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Human Elastin-derived Biomimetic Coating Surface to Support Cell Growth

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***Abstract*—**A new sythetic gene coding for a Human Elastin-Like Polypeptide was constructed and expressed. The recombinant product was tested as coating agent to realize a surface suitable for cell growth. Coatings showed peculiar features and different human cell lines were seeded and cultured. All cell lines tested showed to adhere and proliferate on this substrate that has been shown also to exert a specific effect on cells, depending on cell type.

***Keywords*—**elastin, recombinant protein, coating, cell adhesion.

I. Introduction

THE intrinsic nature of the surface where cells grow has always been recognized of crucial importance with respect to its influence on cell adhesion and the phenotypic expression. More recently, also the physical cues of the adhesion surface have been shown to play a key role in affecting cell behavior [1, 2]. Cell sense the chemical nature of the substrate by their membrane receptors, like integrins and other adhesion molecules that trigger a specific signaling pathway that will contribute to shape the cell phenotype [3]. At the same time, cells have been shown to be responsive to the physical properties of the surface that they come in contact. Topology and structure of the surface to which cells adhere have been shown to be crucial for the subsequent fate of the system [4, 5]. A significant experimental effort is focused on modulation of surface properties for the optimization and control of cell adhesion, morphology, motility, proliferation, and differentiation in both *in vitro* and *in vivo* systems [6 and references therein]. Recent research on the micro and nano patterning techniques further evidenced the importance of the micro and nanometer scale features of the adhesion substrate in affecting cell behavior at interfaces [7]. The research in new biomaterials for engineering of cell adhesion surfaces is still facing a great boost. Despite the enormous progress in this field, there are a number of compelling issues that have not been adequately addressed yet [8]. In particular, to find the ideal combination of a biomaterial and the cells with the proper environmental conditions for realization of a functional biological system resembling the physiological one remains a crucial challenge.

Mimicking the structure of naturally occurring proteins by using artificial protein polymers with specific materials properties is a very promising approach for the development of new bioactive biomaterials. A key concept in this approach is the design and tailoring of biomimetic materials, to promote exchange of stimuli and induce specific cellular reactions [9]. Progress in recombinant DNA technology opened the way to the realization of new protein polymers that can be employed to obtain innovative biomaterials that can interact with the surrounding environment. A very interesting model for such materials is represented by elastin-like polymers, i.e., repetitive artificial polypeptides based on elastin repeated motifs [10].

Elastin represents an important macromolecule for biomaterial design. Under appropriate conditions of concentration, ionic strength and temperature, the protein undergoes the inverse phase transition, a reversible temperature-dependent coacervation process. This behaviour is mainly due to the presence of the hydrophobic repeated domains with the pentapeptidic (VPGXG) motif [11]. Many variants of the repeat have been employed to obtain artificial polypeptides and it has been shown that the resulting recombinant products retain this peculiar feature [12-16].

With the aim of realizing a family of biomimetic protein polymers that can be exploited in the biomedical and biotechnological fields, the design and construction of synthetic genes modeled on domains found in human elastin was undertaken. The first macromolecule, named HELP (Human Elastin-Like Polypeptide) set up was based on part of the sequence of exon 23, coding for a crosslinking domain rich in alanine and lysine and on exon 24, based on the hexapeptidic VAPGVG repeat [17].

Also a second gene, named HELP1, was realized assembling only the sequence coding by exon 24, giving an expression product lacking the alanine/lysine-rich crosslinking domains [18]. The physico-chemical charaterisation of the two recombinant biopolymers has evidenced some differences in their temperature responsive behavior, in particular at low biopolimer and salt concentration (manuscript in preparation).

In the present work, the potential of the recombinantly expressed HELP1 biomimetic macromolecule as a coating agent for cell adhesion and growth was explored. Surfaces prepared with this component have been shown to possess peculiar features and to be suitable for cell attachment and

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proliferation. In addition, an effect on the morphology of some cell lines has been observed.

