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HELP-based matrices for stimuli-responsive controlled release of bioactive compounds

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INTRODUCTION: Direct delivery of bioactive substances to the sites of injury represents a key issue for therapies based on regenerative medicine and tissue repair [1]. Protein derived hydrogels represent an interesting system for this purpose because they possess several features that make them suitable to this purpose. A method for preparation of hydrogel matrices based on Human Elastin-like Polypeptide (HELP) has been set up [2]. HELPs are a family of elastin-like recombinant biopolymers modeled after the most regularly repeated domain in human tropoelastin, retaining peculiar properties as self-assembling and thermoresponsive behavior [3]. In this study we assayed two elastolytic activities from different sources to test their potential to specifically degrade the HELP matrix.

METHODS: Proteolytic activities were obtained from *P. aeruginosa* PAO1 strain and activated human polymorphonuclear neutrophils (PMN). HELP and HELP1 biopolymers degradation by elastolytic activities was analyzed by 10% SDS-PAGE. HELP matrices were prepared as already detailed [2]. A method to follow HELP matrix degradation was set up adding Coomassie blue dye before crosslinking. Matrix degradation was followed monitoring the release of the dye in the supernatant.

RESULTS: First we tested our HELP and HELP1 biopolymers against the two elastolytic activities to assess their susceptibility to proteolysis. (Fig.1).

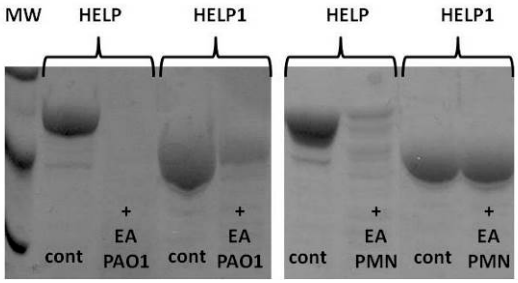

Fig. 1: SDS-PAGE analysis of HELP and HELP1 biopolymers treated with elastolytic activities (EA) derived from P. aeruginosa PAO1 strain and from PMN

The analysis evidenced that HELP biopolymer is more susceptible to degradation that HELP1 This is suggestive of a prominent degradation at the level of the crosslinking domains that are present only in the HELP biopolymer and confirming the presence of elastolytic activity in the samples.
Then we tested the same proteolytic activities against the HELP derived matrices. Both elastolytic activities have been shown to be able to degrade the matrix since it was entirely dissolved within 48 hours while the control counterparts remained intact (Fig. 2).

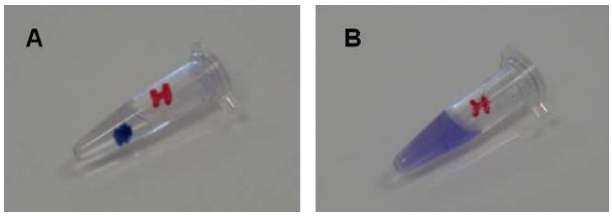

Fig. 2: HELP matrix after 48 hours incubation at 37°C in the absence (A) and in the presence (B) of P. aeruginosa proteolytic activity

DISCUSSION & CONCLUSIONS: The procedure to prepare stable hydrogels from HELP biopolymer exploited the reaction catalyzed by the microbial enzyme transglutaminase. This method can be employed for entrapment of proteic bioactive substances and can be used to realize smart devices for the delivery of biological products. In particular, pathologic conditions that involve an abnormally increased elastolytic activity like for example cystic fibrosis and chronic wounds could represent interesting models to assay the potential therapeutic use of our matrices. Our results show that HELP hydrogels represent a promising tool to realize loadable delivery devices to elicit drug release by a specific stimulus.

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