Microbial Cell Factories

Poster Presentations

Design and production of multi-bioactive recombinant elastin-like polymer: Mimicking the extracellular matrix

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Background

One of the main tasks in the development of tissue engineering is the advance in the design and production of materials designed to act as support for the growing cells and tissues. The evolution in the development of artificial extracellular matrices (ECM) began with the use of biotolerated synthetic materials, mostly polymers, which showed cell attachment and spreading capabilities of a rather unspecific nature. Soon, these materials were improved. Some macromolecules of natural origin were used but more important, more specific functionalities were included in their structure, specially peptide cell attachment sequences.

More recently, the development of genetic engineering has allowed the design and bioproduction of protein polymers. These are mainly made from repeating sequences found in natural proteins, such as elastin, silks, etc., and selected modifications [1]. One of the most promising family of protein polymers is the Elastin-like polymers (ELPs) which have shown an outstanding biocompatibility [2]. These polypeptides are based on the recurrence of certain short monomers that are considered as building blocks in the natural elastin. The most representative polymer of this family is poly (VPGVG), and the vast majority of the ELPs described in the literature are made from selected modifications of this pentamer or its permutation. ELPs are extremely biocompatible and bioprocessable and show and acute smart and self-assembling behavior. Recently, the adaptation of the genetic engineering techniques to this field has opened the possibility of obtaining these polymers with absolute control and absence of randomness in the primary structure. This has allowed the production of multifunctional polymers that can combine physical, chemical and biological functions.

This works was intended as being one step more towards the development of a complex and multifunctional artificial ECM that mimics the versatility and complexity of the natural ECM. We describe herein the use of artificial genes to direct the synthesis of ELPs containing cell attachment sequences (RGD and REDV) of precisely controlled architecture and its bioproduction in *E. coli* [3,4].

Materials and methods

Plasmid Construction

Standard molecular biology techniques were performed to construct two polymer genes and their sequences were verified by automated DNA sequencing. DNA duplex encoding the monomer genes were obtained by Polymerase Chain Reaction (PCR) using two synthetic oligonucleotides. The first monomer gene (**A**) codifying the amino-acid sequence $[(VPGIG)_2$-(VPGKG)-$(VPGIG)_2]_2$-AVTG**RGD**SPASS- $[(VPGIG)_2$-(VPGKG)-$(VPGIG)_2]_2$ and the second one (**B**) $(VPGIG)_2$-(VPGKG)-$(VPGIG)_2$-EEIQIGHIP**REDV**DYHLYP-$(VPGIG)_2$-(VPGKG)-$(VPGIG)_2$-(VGVAPG). After gene cloning, the monomer was obtained by digestion, isolated and subjected to concatenation (controlled ligation). The concatenamer mixture was cloned into a modified expression vector derived

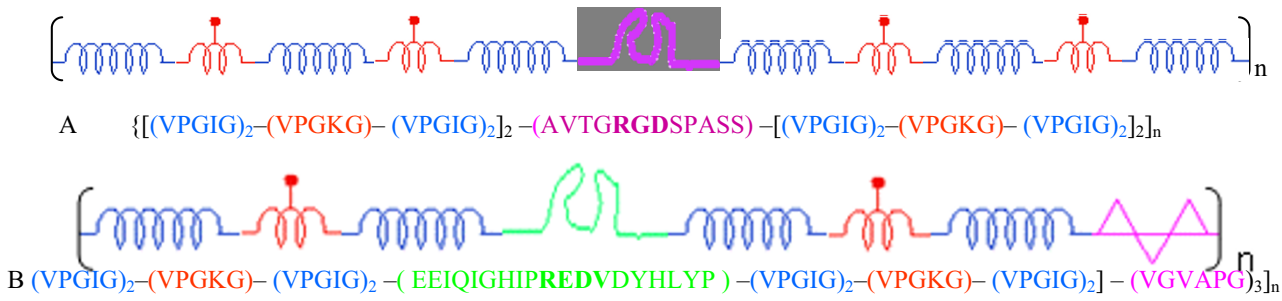

Figure 1
Schematic representation of the polymers A B architecture, identifying each building block with its corresponding amino-acid sequence.

from pET-25(+). Plasmids were selected based on their insert length by PCR colony screening and transformed into the *E. coli* expression strains BL21(DE3) and BLR(DE3). Bacterial growth and gene expression were performed following the manufacturer's instructions. The polymer purification was carried out by several cycles of cold (4°C) and warm (50°C) centrifugation. MALDI-TOF, amino acid analysis and spectroscopic methods proved final polymers high purity and correct sequence. Turbidity experiments and Differential Scanning Calorimetry (DSC) were used to calculate the Transition Temperature (Tt) of the polymers.

Results

The bioproduced polymers comprise different building blocks, each showing a different functionality (see Figure 1). First, the final matrix is to be designed to show a mechanical response comparable to the natural extracellular matrix, so they are produced over a base of an elastin-like polymer of the type (VPGIG)n. Along with the desired mechanical behaviour, this base has shown an excellent biocompatibility. The second building block is a variation of the first. It has a lysine substituting an isoleucine so the lysine γ-amino group can be used for cross-linking purposes while retaining the properties of elastin-like polymers.

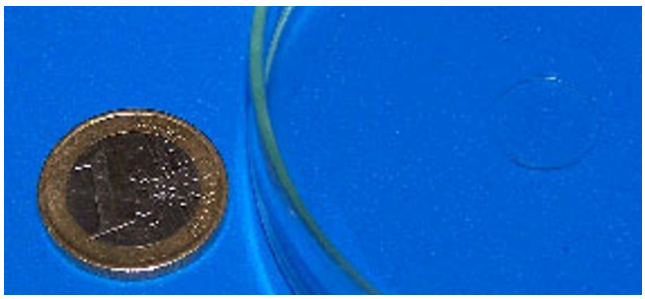

Figure 2
Photograph of a cross-linked matrix of the bioproduced B polymer.

The third group contains cell attachment sequences. In polymer A, this sequence is based the well known RGD, while in polymer B is the CS5 human fibronectin domain, which has the endothelial REDV cell attachment sequence. In addition, polymer B also contains the elastase target sequence VGVAPG to favour its bioprocessability by natural routes.

The polymers and the corresponding matrices (see Figure 2) have been subjected to mechanical tests, physical-chemical, biochemical and "in vitro" analysis. The results clearly confirm the excellent behaviour of this matrix for the pursued purpose, as expected from the combination of functionalities and properties of the building blocks used in its molecular design.

Conclusion

Different recombinant elastin-like protein polymers have been designed and bioproduced in *E. coli* to create advanced scaffolds for tissue engineering. All of them contain, at least, cell adhesion domains of the type RGD or REDV, and specific protease target domains. They can be easily cross-linked to obtain elastomeric hydrogels. These new polymers has been subjected to mechanical tests, physical-chemical and in vitro analysis. The results confirm the excellent behaviour of these matrices for this purpose.

Acknowledgements

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