ON THE SYNTHESIS AND STRUCTURAL CHARACTERISATION OF ELASTIN-LIKE RECOMBINAMER HYDROGEL SCAFFOLDS FOR TISSUE REGENERATION

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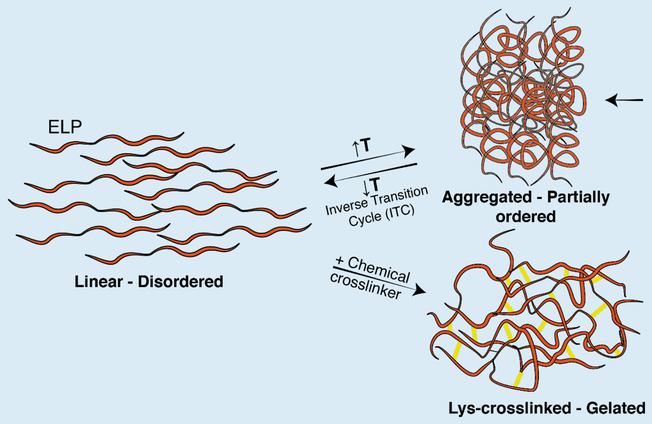
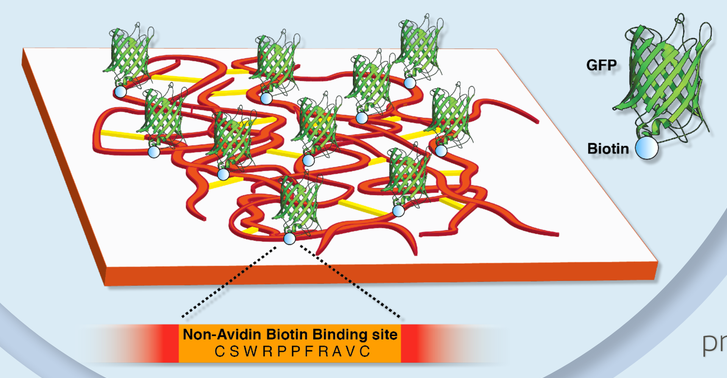
INTRODUCTION



Elastin-like polypeptides (ELPs) are applied in a broad range of biomedical fields, such as drug delivery and tissue engineering. These genetically engineered peptides are designed to **mimic the endogenous elastin** and are characterised by a **repeating $(VPGXG)_n$ motif**, where X could be any **guest residue** except for proline. Due to the periodic occurrence of *cis*-proline residues, such peptides exhibit a **partially ordered behaviour**, undergoing reversible transitions from a disordered state to a *β*-spiral secondary structure. This is known as **Inverse Transition Cycle (ITC)** and occurs under a specific temperature, related to the peptide primary structure. Incorporation of **lysine** residues in low frequency, provides the ability of chemical crosslinking, thus forming a **nanostructured hydrogel** of good mechanical properties, able to function as **biomaterial for tissue scaffolding.**

AIM

- Fabrication of a customisable, biocompatible **hydrogel scaffold** for **cartilage and bone regeneration**
- Genetically synthesised **ELPs** as biopolymeric material
- Controlled **incorporation of biotinylated proteins or peptides** on the ELP molecules (e.g. biotinylated GFP for testing, see figure below) through a **Non-Avidin-related (NAV) biotin binding motif**.
- Docking of biotinylated growth factors to promote differentiation and proliferation of progenitor cells



METHOD OUTLINE

ELP genes design and synthesis (vector: **pET29c**, host: **TOP10 E.coli**)

Overexpression in **BL21** E.coli (after optimization of conditions)

ELP purification (ITC protocol and/or Ni-NTA column)

Chemical **crosslinking** - hydrogel formation

Test with biotinylated GFP

Growth factor docking - **cell seeding**

CHARACTERISATION

SEM
- Surface visualisation
- Topography

TEM
- Bulk characterisation
- Pore size

AFM
- Surface topography
- Mechanical properties (Stiffness, Young's Modulus)

Molecular Dynamics Simulations
- Coarse Grained model
- Aggregation mechanism
- Structural transitions - Inverse Transition Cycle
- *In silico* evaluation step of experimental results

GENE SYNTHESIS

- Artificial ELP genes were *ab initio* synthesised through a **PCR-based strategy** (also known as Recursive PCR, *Prodromou et. al*), which involves multiple partially-overlapping primers (one forward and several reverse ones) and **no template DNA**. Figure 1 schematically summarises the method. This a **single-step** procedure which does not require multiple PCR reactions to produce the final product.

