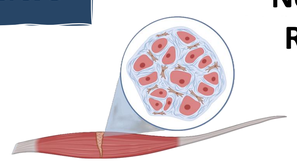
MSC-Conditioned Media: a promising approach for muscle tissue regeneration

Caseiro, A. R.[1,2,3], Pereira, A.[1], Pedrosa, S.P.[4,5], Branquinho, M.V.[2,3,6], Rema, A. [2,3,6], Amorim. I.[6,7,8], Maurício, A.C.M. [2,3,6]

[1]CIVG Centro de Investigação Vasco da Gama, EUVG Escola Universitária Vasco da Gama, Coimbra, Portugal
[2] CECA-ICETA Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto (ICETA), Porto, Portugal
[3] AL4AnimalS Associate Laboratory for Animal and Veterinary Sciences, Portugal
[4] Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal
[5] Biorbis, Unipessoal LDA, Edifício de Biotecnologia da Universidade Católica Portuguesa, Rua Diogo Botelho 1327, Porto 4169-005, Portugal
[6] ICBAS Insitituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Rua Jorge Viterbo Ferreira nr.228, Porto, 4050-313, Portugal
[7] i3S i3S, Instituto de Investigação e Inovação em Saúde, Universidade Porto, Rua Alfredo Allen, 4200-135 Porto, Portugal
[8] IPATIMUP Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Rua Dr. Roberto Frias s/n, Porto, 4200-465, Portugal

SEMIT

Problematic

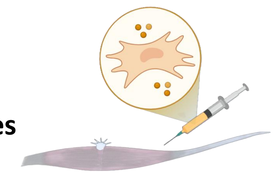


Normal Muscle Regeneration
Fibrotic Tissue



Muscle Injury

MSCs Therapies





Improved Muscle Regeneration

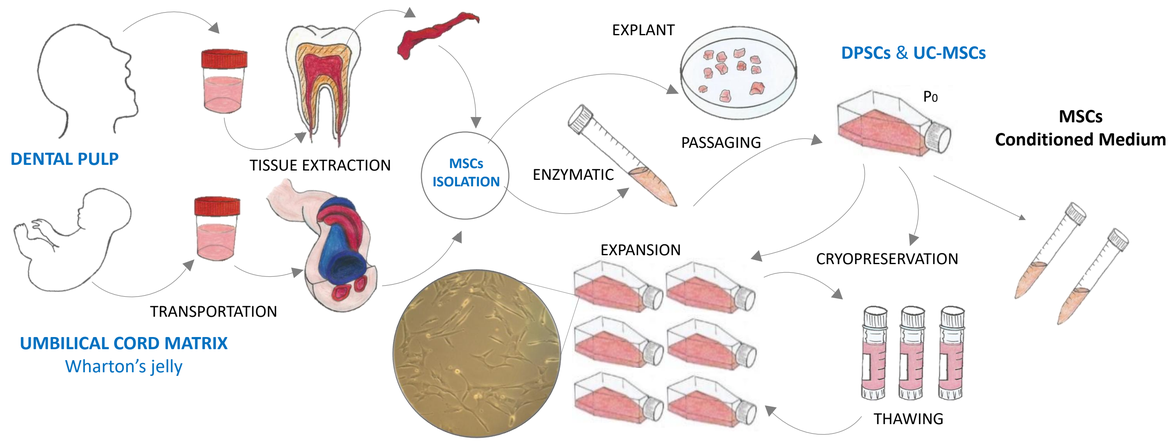
Evidenced BENEFITS of MSCs in muscle regeneration..

- **Scar/fibrosis formation:** Fibrotic tissue replaces muscle fibers, decreasing elasticity and functional capacity.
- **Impaired reinnervation:** Delayed or incomplete nerve regeneration limits muscle control and coordination.

