# Functional assessment of a decellularized bovine muscle organoid with high cell viability and intact ECM

Rossana Topputi

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## Background

Muscle organoids represent advanced in vitro models that closely mimic the architecture and function of native muscle tissue.

Our previous work introduced a muscle organoid derived from the dorsal portion of the bovine diaphragm, characterized by a unique multilayered structure.

In this study we standardized a decellularization protocol to obtain a cylindrical organoid preserving the alternating layered organization of muscle and fibrous tissue.

## Decellularization protocol

Bovine diaphragm samples processed into cylindrical scaffolds (1 cm in diameter)

- Chemical decellularization:

SDC (0.5%, 1%, 2%, 4% for 24 h; 4% for 48 h)

- Enzymatic digestion:

DNase (20 U/µL), 3 h at RT

Trypsin-EDTA, 1 h at 37°C

Washing step:

- Distilled water, 48 h

Protocol optimized to remove cellular components and preserve ECM architecture

## Characterization and functional assessment

- Macroscopic appearence

Treated tissues turned whitish

- Histological analysis

Partial muscle fiber loss (H&E);
Complete nuclei removal (DNA quantification);
Presence of ghost and filled ghost fibers

- Protein analysis

Significant reduction of actin

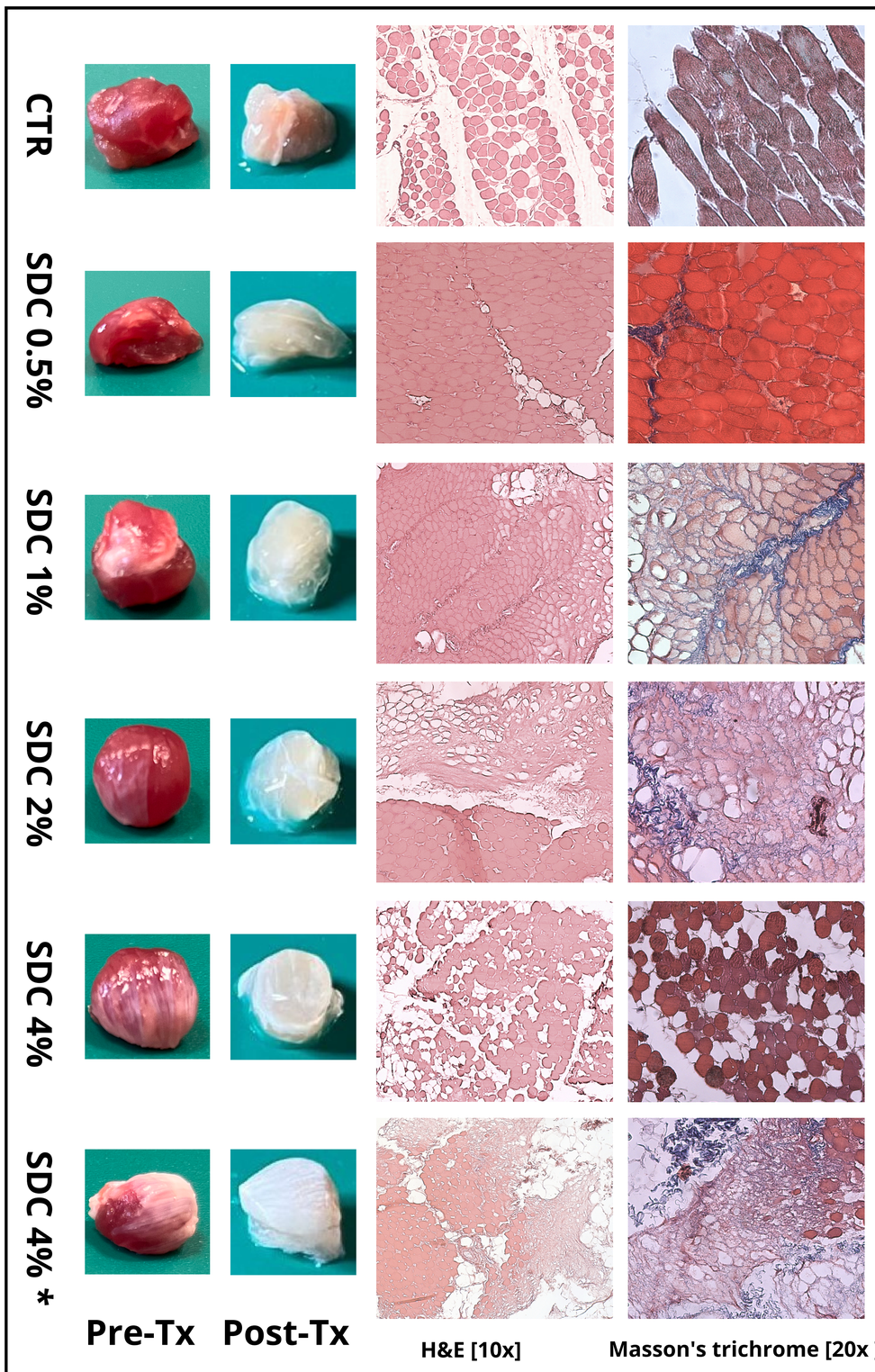
- ECM preservation

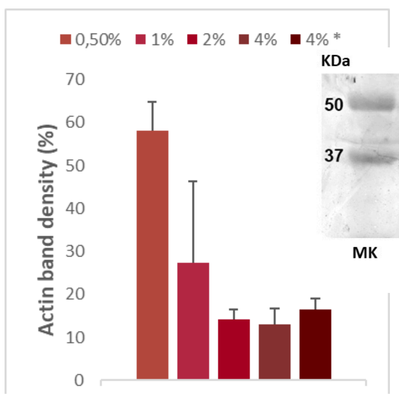
Collagen and GAGs mainteined

- Biocompatibility

No cytotoxicity with C2C12 cells;
High cell viability.

→ **Suitable for recellularization**

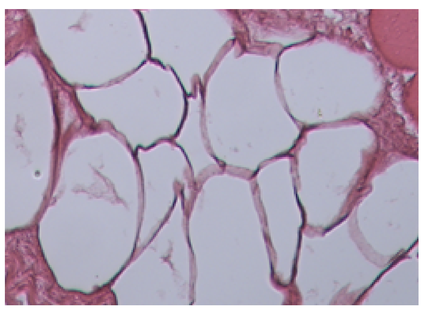


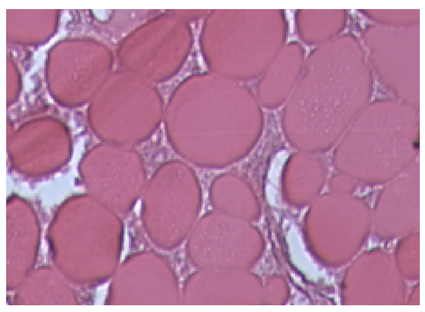

**Quantification of actin band intensity in SDS-PAGE gel**

| Sample | DNA concentration (ng/µL) |
|---|---|
| Control | 538.3 |
| SDC 0.5% | 4.0 |
| SDC 1% | 2.0 |
| SDC 2% | 7.1 |
| SDC 4% | 6.6 |
| SDC 4% (48h) | 2.5 |

