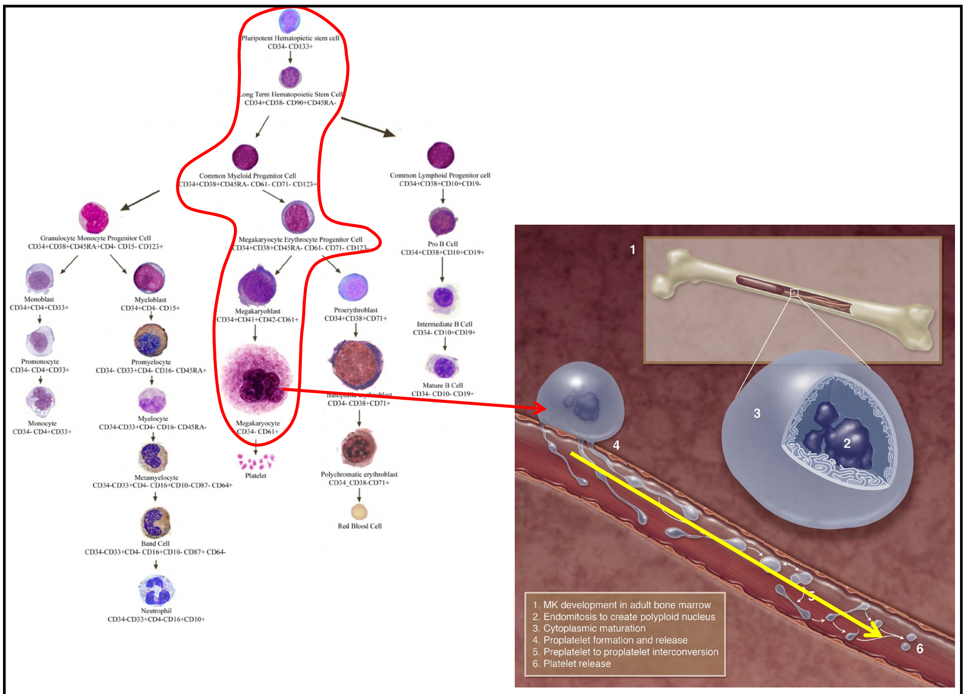

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Platelet and organelles

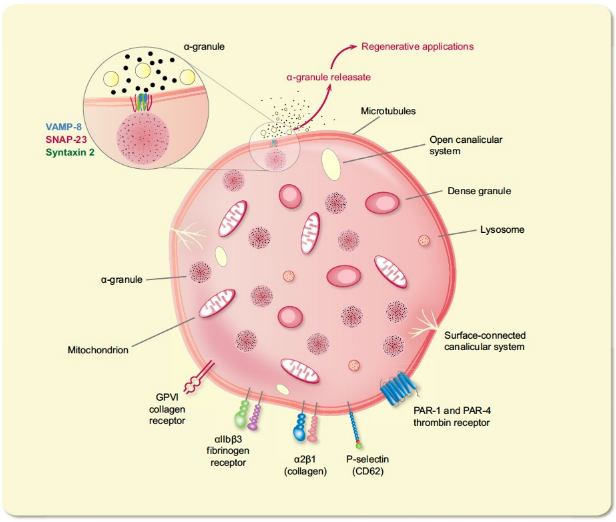

FIGURE 1 Schematic of a platelet together with organelles, highlighting key surface receptors, aggregation factors and an overview of known α-granule releasate factors. These contain adhesive proteins, clotting factors and their inhibitors, fibrinolytic factors and their inhibitors, proteases and antiproteases, growth and mitogenic factors, chemokines, cytokines, membrane glycoproteins and antimicrobial proteins. The platelet releasate may be further used as a biomaterial in numerous applications of regenerative medicine

Scully et al. 2018, Acta Physiol.e13071

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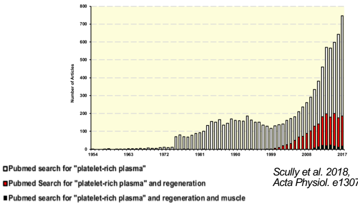
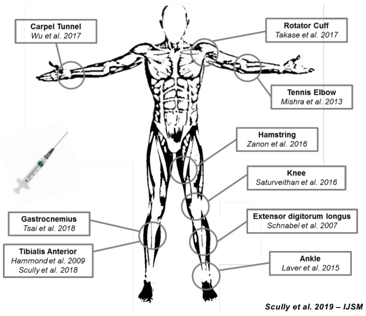
Platelet-mediated skeletal muscle regeneration



Scully et al. 2018, Acta Physiol. e13071



Scully et al. 2019 – IJSM

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Platelet-mediated skeletal muscle regeneration

Front. Med.
https://doi.org/10.1007/s11684-017-0551-6 REVIEW

Platelet-rich plasma: combinational treatment modalities for musculoskeletal conditions

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[1]Regenerative Medicine Laboratory, BioCruces Health Research Institute, Cruces University Hospital, 48903 Barakaldo, Spain; [2]Department of Medicine and Science of Aging, University G. d'Annunzio, Chieti-Pescara, 66013 Chieti Scalo, Italy

Curr Rev Musculoskelet Med (2015) 8:145–153
DOI 10.1007/s12178-015-9259-x

MUSCLE INJURIES (SJ MCNEILL INGHAM, SECTION EDITOR)

Platelet-rich plasma for muscle injuries: game over or time out?