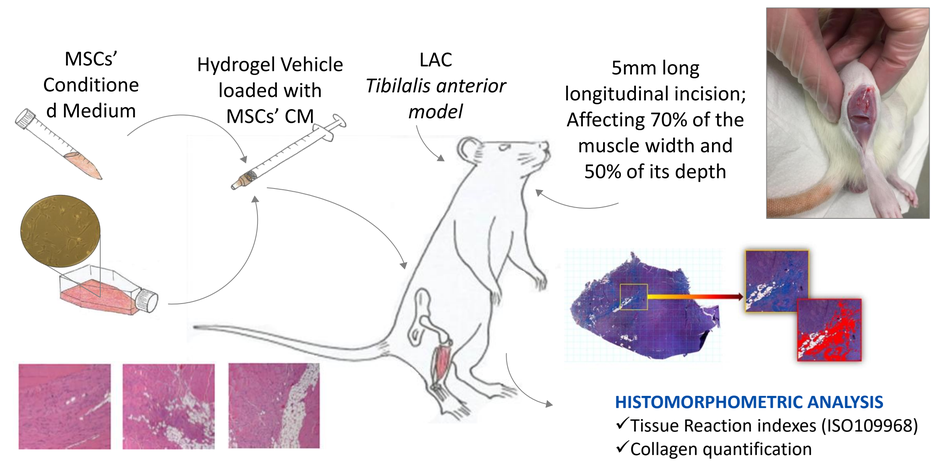
- **Decreased fibrosis:** MSCs modulate TGF-β1 and other factors to reduce scar tissue formation, restoring elasticity.
- **Improved vascularization:** MSCs secrete pro-angiogenic factors (e.g., VEGF), supporting blood vessel growth and energy delivery.

Methodology

MSC-DERIVED **CONDITIONED MEDIUM**

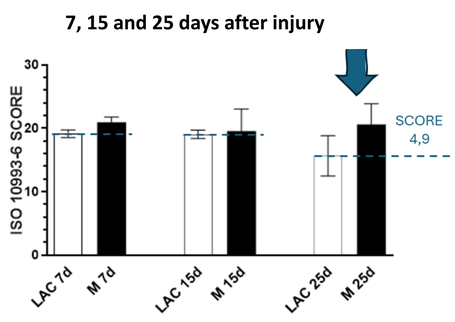


IN VIVO **ASSAYS** – RAT LACERATION MODEL



Results

LOCAL TISSUE RESPONSE AFTER IMPLANTATION



SCORE (based on ISO 10993-6)	
0,0-2,9	No reaction
3,0 - 8,9	Slight reaction
9,0 - 15,0	Moderate reaction
>15	Severe reaction

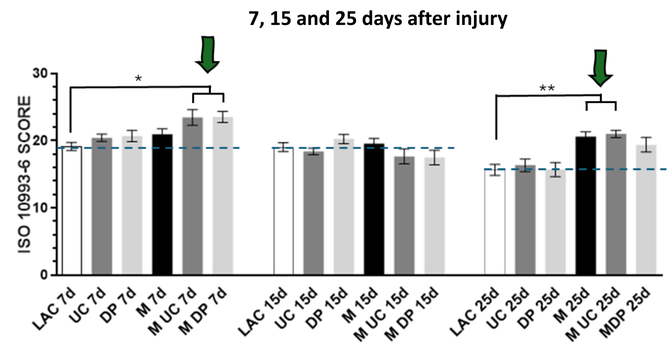


Fig 1. Left graph: ISO 10993-6 scores for untreated laceration (LAC) and lacerations treated with Matrigel Matrix (M) at 7, 15, and 25 days. Right graph: ISO 10993-6 scores for untreated laceration (LAC), Matrigel Matrix (M), dental pulp (DP) and umbilical cord (UC) conditioned medium, Matrigel plus dental pulp conditioned medium (M DP), and Matrigel plus umbilical cord conditioned medium (M UC) at 7, 15, and 25 days. Symbols "*" indicates statistical significance relative to the untreated group: * p<0.05; ** p<0.01.

