Perivascular stem/progenitor cells, up-and-coming tissue regenerating route

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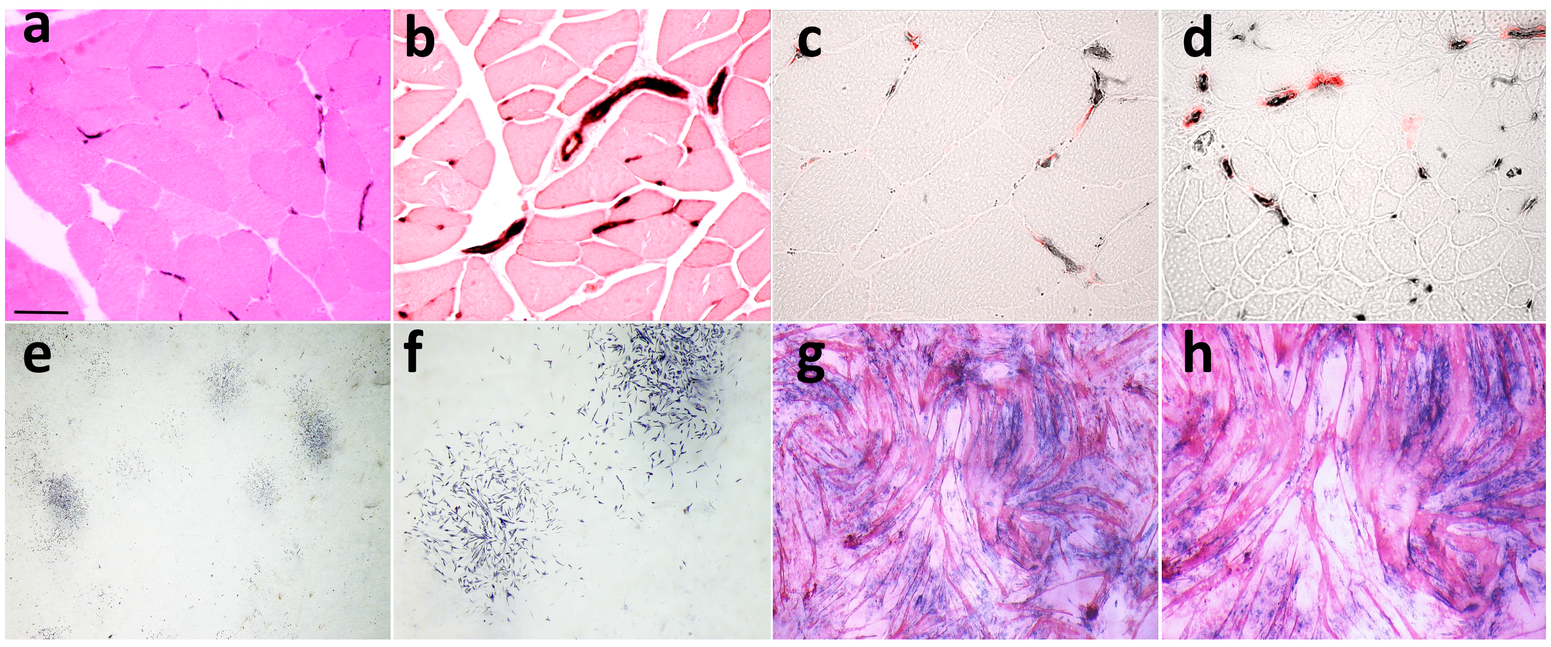
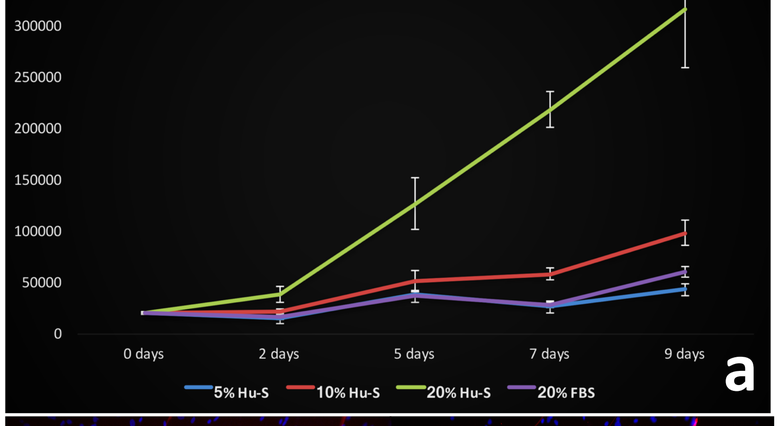
Cesare Gargioli[1], Jacopo Baldi[2], Carmine Zoccali[2], Pina Chichierchia[3], Fabio Erba[3], Maria Laura Foddai[4] and Roberto Biagini[2]