Michael J. Mosca · Scott A. Rodeo

Platelet-rich fibrin in arthroscopic repair of massive rotator cuff tears
A prospective randomized pilot clinical trial

Pain Physician 2017; 20:E345-E356 • ISSN 2150-1149

Narrative Review

Platelet Rich Plasma in Musculoskeletal Pathology: A Necessary Rescue or a Lost Cause?

Annu Navani, MD[1], Gang Li, MD, PhD[2], and Joshua Chrystal, DC, MD[3]

frontiers in Chemistry — MINI REVIEW, published: 31 October 2017, doi: 10.3389/fchem.2017.00089

Platelet-Rich Plasma Derived Growth Factors Contribute to Stem Cell Differentiation in Musculoskeletal Regeneration

Yun Qian[1,2†], Qixin Han[3†], Wei Chen[1,2†], Jialin Song[1,2], Xiaotian Zhao[4], Yuanming Ouyang[1,2*], Weien Yuan[3*] and Cunyi Fan[1*]

Muscle repair: platelet-rich plasma derivates as a bridge from spontaneity to intervention

Mikel Sánchez[a,b], Eduardo Anitua[c,d], Diego Delgado[b], Pello Sánchez[b], Gorka Orive[d], Sabino Padilla[c,d,*]

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Brossi et al. BMC Veterinary Research (2015) 11:98
DOI 10.1186/s12917-015-0403-z
BMC Veterinary Research

RESEARCH ARTICLE — Open Access

Platelet-rich plasma in orthopedic therapy: a comparative systematic review of clinical and experimental data in equine and human musculoskeletal lesions

Patricia M Brossi, Juliana J Moreira, Thaís SL Machado and Raquel YA Baccarin*

Format: Abstract

Am J Sports Med. 2017 Mar;45(4):945-953. doi: 10.1177/0363546516677547. Epub 2016 Dec 27.

The Use of Platelet-Rich and Platelet-Poor Plasma to Enhance Differentiation of Skeletal Myoblasts: Implications for the Use of Autologous Blood Products for Muscle Regeneration.

Miroshnychenko O[1], Chang WT[1], Dragoo JL[2].

Orthop J Sports Med. 2016 Jul; 4(7 suppl4): 2325967116S00143. PMCID: PMC4968283
Published online 2016 Jul 29. doi: 10.1177/2325967116S00143

Customized Platelet-Rich Plasma for Skeletal Muscle Injuries

Justin James Hicks,[1] Hongshuai Li, MD, PhD,[2] Marc J. Philippon, MD,[3] Shepard R. Hurwitz, MD,[4] Johnny Huard, PhD,[5] and MaCalus Vinson Hogan, MD[6]

Clinical Review: Current Concepts — Musculoskeletal Medicine

Applications of Platelet-Rich Plasma in Musculoskeletal and Sports Medicine: An Evidence-Based Approach

Rosalyn T. Nguyen, MD, Joanne Borg-Stein, MD, Kelly McInnis, DO

Format: Abstract

AJR Am J Roentgenol. 2011 Mar;196(3):628-36. doi: 10.2214/AJR.10.5975.

Musculoskeletal applications of platelet-rich plasma: fad or future?

Lee KS[1], Wilson JJ, Rabago DP, Baer GS, Jacobson JA, Borrero CG.

Author information

Platelet Rich Plasma Use in Allograft ACL Reconstructions: Two-Year Clinical Results of a MOON Cohort Study

Robert A. Magnussen, MD, **David C. Flanigan, MD**, **Angela D. Pedroza, MPH**, **Kate A. Heinlein, BS**, and **Christopher C. Kaeding, MD**
Department of Orthopaedics, OSU Sports Medicine, Sports Health and Performance Institute, The Ohio State University, Columbus OH

Acta Physiol (Oxf). 2018 Jul;223(3):e13071. doi: 10.1111/apha.13071. Epub 2018 Apr 19.

Platelet biology in regenerative medicine of skeletal muscle.

Scully D[1], Naseem KM[2], Matsakas A[1].

Author information

Platelet rich plasma therapy: A comparative effective therapy with promising results in plantar fasciitis

Mukesh Tiwari MS (Orthopedic)[a,], Rakesh Bhargava MS (Orthopedic)[b]*

[a]Associate Professor, Dept. of Orthopaedics, NIMS Medical College, Shobha Nagar, Jaipur, Rajasthan, India
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Controversial literature

- Inconsistent results
- Standardisation of preparation
- Timing of application

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TABLE 1 Platelets and skeletal muscle regeneration