II. Materials and Methods

A. Expression and purification of HELP1

The production of the recombinant HELP1 biopolymer has been detailed previously [18]. Briefly, *E. coli* NEB Express *Iq* co-transformed with pHELP1 vector and pLysS plasmid were cultured at 37°C with shaking in TB broth to OD_{600} of 1 and then induced with IPTG to a final concentration of 0.1mM. The bacteria were collected by centrifugation after 4 hours of further growth. The expressed product was extracted from the frozen pellet by re-suspension in a non-denaturant extraction buffer and by 3 passages through a high pressure homogenizer (GEA Niro-Soavi) at 1300 bar. The supernatant was clarified by centrifugation at 4°C. The recombinant biopolymer was then selectively precipitated by salt addition and incubation at 42°C. The pellet was recovered by centrifugation at 37°C and re-dissolved in cold water. The sample was further purified by two more identical cycles of precipitation and re-suspension. The material was finally frozen and lyophilized for long term storage.

B. Preparation of HELP1 coatings

HELP1 lyophilized sample was dissolved in bi-distilled cold water at a concentration of 0.05% (w/v) and sterilized by a 0.22μm filter. Wells (diameter 4mm) of microvessels (IBIDI, μ-slides angiogenesis, ibiTreat, Germany) were filled with 8 μl of the sterilized HELP1 solution and let dry under sterile hood flow at room temperature (22°C). Control wells were filled with the same volume of sterile water.

C. HELP1 surface characterisation

The bottoms of the wells coated with HELP1 were analysed under inverted phase contrast microscope (Leica) and photographed. For scanning electron microscopy and microanalysis, the well bottoms were excised from the microvessel, mounted on the specimen holder with electro-conductive tape, sputter-coated under vacuum with gold and subsequently analyzed.

D. Cell lines and culture conditions

EaHy926, A549 and MCF-7 cell lines were maintained in standard conditions, using DMEM supplemented with 10% FCS, penicillin and streptomycin and incubated at 37°C under 5%CO_2. Cells were seeded at 5000 cells per well in a final volume of 50 μl at least in triplicate for each time point.

E. Viability and proliferation assay

Cultures were tested with WST-1 reagent (Roche) following the manufacturer's instructions at 24, 72 and 168 hours after seeding.

III. Results and Discussion

HELP1 surface coating and characterization

In the present study, the HELP1 coated surfaces were prepared from a biopolymer solution in water. Previous observations showed that the solubility of HELP1 at room temperature (22°C) was about 0.5% (w/v). Thus, a 10-fold lower concentration was chosen as a starting condition.

Using microwells and small volumes, the water was allowed to evaporate under hood flow and the coating was dried in a short time, typically within tens of minutes. Under these conditions, the coated surface resulted to have a peculiar appearance observable by phase contrast microscope, as shown in Fig. 1. A defined pattern of concentric circles was evident, suggesting an alternating succession of ridges and grooves, especially in the central area. It is likely that the peculiar feature of the coating is related to the self-assembling properties of HELP1.

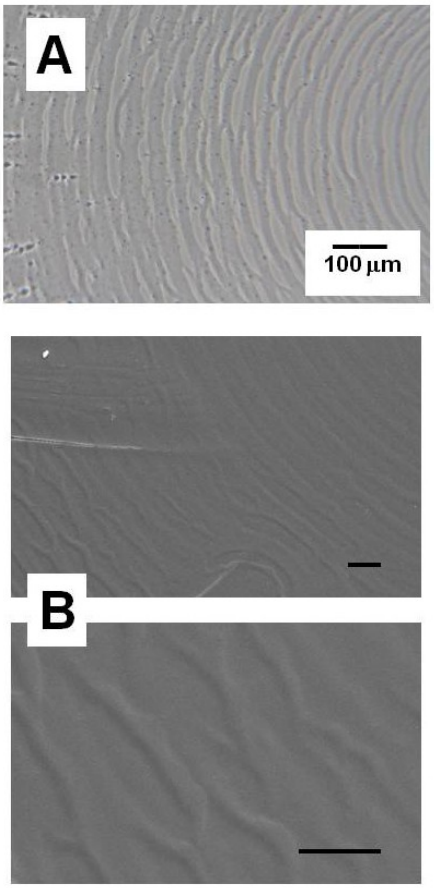


Fig. 1 HELP1 coated surface. A, phase contrast microscopy; B, scanning electron microscopy, bar is 50 μm

The SEM analysis confirmed the arrangement seen by phase contrast (Fig. 1) and microanalysis showed that HELP1 biopolymer was present all over the surface.