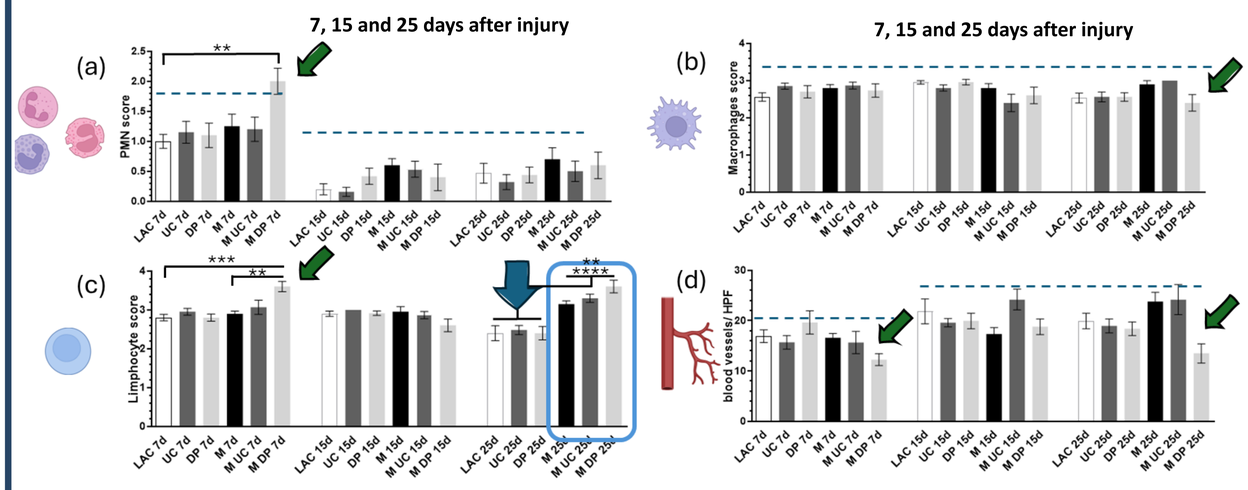
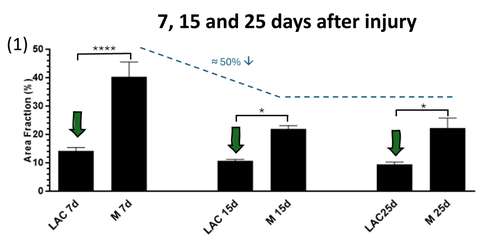
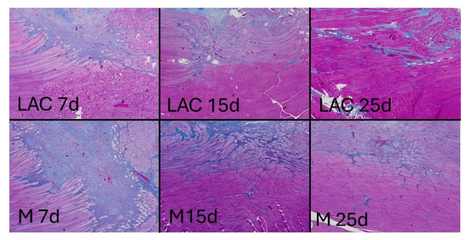
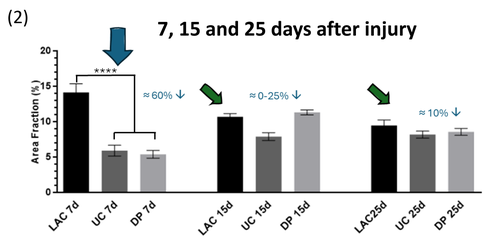


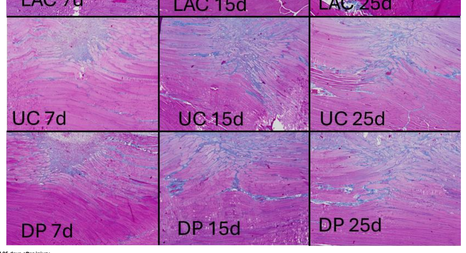
Fig 2. (a) Polymorphonuclear cell scores for untreated laceration (LAC), Matrigel Matrix (M), dental pulp (DP) and umbilical cord (UC) conditioned medium, Matrigel plus dental pulp conditioned medium (M DP), and Matrigel plus umbilical cord conditioned medium (M UC) at 7, 15, and 25 days. (b) Macrophage scores for the same experimental groups and timepoints. (c) Lymphocyte scores for the same groups and timepoints. (d) Blood vessels per high power field (hpf) for untreated laceration (LAC), Matrigel Matrix (M), dental pulp (DP), umbilical cord (UC) conditioned medium, Matrigel plus dental pulp conditioned medium (M DP), and Matrigel plus umbilical cord conditioned medium (M UC) at 7, 15, and 25 days. Symbols "*" indicates statistical significance * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001.

