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BIOMIMETIC POYPEPTIDES: A NEW STRATEGY FOR MUSCLE TISSUE REGENERATION

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Abstract — Despite recent progress in regenerative medicine, functional muscle tissue restoration still represent a challenge, being unable to self- restore significant tissue loss, as consequence of trauma, congenital defects, tumor ablation or denervation. The creation of new muscle through tissue engineering represents an alternative for the replacement of tissue after severe damage. Among the many materials available, those of natural origin are preferable for their biocompatibility and their capacity to resemble the native physiologic environment of cells. A new opportunity is represented by the adoption of a biomimetic approach that allows to realize compounds that are in –between natural and synthetic origin with limited variability and controlled features. Human elastin-like polypeptides that have been designed and produced in our laboratory represent an innovative and promising tool to be exploited in muscle tissue engineering.

Index Terms — TRANS2CARE, biomimetic strategy, elastin, recombinant protein

1 BACKGROUND

Perhaps the most appealing opportunity is represented by gaining inspiration from nature for the precise "tailoring" of biomaterials with finely tuned unique functional properties. The assembling of functional protein domains lead to the creation of new, artificial bio-inspired polypeptides. A very interesting and promising model is represented by Human Elastin-Like Polypeptides (HELP), the repetitive artificial polypeptides based on penta- or hexa- peptidic motifs that characterize elastin [1]. These protein polymers retain several peculiar biophysical properties as, for example, the reversible inverse phase transition, changing the solubility and aggregation state in response to temperature variation.

2 OBJECTIVES

We will employ HELPs biopolymers to realize biomimetic surfaces with different mechanical properties to favour muscle cell culturing. The biomimetic environment is expected to enhance the applicative potential of the cells for skeletal muscle regeneration. Our main aim is to evaluate the capacity of HELP polypeptides to support cell adhesion, growth and differentiation in vitro. Particular attention will be dedicated to the differentiation process that lead to myotube formation, expression of an organized contractile apparatus and assembly of the excitation-contraction coupling apparatus.

3 APPROACH & METHODS

General approach

We have already shown that the properties of HELPs can be exploited to prepare biomimetic surfaces. These surfaces are well tolerated by cells that adhere and grow, remaining viable. Their differentiation potential has been shown to be unaltered. HELP polypeptide in particular can be used also to realize three-dimensional biomimetic environments that allow viable cell encapsulation. Moreover, the functionality of these biopolymers can be further expanded, embedding in the same macromolecule a bioactive domain.

Methods

The two prototypes of recombinant HELP biopolymers, as well as a biopolymer recently synthesized (HEPLc), will be tested in combination with myoblast C2C12 cells. Cell viability, proliferation and differentiation will be studied. In particular, the myotube formation potential of C2C12 cultured on these elastin biomimetic subtrates will be evaluated, in conjunction with the expression of differentiation markers, such as myogenin and skeletal muscle myosin and components of the calcium signalling machinery. In the next step, the cells will be seeded and/or embedded into 3D HELP scaffolds to boost cell differentiation towards a fully competent contracting muscle cell.

4 RESULTS

Skeletal muscle fibers, in vivo, derive from a highly coordinated sequence of molecular and cyotogical changes, ultimately leading to the expression of the contractile, multinucleated phenotype (Fig. 1).

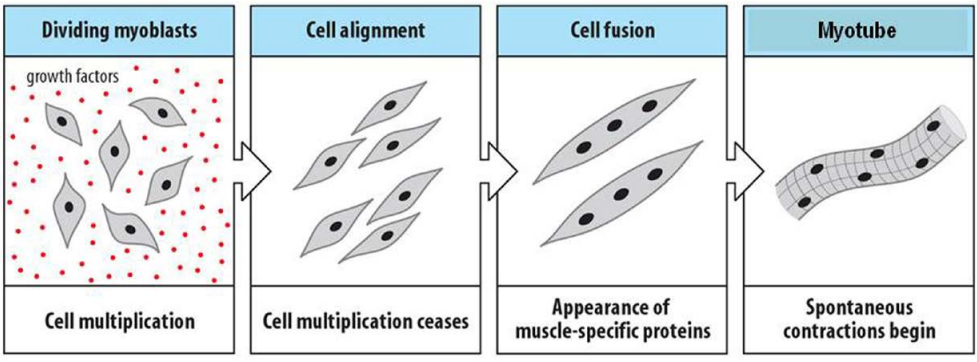

Figure 1. Differentiation pattern and steps for skeletal muscle fibers formation

C2C12 myoblasts can be induced to recapitulate, in vitro, most of these steps, thus representing a useful model to test the suitability of new biomaterials to stimulate muscle formation.
We compared the proliferation of C2C12 myoblasts seeded onto glass coverslips in the presence and in the absence of three HELP biopolymers (HELP, HELP1 and HELPc). WST-1 assay showed intense proliferation in all conditions tested after 24, 48 and 72h in culture, and a significant (***p < 0.001 **p < 0.01) increment of cell number on the HELP biopolymers treated glass coverslips at 48 and 72h.

