ELASTIN-BASED BIOPOLYMERS FOR BIOMEDICAL AND BIOTECHNOLOGICAL APPLICATIONS.

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Abstract — Perhaps the most appealing opportunity is represented by gaining inspiration from nature for the precise tailoring of biomaterials with finely tuned unique functional properties. A very promising model is represented by Human Elastin-Like Polypeptides (HELP), repetitive artificial polypeptides based on penta- or hexa- peptidic motifs that characterize elastin. These protein polymers retain several peculiar biophysical properties as, the reversible inverse phase transition, changing the solubility and aggregation state in response to temperature variation. The smart nature of this class of compounds makes them attractive for many applications in the biomedical and biotechnological fields, in particular for biomaterial development. The Trans2Care project aiming to translating scientific and technological expertise into products for the biomedical field represents an exciting and challenging environment to best exploit the potential these biomimetic macromolecules.

Index Terms — recombinant protein, elastin, biomimetic strategy, advanced biomaterials

1. INTRODUCTION

1.1. **Elastin, a model for protein-based artificial biopolymers**

Bio-mimicry, the concept of taking inspiration from nature is perhaps among the most appealing strategies to create customized biomaterials with finely tuned peculiar features.
Elastomeric proteins, well represented among Vertebrates are among the components that received considerable attention. Elastin, one of the components of the extracellular matrix, possesses rubber-like elasticity undergoing deformation without rupture and provides an important model for biomaterials design. Another peculiar extensively studied characteristic of elastomeric proteins is coacervation. Under appropriate conditions of concentration, ionic strength and increasing temperature, the protein is known to separate from solution as a second phase. It has been shown that this behavior is mainly due to the presence of the hydrophobic (VPGXG) pentapeptide, typical of the mammalian protein.
In 1974, Urry *et al.* first developed artificial polypeptides mimicking these repeated hydrophobic motifs characterizing bovine tropoelastin. [1].
In the human protein, the most structurally regular sequence is represented by the repetition of the (VAPGVG) hexapeptidic motif. It has been shown that the VAPGVG motif is biologically functional.
By a molecular biology approach, a regularly repeated domain from human tropoelastin was chosen as a basic modulus to obtain the Human Elastin-Like Polypeptides (HELPs). These artificial proteins can be employed in the production of innovative micro- and nano-structured biomaterials with a huge potential for employment in the biotechnological and biomedical fields.

1.2. **HELP biopolymers family**

In our lab the HELP macromolecule, based on the most structurally regular module of human tropoelastin was developed and expressed in bacteria [2]. The polypeptide is soluble in aqueous solution below a critical temperature, whereas it forms aggregates above this temperature. This process, known as “inverse” phase transition, is fully reversible.
A second synthetic gene was developed, and a polypeptide, named HELP1, based only on the most consistently repeated peptide sequence of human tropoelastin was obtained (Figure 1).

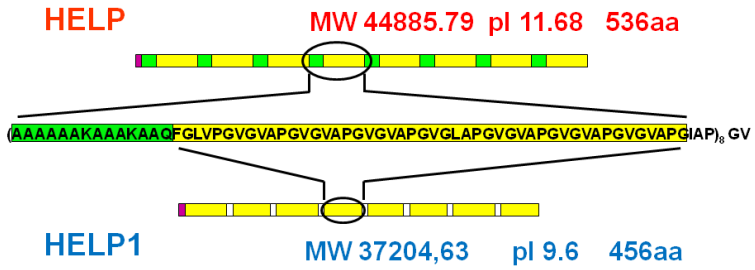

Figure 1.
Schematic representation of the two HELP and HELP1 prototypes.

Overall, the work carried on in our lab is aimed at the production of a family of artificial polypeptides that exhibit different and variable physicochemical properties. The two prototype macromolecules HELP and HELP1 that differ in their primary structure have been characterized from the physic-chemical point of view, showing a different behaviour in relation to the different environmental conditions [3]. Both biopolymers are able to give reversible hydrogels under defined conditions by raising temperature (30-37°C).
Relatively large yields have been recently achieved upon optimization of biopolymer expression and of the purification steps, in the range of hundreds of milligrams of purified product per liter of bacterial culture. [4]
A key feature of these recombinant products is related to the modular structure of the synthetic genes that allows the tailoring of the macromolecule for a specific application.
In this light, the HELPs can be viewed as prototypes whose functionality can be implemented by simply fusing peptidic regions to the C-terminal part of the biopolymers. The addition of a bioactive domain of choice will result in the conferring specific function to the whole product.

2. BIOTECHNOLOGICAL AND BIOMEDICAL APPLICATIONS

Many recent examples show the potential of these polypeptides for a wide range of biomedical applications, and to build structures with highly controlled properties and behaviour [5].
The thermoresponsive self-assembly properties of these macromolecules are of particular interest to develop functional biomaterials, especially in the field of targeted delivery and controlled release of active molecules.

A method for preparation of 3D matrices with hydrogel features has been patented recently [6] and prosecution procedures are currently ongoing in Europe and USA (Figure 2).

Figure 2.
Stable HELP hydrogel matrix

This method represents a valuable option to preserve cell viability and functions as it avoids the use of harsh chemical reagents commonly used for preparation of matrices [7]. A huge potential for biomaterial development is foreseen. For example, one interesting feature is represented by the possibility to encapsulate cells in the gel maintaining their viability.
As shown in Figure 3, cells spheroids can grow within the gel.

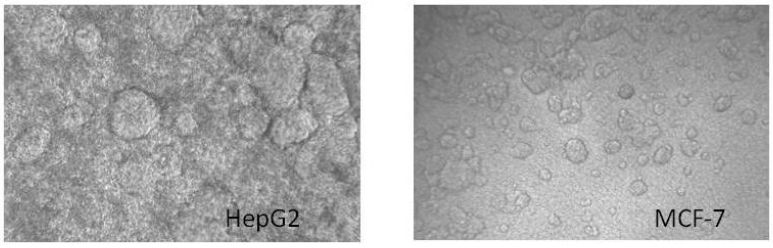

Figure 3.
Viable cell encapsulation in 3D HELP matrix

3. AIMS IN TRANS2CARE PROJECT

Proteinaceous material represents a promising alternative to chemically synthesised, traditional biomaterials still commonly employed in the biomedical field. HELPs represent promising innovative macromolecules and show high potential for a wide range of applications. Thus, we intend to exploit the Trans2Care partners' expertise and knowledge to:

a) exchange ideas between traditionally distinct research areas and bringing together the competences to devise the employment of HELP biopolymers for specific demand;
b) provide the HELP compounds to the partners to setup experimental work;
c) develop commercial applications of HELPs and biomaterials derived.

In particular, collaboration with:

- University of Nova Gorica (PP3), on development of innovative environmental biosensors
- Treviso Tecnologia (PP5), on supporting technology transfer of existing and future HELP products
- ZTM (PP10), on development of advanced cell growth and delivery systems.

4. REFERENCES

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