Different parameters were assayed in order to evaluate if and how they affect the coating obtained. Biopolymer concentration, temperature and in particular the nature of the surface have been shown to be the critical variables and are currently under investigation.

The experimental setting allowed a highly reproducible preparation of HELP1 coated surfaces that have been shown to be quite stable. The surfaces were covered with cell culture medium and kept at room temperature (22°C) for one week without significant loss of biopolymer (data not shown).

Cell cultures on HELP1 coating

Peptide-based biopolymers are considered promising compounds for tissue engineering, regenerative medicine and related fields, mainly due to their hydrophilic networking propensity, expected good biocompatibility, favorable degradation rate and products, very low cytoxicity and immunogenicity [19]. Despite in the last ten years there has been a considerable body of work conducted on elastin-like polypeptides and their applications, there are still rather few examples of their employment in supporting cell growth [20 and references therein]. The focus of this study was to test the patterned HELP1 coating with different cell lines of human origin. Since elastin is abundant where elasticity is of major importance as in blood vessel walls, HUVEC derivative Ea.Hy926 were chosen as a endothelial cell model in the perspective of application of our biomaterial to vascular tissue restoration. These cells were grown both on standard treated plastic as control culture and on the HELP1 patterned surface. Notably, as can be seen in Fig. 2 (A and B) the Ea.Hy926 cells seeded on HELP1 showed a clear response toward the coated surface in that the appearance of the culture resembled the concentric rings pattern observed. Moreover, at a single cell level, it was clearly evident a change in morphology related to adhesion and alignment on the surface, when compared to control cells grown on standard treated plastic.

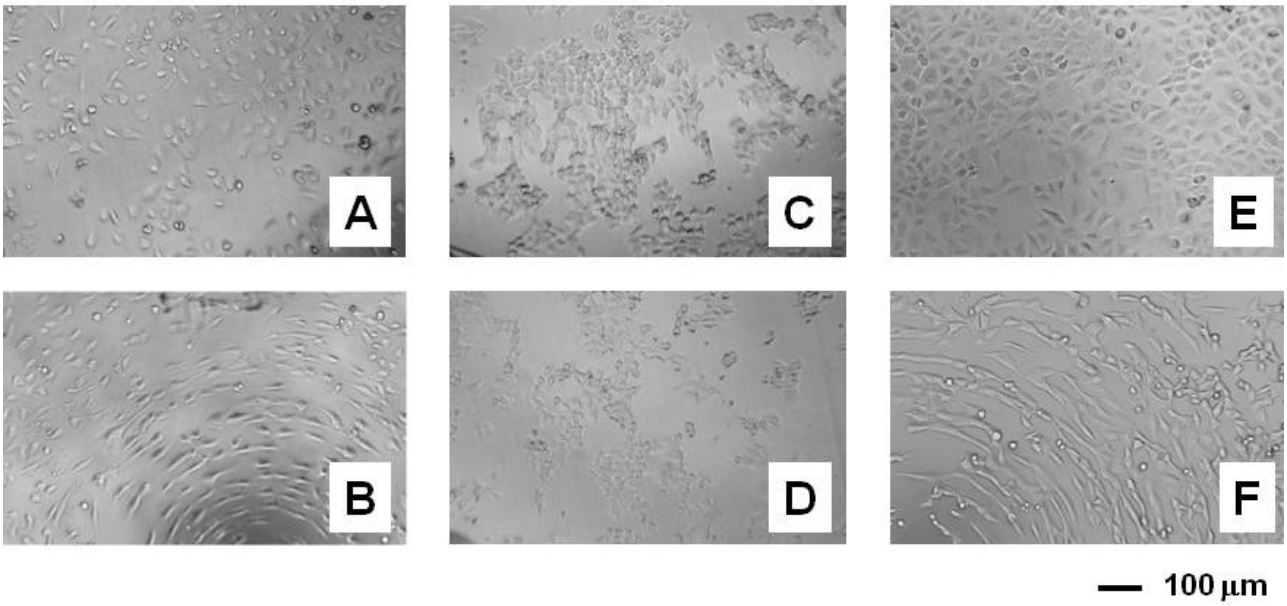


Fig. 2 Phase contrast microscopy of Ea.Hy926 (A and B), MCF-7 (C and D) and A549 (E and F) cells cultured on control standard treated plastic (A, C, and E) and on HELP1 coating (B, D, and F) at 72 hours after seeding.