Reference	Species	Intervention	Findings
9	Rat	PRP on a flexor sublimis lesion	↑ Leucocyte infiltration; ↑ early inflammatory response post-muscle injury
2	Rat	PRP on flexor sublimis incision	↑ mRNA of pro-inflammatory cytokines, MRFs & IGF-1Eb; ↓ myo-miR-133a
70	Rat	PRP on tibialis anterior under muscle strains	↑ Myogenesis ↓ Time-to-recovery after a muscle strain
16	Rat	PRP on gastrocnemius muscle injury	↓ Pain/claudication score
15	Rat	PRP in gastrocnemius contusion	↓ Oxidative stress and ↑ enzymatic antioxidants in injured skeletal muscle
82	Rat	PRP-derived growth factors on rat muscle satellite cells	↑ Proliferation and osteogenic differentiation ability of satellite cells from rat masticatory muscle
54	Rat	Rat releasate on rat gastrocnemius muscle cells in vitro	↑ Proliferation; ↑ cyclin A2, B1, cdk1, cdk2 and PCNA of protein expression (dose-dependently)
17	Rat	TGF-β1 neutralization in PRP on a cardiotoxin-induced muscle injury model	↑ Muscle regeneration; ↓ fibrosis; ↑ angiogenesis; prolonged satellite cell activation; ↑ M2 macrophages to the injury site
78	C2C12 myoblasts and Rat	(i) Human releasate on C2C12 murine myoblasts; (ii) Rat PRP on rat rotator cuff tear	↑ Proliferation; inhibited myogenic differentiation; ↓ expression of adipogenic genes and lipid droplet formation in vivo
69	Mouse	Muscle contusion injury and PRP at different time points	PRP injection 7 d after injury ↑ exercise time; ↓ fibrotic tissue; PRP at 1 and 4 d after injury ↓ exercise time; ↑ fibrotic tissue
94	Mouse	Gelatin hydrogel with platelet releasate in wound healing	↑ Levels of angiogenesis ↑ Wound healing rate
34	Mouse	Human releasate on muscle-derived progenitor cells	↑ Proliferation of hMDPCs; PDGF further increases the proliferative effects of PRP
80	Rabbit	Rabbit PRP with ASC extracts on rabbit myogenic progenitors and human fibroblast culture	ASCs extracts had a stronger effect on proliferation of MPCs than PRP
61	Human athletes	PRP in grade II muscle lesions	↓ Pain in all patients and improved muscle function in 85% of patients after first injection ↓ VAS 2 wk post-treatment. 100% return to sport activities after 35 d (non-controlled study)
62	Human athletes	PRP in acute muscle injury	93% ↓ pain after 28 d vs 80% in control; ↑ range of motion and strength
63	Human patients	PRP in proximal hamstring injuries	↓ VAS and NPRS scores
96	C2C12 myoblasts	Human PRP lysate on C2C12 murine myoblasts	↑ C2C12 proliferation up to 20% PL but mildly cytotoxic at 100%; ↑ C2C12 scratch wound closure
48	Human (*ex vivo*)	(i) PRP (ii) releasate with depleted TGF-β1 and myostatin (iii) PPP; in human skeletal muscle myoblasts	PPP and releasate with depleted TGF-β1 and myostatin induced myoblast differentiation; ↑ myoblast proliferation with PRP

Scully et al. 2018, Acta Physiol.e13071

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Different stages and steps in preparation of platelet-based applications

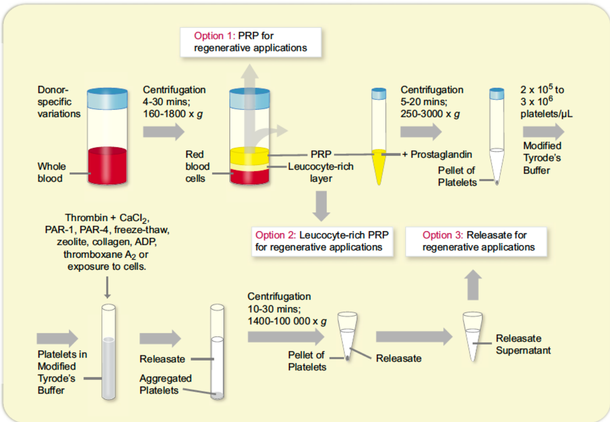

FIGURE 4 A schematic diagram showing the different stages in preparation of platelet-based applications, highlighting possible steps for experimental variations based on published evidence (Table 3). Variations may include donor-specific variability, variability in centrifugations, use of different platelet-based applications (eg, PRP or leucocyte-rich PRP or platelet releasate), platelet concentration, buffer of resuspension, platelet agonists used for activation and the storage conditions

- Different centrifugation speeds
- Different platelet concentrations
- Different platelet agonists

Scully et al. 2018, Acta Physiol.e13071

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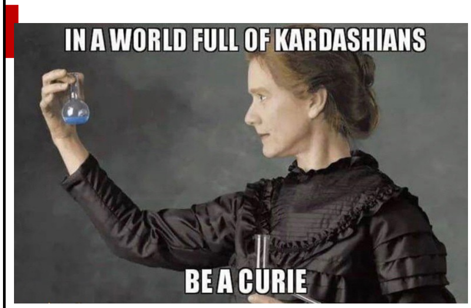
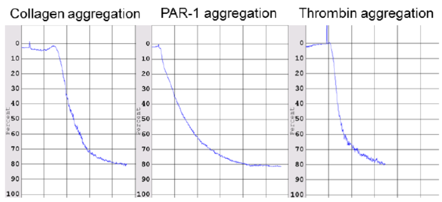
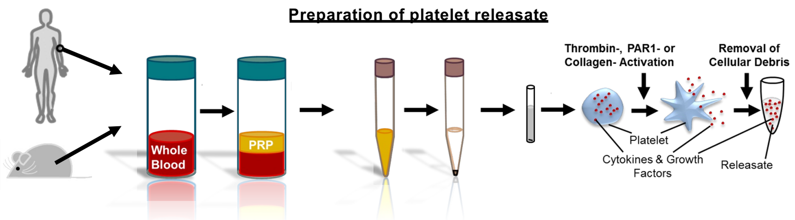
Experimental Approach