[1]Department of Biology, Tor Vergata University; [2]Oncological Orthopaedics, Regina Elena National Cancer Institute; [3]Muscle-Skeletal Tissue Bank, Regina Elena National Cancer Institute; [4]Transfusion Medicine, Regina Elena National Cancer Institute.

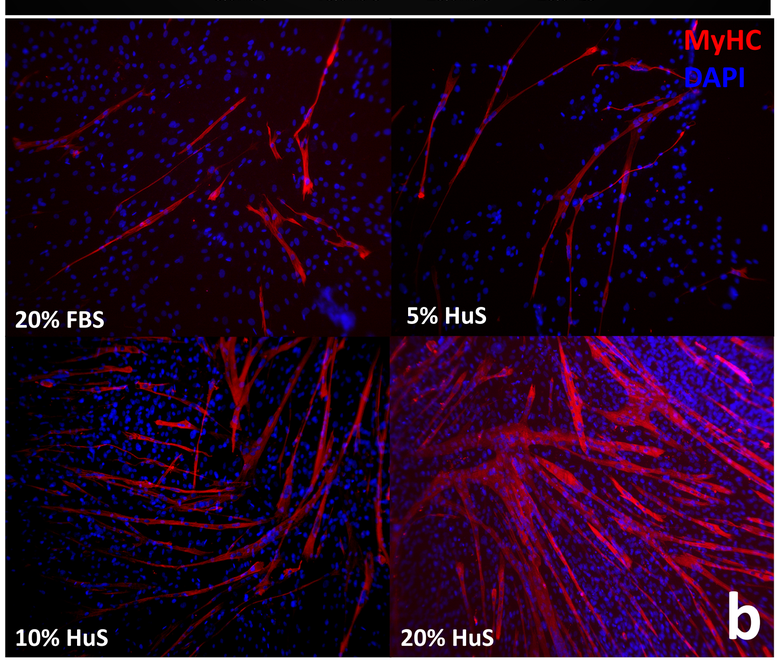
INTRODUCTION. Skeletal muscle can self repair and regenerate in response to damage: but, it is unable to restore significant loss of tissue as it occurs in extensive traumatism or surgery. Reconstructive strategies, such as autologous muscle transplantation and intra-muscular injection of muscle progenitor cells yield only modest therapeutic outcomes, hence new interdisciplinary scientific approaches, based on tissue engineering, are developing to replace damaged or ablated skeletal muscle. Here we report a novel strategy based upon the use of mouse or human mesoangioblasts in a cell-compatible PEG-fibrinogen (PF) hydrogel biomaterial succeeding in replace mouse tibialis anterior (TA) *in vivo* (Fuoco et al., 2015). These data establish a new paradigm for skeletal muscle tissue engineering, showing that it is possible to create an artificial muscle morphologically and functionally very similar to a normal one. Nevertheless the challenge is to scale up this technology for human patients application. For this purpose we isolate vessel associated muscle progenitor cells for alkaline phosphatase (AP) expression so called pericytes, showing great myogenic capabilities while supporting angiogenesis. Pericytes are a wider population expressing several markers (CD146, NG2, PDGFRb and AP), revealing as potent cell source able to generate muscular tissue and at the same time to promote angiogenesis. Hence, exploiting their capability we are attempting to build pericytes derived artificial muscle human-like size combining different technology.