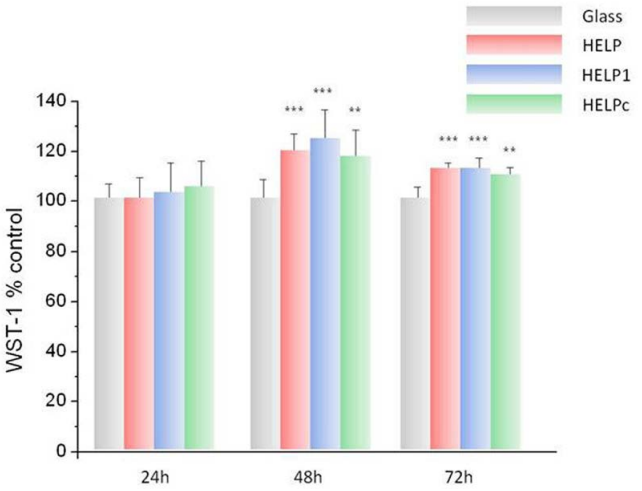

Figure 3. C2C12 myoblasts cultured on different substrates.

We assayed the ability of HELP polymers to stimulate cell alignment and fusion into myotubes.
Cells were induced to differentiate by decreasing the serum concentration. After 48 h, a significant increase of myotubes was detected in cells seeded onto HELP biopolymers, in comparison with cells grown onto glass coverslips (Fig.3).

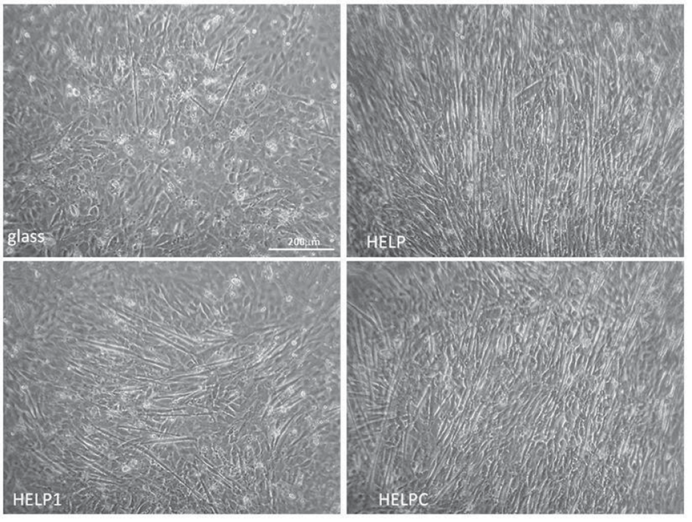

Figure 2. C2C12 myoblasts proliferation assessed by WST-1 assay.

5 POTENTIAL NEW PRODUCTS

- Product: The HELP polypeptides have been designed to be further modified by addition of a functional, bioactive domain that can confer a new functionality to the whole macromolecule. These domains could be easily embedded in the HELP scaffold to obtain a new hybrid macromolecule from which functional biomaterials could be obtained under different formulations as for example hydrogels, coatings, microspheres, films and others. This kind of materials hold a great potential for development of new functional devices for cell and drug delivery.
- Patent: 3D matrices of human elastin-like polypeptides and method of preparation thereof", application No: EP2419442, Apr 15th, 2010.

6 CURRENT COLLABORATIONS

6.1 With other researchers

Dr. Gianni Ciofani, Istituto Italiano di Tecnologia, Pontedera, Italy
Prof. Diego Mantovani, Université Laval, Laboratoire de Biomatériaux et Bioingénierie, Québec, Canada
Prof. Marta Cerruti, McGill University, Materials Engineering, Montreal, Canada
Dr. Francesca Boccafoschi, Università degli Studi del Piemonte Orientale, Dipartimento di Medicina Clinica e Sperimentale, Novara Italy
Prof. Heinz Redl, Ludwig Boltzmann Institute for experimental and clinical Traumatology / AUVA Research Center, Wien, Austria
Dr. M. Letizia Focarete Università di Bologna, Dipartimento di Chimica Bologna
Dr. Artemis Stamboulis, University of Birmingham, School of Metallurgy and Materials

6.2 With SMEs

Educell d.o.o. Trzin, Slovenia

6.2 With Hospitals

Prof. Zoran Arnež, Chirurgia Plastica e Ricostruttiva, Ospedale di Cattinara, Trieste
Prof. I. Milosev, Valdoltra Orthopaedic Hospital, Ankaran, Slovenia

7 CONTACT OR COLLABORATIONS NEEDED

Future collaboration with industrial partners is needed.

8 COMMUNICATION TOOLS

- Results of the work are disseminated through high quality scientific publications.
- Ciofani, G., Genchi, G.G., Liakos, I., Athanassiou, A., Mattoli, V., Bandiera, A. (2013),
- Human Recombinant Elastin-Like Protein Coatings for Muscle Cell Proliferation and Differentiation, Acta Biomater., 9, 5111-5121.
- Çelebi, B., Cloutier, M., Balloni, R., Mantovani, D., Bandiera, A. (2012)

- Human elastin- based recombinant biopolymers improved mesenchymal stem cell differentiation Macromol. Biosci., 12, 1546-1554.
- Bandiera A., Sist P., Urbani R. (2010)
- Comparison of Thermal Behavior of Two Recombinantly Expressed Human Elastin-Like Polypeptides for Cell Culture Applications.
- Biomacromolecules, 11, 3256–3265.

9 FUNDS NEEDED

9.1 For basic research (investigation of biological mechanisms): 30.000 €

9.2 For applied research (solutions for real-world problems): 50.000 €

10 CONCLUSION

The results presented here open a promising perspective for development of new strategies for muscle tissue regeneration. The biomimetic elastin polypeptides have been shown to be cytocompatible with myoblasts and the propensity to direct them to a more differentiated phenotype has been observed. These studies hold the potential to solve important unmet medical needs, as those related to muscle tissue restoration and regeneration.

ACKNOWLEDGEMENT

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