A threshold of at least 80% aggregation



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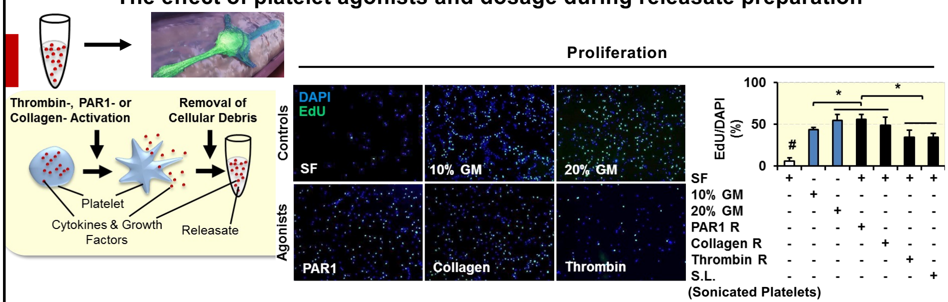
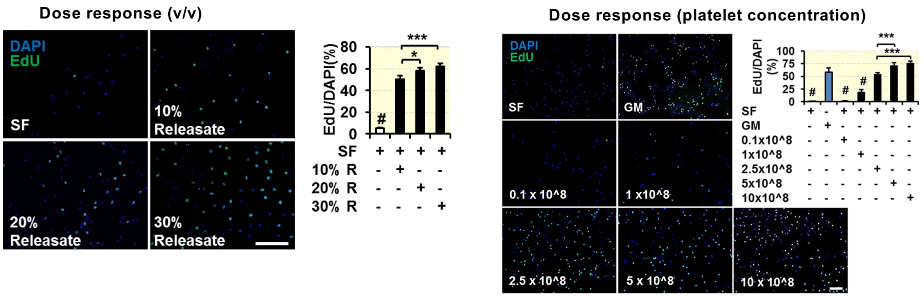
The effect of platelet agonists and dosage during releasate preparation





More platelets and more releasate → more proliferation

Scully et al. 2018, Acta Physiol.e13207

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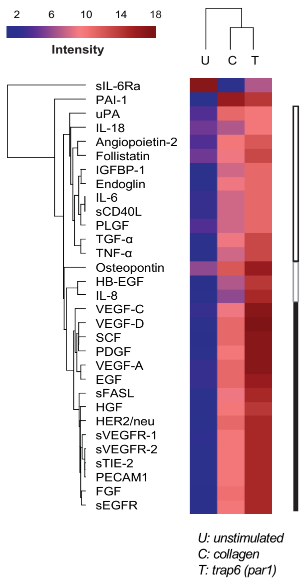
Key points

- PAR1- and Collagen- activated platelet releasate induce stronger myoblast proliferation than thrombin or sonication
- 10-30% releasate (v/v) shows a dose-response on the proliferation of myoblasts
- Different platelet concentrations exhibit a dose-response on myoblast proliferation

Releasate composition



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Overview of effects of releasate components on myoblast proliferation and differentiation based on published evidence

Reference	Factor	Experimental evidence	Proliferation	Differentiation
1 2	Epidermal growth factor receptor (sEGFR)	Blocking EGFR causes a loss of proliferation of satellite cells. sEGFR down-regulation triggers human myoblast differentiation	↑	↓
3	Fibroblast growth factor (FGF)	A strong MAP kinase agonist, is both a potent mitogen and inhibitor of myogenic differentiation	↑	↓
4 5	Follistatin	Myostatin antagonist, Follistatin, improves skeletal muscle healing. Follistatin alters myostatin gene expression in c2c12 muscle cells	↑	
a) 6 b) 2	human epidermal growth factor receptor 2 (ErbB2) (sHER2/neu)	a) (ErbB2) is not upregulated in proliferation or differentiation, b) however epidermal growth factor receptor (EGFR) down-regulation triggers human myoblast differentiation	-	↓
7	Hepatocyte growth factor (HGF)	2 ng/ ml promoted cell division but reduced myogenic commitment and fusion, 10 ng/ml HGF reduced proliferative capability, but increased differentiation	↑↓	↓↑
8	Soluble Interleukin 6 receptor alpha (sIL-6Ra)	IL-6 expression ↑ hypertrophic muscle growth regulating myoblast proliferation and migration	↑	↑
9	Osteopontin	A pro-fibrotic factor in skeletal muscle and myoblasts	-	(↑ Myogenin)
a) 10 b) 11	PDGF-AB/BB	a) Platelet-derived Growth Factor-BB stimulates growth and inhibits differentiation b) PDGF-AA and PDGF-AB have little or no effect on proliferation and differentiation	PDGF-BB ↑ PDGF AB -	PDGF-BB ↓ PDGF AB -
12	Stem cell factor (SCF)	SCF increases skeletal muscle stem cell number	↑	
13	Soluble Angiopoietin-1 receptor (sTIE-2)	Angiopoietin1 (Ang-1), Tie1, and Tie2 mRNA increased during differentiation.	-	↑
14	Vascular Endothelial Growth Factor Receptor-1 (sVEGFR-1)	VEGF acting on VEGFR1 was increased during proliferation and differentiation.	↑	↓
14	(sVEGFR-2)	VEGFR2 was expressed minimally during proliferation and increased during differentiation.	↓	↑
15	Angiopoietin-2	Enhanced differentiation and survival, no influence on proliferation or migration	-	↑
2	EGF	EGF stimulates myoblast proliferation in growth but not in differentiation medium	↑	↓
16	Endoglin(CD105)	Increased proliferation and migration and counteracts TGF-1	↑	↓
17 18	Heparin-binding epidermal growth factor (HB-EGF)	Inhibiting HB-EGF rendered myotubes sensitive to apoptotic cell death and is upregulated in differentiation	-	↑
a) 19 B) 3	Insulin-like growth factor-binding protein 1 (IGFBP-1)	a) IGFBP-1 enhances insulin signalling b) A weak activator of the MAP kinase cascade, is mitogenic but ultimately enhances the differentiated phenotype	↑	↑
8	IL-6	IL-6 expression ↑ Hypertrophic Muscle growth IL-6 Regulates Myoblast Proliferation and Migration	↑	↑
20	IL-8	Interleukin 8 (IL-8) acts as an angiogenic factor	-	-
21	IL-18	IL-18 ↑ angiogenesis	-	-
22 23	PAI-1	Paracrine PAI-1 is involved in glucocorticoid-induced muscle wasting, negative role of PAI-1 in muscle regeneration with increase in fibrosis	↓	↓
24	PLGF	Placenta growth factor (PLGF) to enhance vascularization	-	-
25	Transforming Growth Factor-α (TGF-α)	TGF-α did not C2C12 differentiation. Overexpressing in mice causes a smaller fibre cross-sectional area.	?	-
a) 26 b) 27 c) 28	Tumour necrosis factor (TNF-α)	a) Recombinant TNF-α in differentiation medium stimulated myogenesis at 0.05 ng/ml, but inhibited it at 0.5 and 5 ng/ml b) TNF-α inhibits myogenic differentiation of C2C12 cells through NF-kB. c) Myotube atrophy was induced by TNF- α	?	↓
29	uPA	Urokinase-type plasminogen activator induces myoblast fusion and differentiation	-	↑
30	VEGF-A	b) VEGF causes myoblast proliferation	↑	↑
31	VEGF-C	VEGF-C plays a role in angiogenesis and lymphangiogenesis in a murine model of hind limb ischemia	?	?
32 33	VEGF-D	VEGF-D is the most potent (angiogenesis and lymphangiogenesis) member of the VEGFs when delivered via an adenoviral vector into skeletal muscle	-	?