It seemed therefore interesting to test the behavior of cells of different origin, like the human epithelioid MCF-7 cell line. In this case, as can be seen in Fig. 2 (C and D), no significant difference in adhesion and spreading behavior is appreciable between control and HELP1 treated surface.

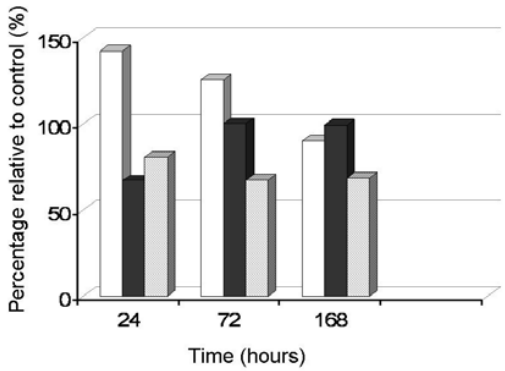
Human endothelial-like Eahy926 cell line was derived from fusion of human umbilical vein endothelial cells with human cell line A549 of epithelial origin [ref]. Thus, also the A549 parental cell line on HELP1 coating was probed. In Fig. 2 (E and F) the result is shown and similarly to that observed for Ea.Hy926 cells, the A549 culture show a response when the surface is coated with HELP1 biopolymer. Therefore, these data suggest that the observed effect may be related to some features that are present in the A549 phenotype rather than to the endothelial or epithelial origin of the cells.

There are some considerations that can be done. All the cell types assayed have been shown to adhere well on our coating. This suggests that all the cells tested recognized the substrate from the chemical point of view. The difference in the cell response appeared to reside in the capability of cells to somehow discriminate in the physical structure of the

substrate. It is likely that cells align along the grooves in the direction were they encounter the least resistance, as already described for other biological system [5], however more investigations are needed to further elucidate this issue.

The HELP1 coating has been shown to elicit effects on behavior of a specific cell type. It is likely that these effects are mediated by cell surface molecules whose expression is tissue-specifically modulated. Further investigations on membrane receptors and molecules involved in this process will contribute to better clarify this issue.

In these experiments, it has been observed that when cells responded to HELP1, the effect was ready detectable few hours after seeding and was lasting for the experiment time (data not shown). The three cell lines where analyzed for viability and proliferation at 24, 72 and 168 hours after seeding. At 96 hours medium was changed. The results are shown in fig.3.



. Fig. 3 Proliferation assay of the different cell lines cultured on HELP1 coated surfaces as percent of the control culture on standard treated plastic. Ea.Hy926 (white bar), MCF-7 (black bar) and A549 (grey bar) at different times.

The viability and proliferation assays performed on the cultures on HELP1 coated surface showed that Ea.Hy926 cells gave a signal that resulted even higher than that of the control counterpart. This suggests that HELP1 coating may have a positive effect on Ea.Hy926 cell proliferation. The cultures after 72 hours show a tendency to reach a plateau value lower than that of the controls. Given that this assay, being related to metabolism, did not distinguish between viability and proliferation, the data could reflect the fact that responsive cultures did not occupy all the surface, but have preferential areas of proliferation. In these sites, cells became more crowded, reaching confluence very quickly and stop dividing. All the cultures showed good vitality after one week in culture.

IV. Conclusion

The data show that the new recombinant HELP1 macromolecule is suitable as coating agent. Cells of different origin are able to adhere and grow with negligible cytotoxicity. Moreover, depending on cell type, HELP1 coating has been shown to elicit a cell response. These findings point to the potential of biopolymers from synthetic genes to be applied in development of smart biomimetic surfaces for cell growth. Characterization and understanding of surface and cell interactions will be critical in controlling cell adhesion and proliferation. On the other hand, studies of *in vitro* interactions between cells and adhesion surface in a well defined, controlled environment offer the opportunity to better elucidate cell biological behavior. In this context, the field of biomimetic materials is likely to supply important new knowledge to molecular and cell biology.

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