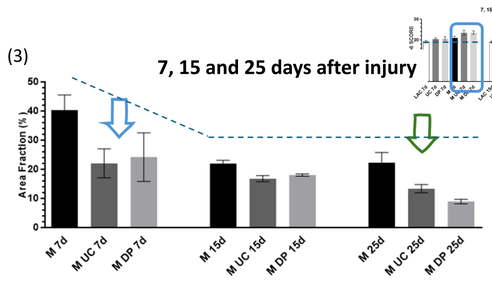
MUSCLE REGENERATION: **FIBROTIC RESPONSE**











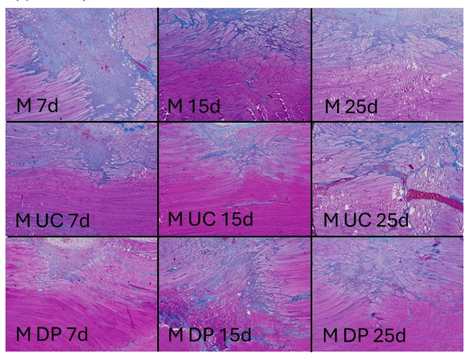


Fig 3. (1): Collagen area (μm²) was quantified relative to total image area to calculate the Collagen Area Fraction (%), normalized to baseline collagen in healthy skeletal muscle TA, for untreated laceration (LAC) and lacerations treated with Matrigel Matrix (M) at 7, 15, and 25 days. (2): The same quantification approach was applied to untreated laceration (LAC), dental pulp (DP), and umbilical cord (UC) conditioned medium groups at matched timepoints. (3)t: The identical analysis was performed for Matrigel plus dental pulp conditioned medium (M DP) and Matrigel plus umbilical cord conditioned medium (M UC) at 7, 15, and 25 days. Representative micrographs for all conditions are presented beneath each graph. Symbols "*" indicate statistical significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001.

Conclusion

- ✓ISO methodology highlights the need for integrated evaluation of tissue responses in context, not just isolated material responses.
- ✓In non-critical muscle injuries, a slightly more pronounced tissue reaction from matrix is not detrimental to regeneration.
- ✓Functional muscle recovery is mainly linked to reduced development of non-contractile fibrotic tissue, achieved by CM from umbilical cord and dental pulp MSCs.
- ✓Despite distinct secretome profiles, *in vivo* responses to CM from both sources were similar, showing no significant differential effect.
- ✓Further studies are needed to understand how different secretome profiles can drive similar biological responses.
- ✓Findings support previous evidence that secretome-mediated effects involve more than just the soluble fraction, indicating complex layers of angiogenic and reparative functions.

This work was supported by the Portuguese Foundation for Science and Technology (FCT)(Doctoral Fellowship SFRH/BD/101174/2014) and Strategic Project PEst-OE/AGR/UI0211/2011 and project UID/06432/2025 (https://doi.org/10.54499/UID/06432/2025), and the project BIOIMP_ACE_MAS_6_E, co-funded by the EU through the Interreg VI-A Spain-Portugal Programme (POCTEP) 2021-2027. The authors also acknowledge the support from EUVG and CIVG founding entity Associação Cognitária Vasco da Gama.

TERMIS EU-Chapter 2026 PALMA DE MALLORCA 21-24 APRIL / SPAIN