"↑/↓": indicate increase/decrease respectively, "-": indicates no effect and "?": indicates that we were not able to identify any relevant reference

Scully et al. 2018, Acta Physiol.e13071

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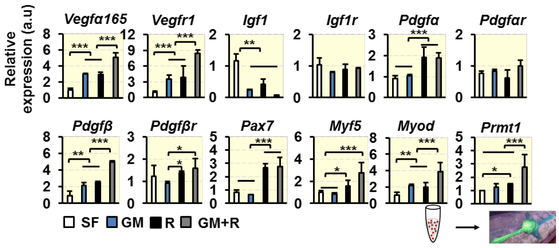
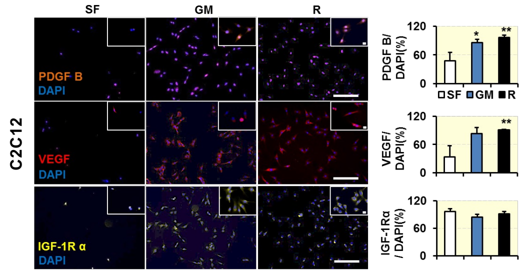
The effect of releasate on gene and protein expression patterns of myoblasts

Myoblast mRNA expression with releasate



Pdgfα, Pdgfβ, Vegfα165 and Prmt1 mRNA are increased with platelet releasate

Myoblast protein expression with releasate



Releasate increases PDGF and VEGF protein expression in proliferating myoblasts

Scully et al. 2018, Acta Physiol.e13207

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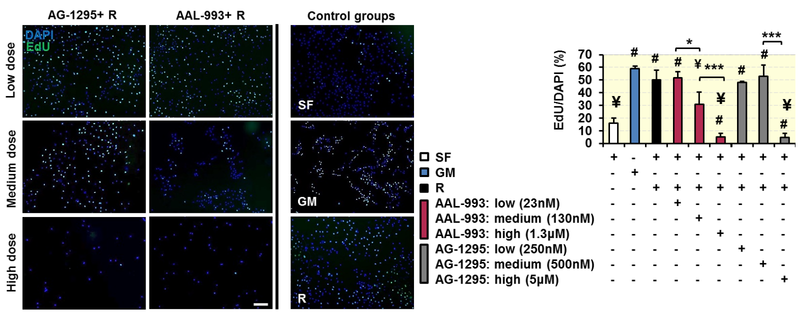
Platelet releasate drives myoblast proliferation through PDGF and VEGF

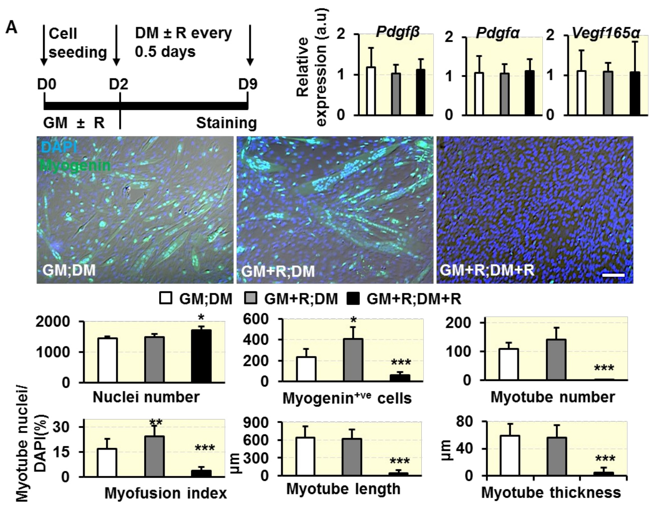
Inhibition of VEGF and PDGF



AAL-993 is a potent inhibitor of VEGF receptors
AG-1295 is a potent inhibitor of PDGF receptor