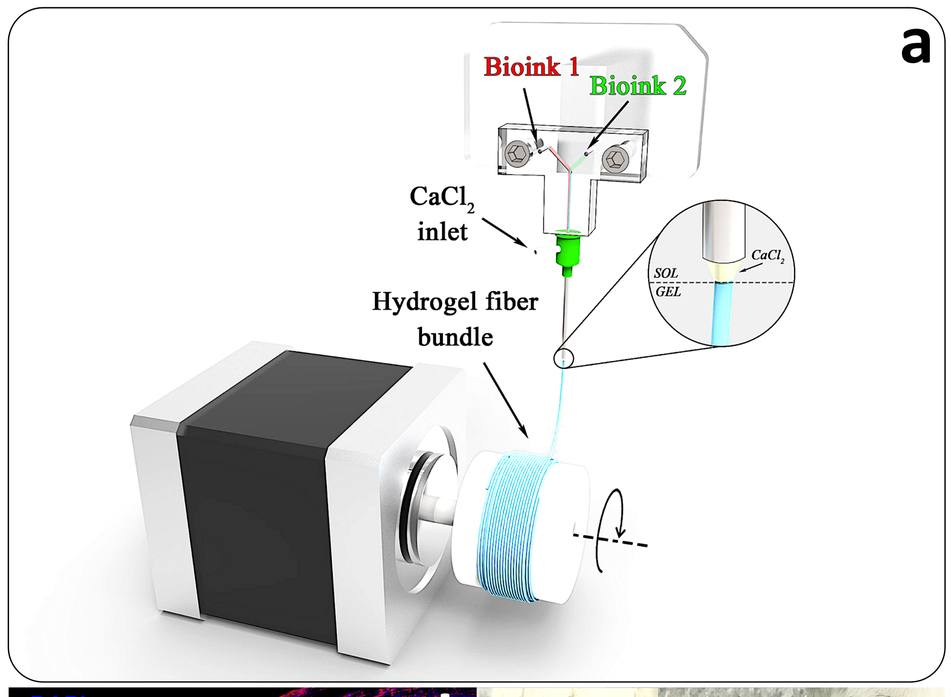
AP expression on skeletal muscle sections and isolated human pericytes. **(a-b)** AP staining (black) on human (a) and mouse (b) muscle sections counterstained with eosin revealing pericytes localization around blood vessel. **(c-d)** Smooth Muscle Actin (SMA) immunofluorescence (red) superimposed on AP staining phase contrast images from human (c) and mouse (d) muscle sections revealing pericytes laying beneath vessel wall smooth muscle layer. **(e-f)** AP staining (blue) on colonies formed after human muscle derived pericytes isolation, (f) enlarged view. **(g-h)** AP staining (blue) and immunohistochemistry against Myosin Heavy Chain (MyHC) (red) showing spontaneous myogenic differentiation of human muscle derived pericytes, (h) higher magnification.