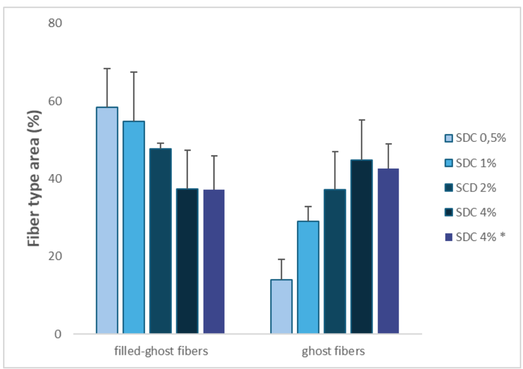
**Extracted DNA concentration (ng/µL)**



**Ghost fibers**



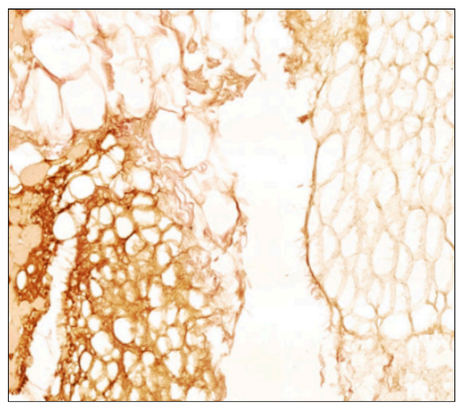
**Filled-ghost fibers**



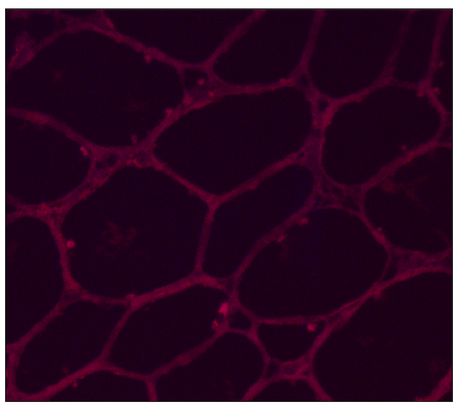

**Mean area percentage of filled-ghost and ghost fibers after treatment**



***Collagen I***

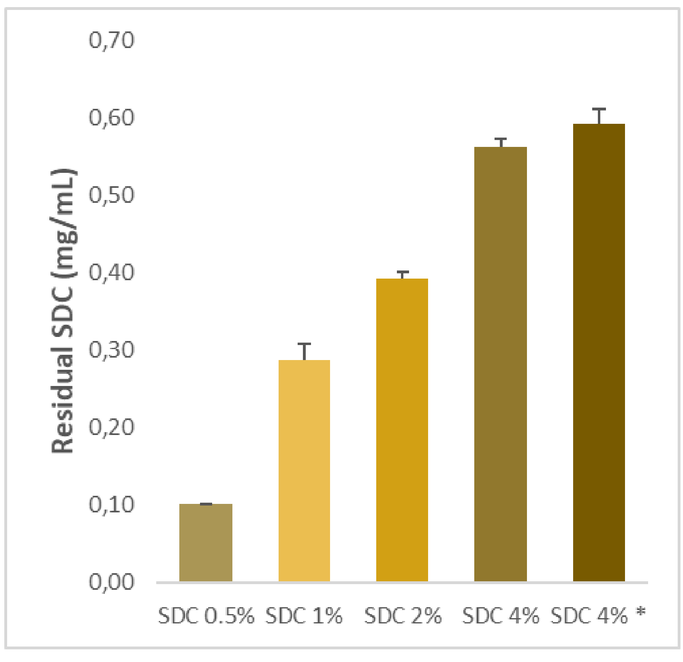


***Myosin***

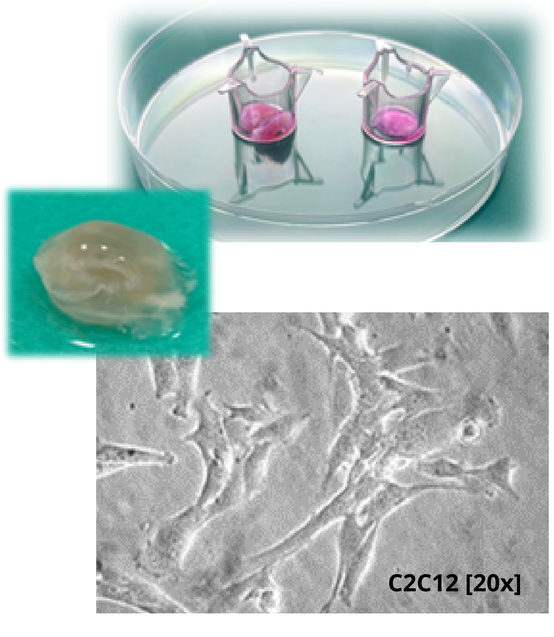


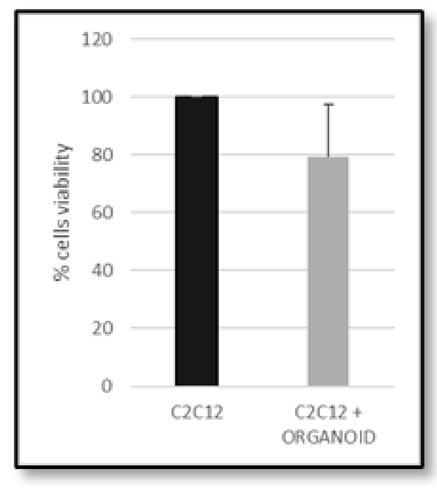
***F- Actin***

**Detection of ECM and myofibrillar components**

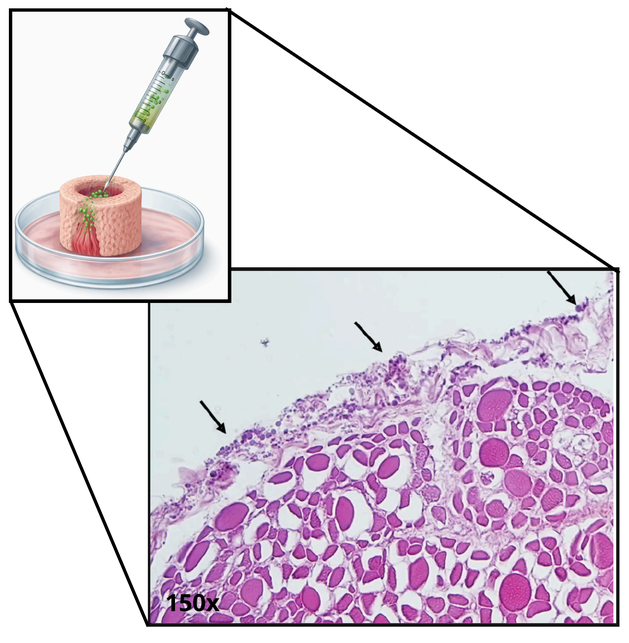



**Residual SDC after treatment**




**MTT assay: Biocompatibility assessment**




**Cells injection**

## OUTCOMES

The obtained scaffold support efficient recellularization and long-term cell viability. It represents a promising platform for future muscle research, drug testing, and regenerative medicine.

## WORK IN PROGRESS

The scaffold will be used for recellularization with both healthy and pathological muscle cells, aiming to establish a reliable platform for preclinical studies of muscle disorders.

## Reference

R.Topputi, E.E. Akyurek , M. Erba, A. Carolo, L. Melotti, G. Ventriglia, E. Casalino, M.V. Patruno, R. Sacchetto, T. Martinello