14

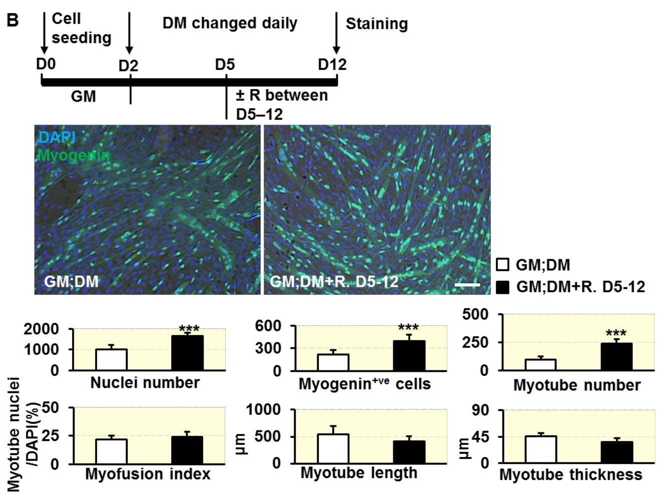
Temporal effects of platelet releasate on myoblast differentiation



Early addition of releasate upregulates myoblast differentiation
Continuous addition of releasate inhibit myoblast differentiation

15

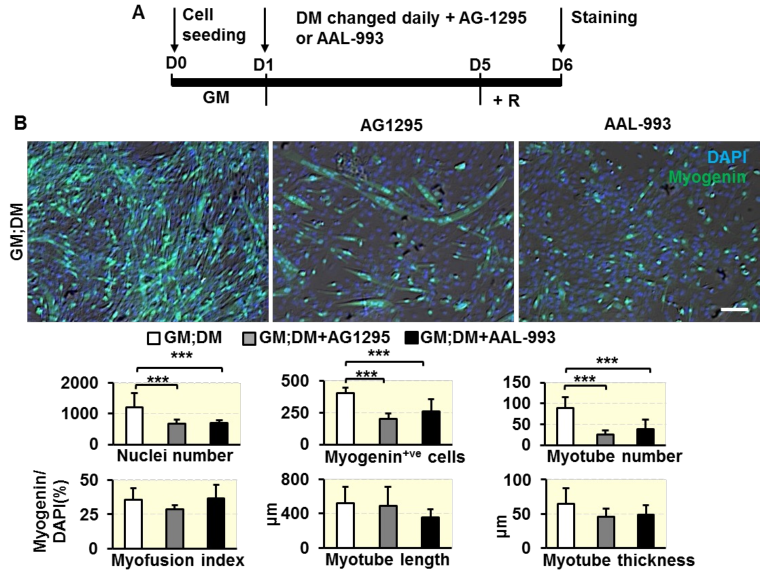
Temporal effects of platelet releasate on myoblast differentiation



Addition of platelet releasate after myoblast fusion is beneficial for myogenic differentiation

16

PDGFR and VEGFR inhibition reduces myoblast differentiation



Inhibition of differentiation is not rescued by releasate in terminal differentiation

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Key points

1. Temporal effects of platelet releasate on myoblast differentiation
2. Early addition of releasate upregulates myoblast differentiation
3. Addition of platelet releasate after myoblast fusion is beneficial for myogenic differentiation
4. Continuous addition of releasate inhibits myoblast differentiation
5. PDGFR and VEGFR inhibition reduces myoblast differentiation

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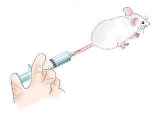
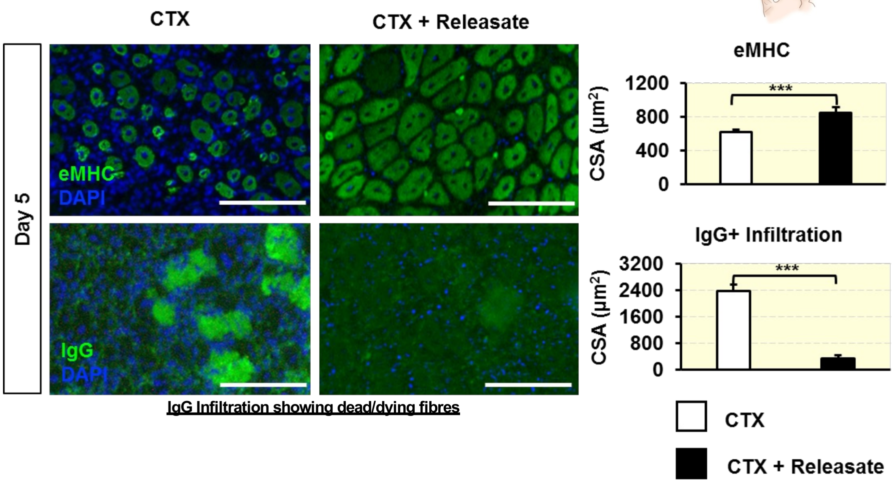
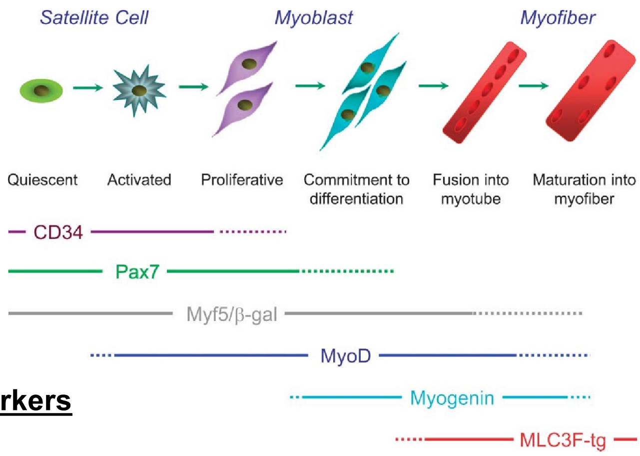
In Vivo work on skeletal muscle regeneration:





Platelet releasate accelerates skeletal muscle regeneration of tibialis anterior *in vivo*

19



Muscle Stem Cell Markers

Pax7 – quiescence

MyoD - activation/ proliferation

Myogenin- differentiation

Zammit et al. 2006– J Histolchem Cytochem) 54

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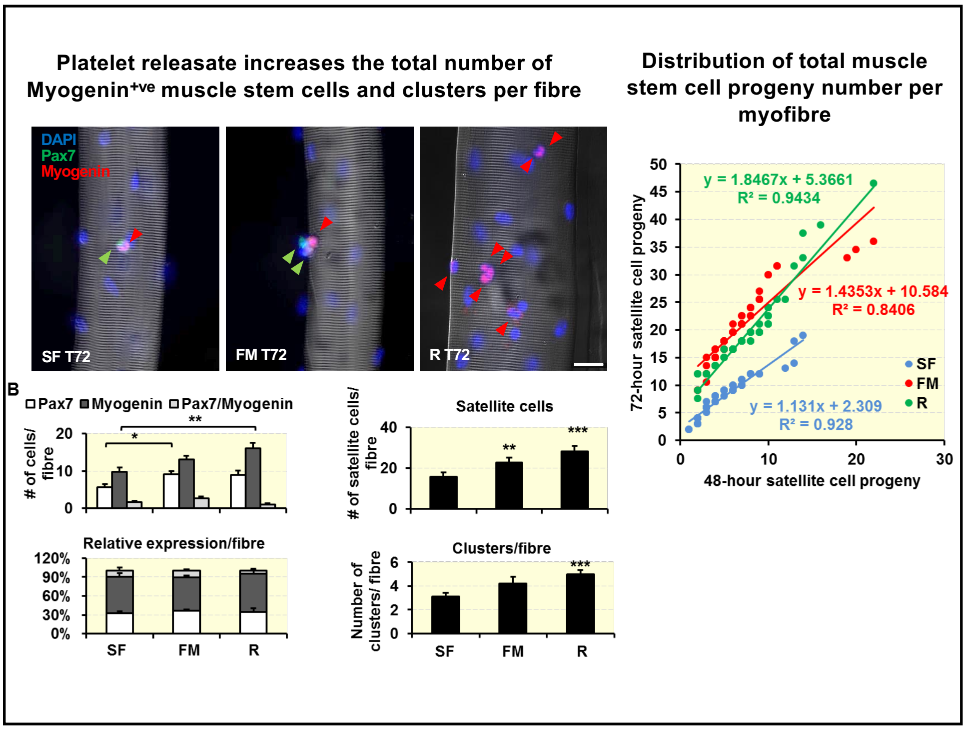
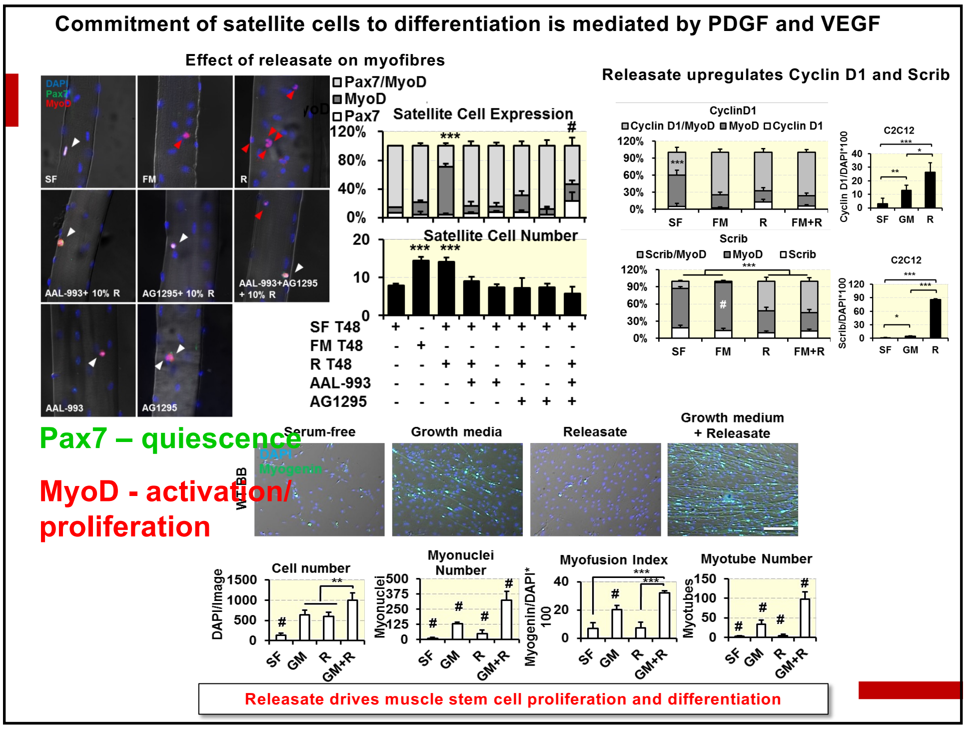