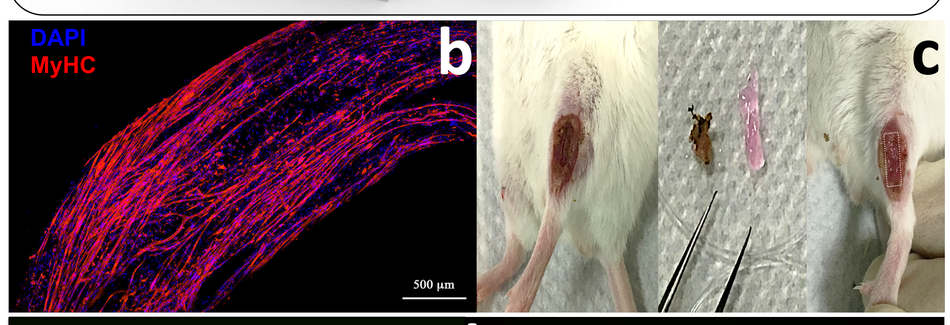


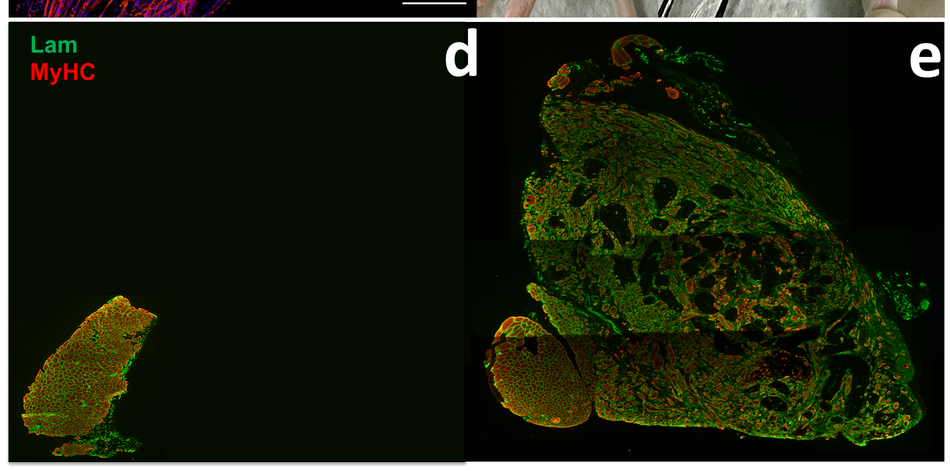
Ascertained the myogenic potential of muscle derived human pericytes and with the perspective to employ this cell population in clinic for cell based therapies apt to skeletal muscle tissue reconstruction upon massive tumor ablation. We tested the effect of human derived serum obtaining astonishing results: revealing the enhanced proliferating and differentiating activity comparing with 20% fetal bovine serum (FBS), standard concentration used for pericytes expansion *in vitro*. Moreover exploiting modern techniques likewise 3D printing, assembled with custom made microfluidic printing head, we were able to generate i*n vitro* myo-structure for replacing ablated or damaged muscle.



Behavior of human muscle derived pericytes upon different concentration of human derived serum. **(a)** Growth curve comparing the effect of different human derived serum (HuS) concentration with the standard 20% (FBS), showing the increased proliferating activity in 20% HuS). **(b)** Immunofluorescence against Myosin Heavy Chain (MyHC) (red) showing spontaneous pericytes myogenic differentiation upon exposure to different human derived serum concentration, nuclei were counterstained by DAPI (blue). The results highlighted the remarkable amelioration of pericytes myogenic capability upon 20%Hus exposure.







Microfluidic based 3D printing technology for artificial skeletal muscle reconstruction. (a) Schematic representation of custom made 3D printing system based on PF and Alginate extrusion. **(b)** 3D printed obtained myo-structure labeled by immunoreaction against Myosin-Heavy Chain (MyHC) (red), nuclei were counterstained by DAPi (blue). **(c)** Macroscopic images showing surgical mouse anterior tibialis (TA) ablation(left), comparison between removed TA tissue and implanted myo-structure (center) and positioned myo-structure replacing ablated TA (right). **(d, e**) Immunofluorescence analysis on TA transversal section reconstruction from control TA (d) vs replaced one with 3D printed myo-structure (e). Muscle fibers were stained for MyHC (red) while basal lamina was stained for laminin (green).

CONCLUSIONS. These data represent a ground breaking advance over previous attempts to generate an artificial skeletal muscle tissue *in vivo*. Starting from these observations, as proof of concept, we are strengthening this novel approach for regeneration and/or reconstruction of skeletal muscle tissue segments human-like size in order to translate this technique to clinical application. For this purpose human derived pericytes have been isolated from muscle biopsies revealing a remarkable spontaneous myogenic potential *in vitro*. Moreover by employing human derived serum we observed a remarkable amelioration of pericytes proliferation and differentiation, further exploring the possibility to grow autologous cells in autologous serum avoiding animal contaminant and any rejection possibility in case of future clinical application. Furthermore, with the perspective to combine and to exploit new emerging technology likewise microfluidic based 3D printing for human-like size muscle reconstruction.

Fuoco et al., In vivo generation of a mature and functional artificial skeletal muscle. EMBO Mol Med. 2015 Feb 25.