Platelet releasate increases the total number of Myogenin+ve muscle stem cells and clusters per fibre
Distribution of total muscle stem cell progeny number per myofibre
DAPI
Pax7
Myogenin
SF T72
FM T72
R T72
B
Pax7 Myogenin Pax7/Myogenin
# of cells/ fibre
Satellite cells
# of satellite cells/ fibre
Relative expression/fibre
Clusters/fibre
Number of clusters/ fibre
SF
FM
R
y = 1.8467x + 5.3661
R² = 0.9434
y = 1.4353x + 10.584
R² = 0.8406
y = 1.131x + 2.309
R² = 0.928
72-hour satellite cell progeny
48-hour satellite cell progeny

21



Commitment of satellite cells to differentiation is mediated by PDGF and VEGF
Effect of releasate on myofibres
Pax7/MyoD
MyoD
Pax7
Satellite Cell Expression
Satellite Cell Number
SF T48
FM T48
R T48
AAL-993
AG1295
Releasate upregulates Cyclin D1 and Scrib
Pax7 – quiescence
MyoD - activation/ proliferation
Serum-free
Growth media
Releasate
Growth medium + Releasate
Cell number
Myonuclei Number
Myofusion Index
Myotube Number
Releasate drives muscle stem cell proliferation and differentiation

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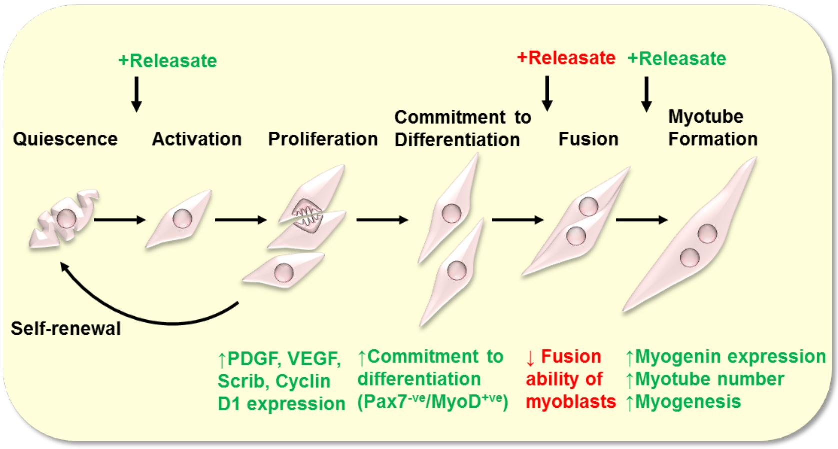

Platelet releasate stimulates muscle stem cell proliferation and differentiation through the PDGF/VEGF/MyoD/Scrib-axis

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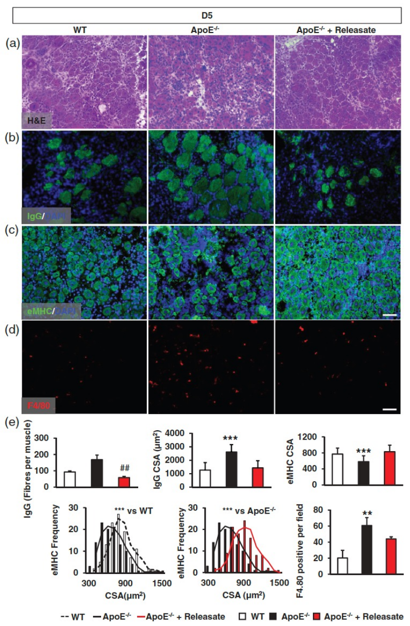
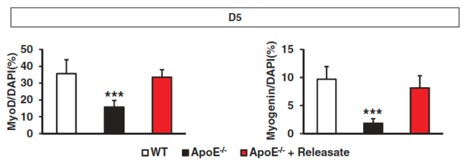
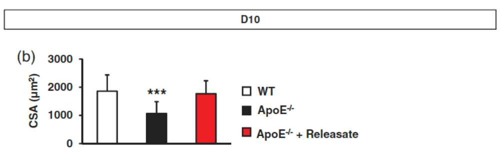
Impaired skeletal muscle regeneration of ApoE–/– mice is rescued by platelet releasate



Activation and differentiation of skeletal muscle stem cells is impaired in ApoE–/– mice and can be alleviated by treatment with platelet releasate



Impaired skeletal muscle regeneration of ApoE–/– mice 10 days after injury is rescued by platelet releasate



Barlow et al. 2021, Exp Physiol. 106

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Conclusions

1. We have shown *in vitro* and *ex vivo* data on PAR-1 -activated platelet releasate dose-dependently stimulating myoblast proliferation
2. We have identified two essential components of releasate being essential for both proliferation and differentiation using mRNA, protein and inhibitory analysis
3. We have shown a temporal effect of platelet releasate on myotube formation
4. We have identified the effect of platelet releasate on a myoblast lineage progression marker *ex vivo*; Scrib
5. Platelet secretome normalises the compromised muscle regeneration in a mouse model of hyperlipidaemia

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The Role of Platelet Secretome in Skeletal Muscle Biology and Satellite Cell Function

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