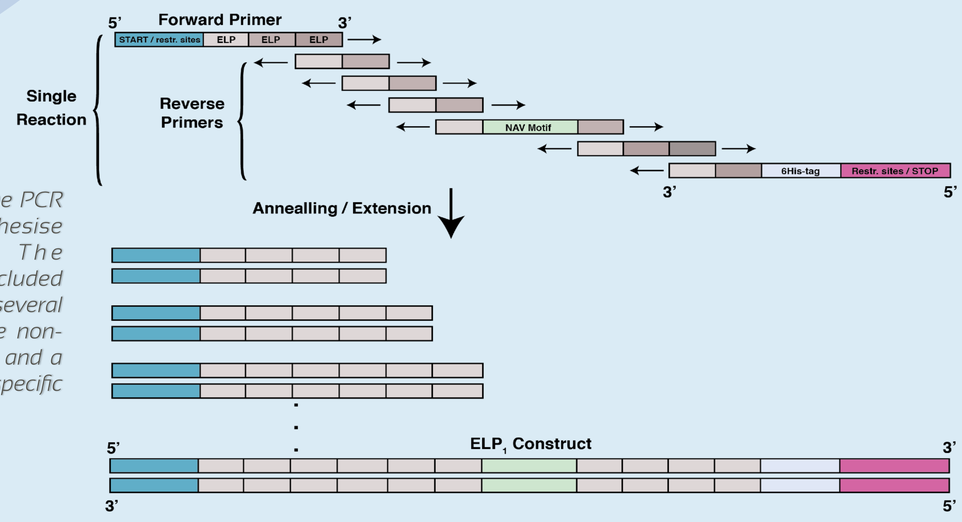


Figure 1: Schematic of the PCR method used to synthesise initial ELP genes. The component primers included start and stop codons, several ELP tandem repeats, the non-avidin biotin binding site and a 6His-tag as well as specific restriction sites.

- Two additional repeats of the whole gene synthesised by the above method were introduced, using a method known as **Recursive Directional Ligation (RDL)** (Figure 2). Two ELP_1 monomers were cut from their vector plasmids, one on the 5' and one on the 3' end, and ligated together to form an ELP_2 dimer. One more repeat was introduced in a similar way to form the final ELP_3 construct. TOP10 *E. coli* strains were used as hosts.

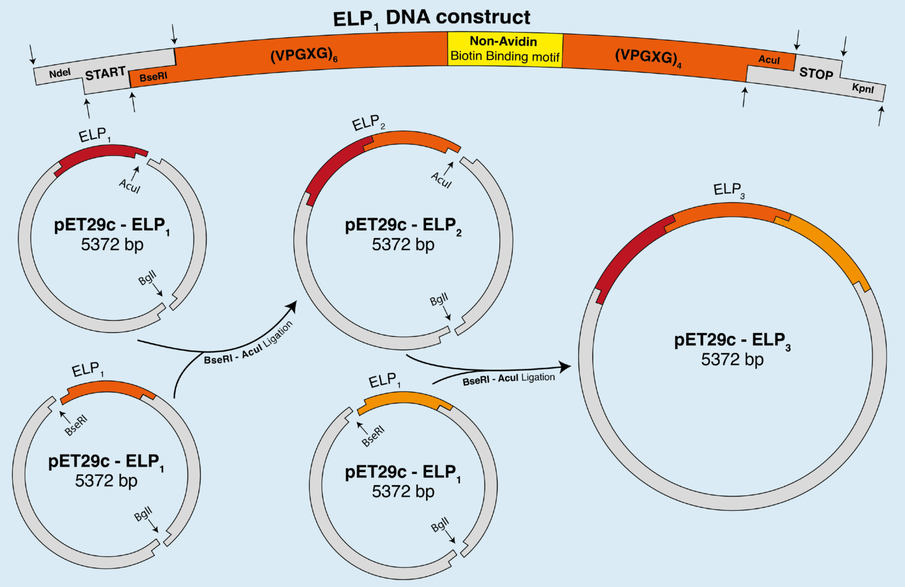


Figure 2: Schematic of the Recursive Directional Ligation method, used to insert two extra tandem repeats of the initial ELP gene. Note the restriction enzyme combinations for each step of the procedure.

CURRENT RESULTS

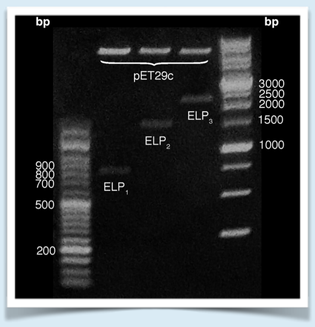


Figure 3: NdeI/KpnI digestion of plasmids containing one, two and thee NAV-ELP gene repeats

- We have successfully constructed **two ELP genes** (one containing the NAV motif and one control peptide without it) by the methods described **(Figure 3)**.
- Sequencing results show consistency with the predicted DNA sequence.
- The NAV-ELP and control-ELP constructs, code for peptides of **~57kDa** and **~49kDa** respectively.

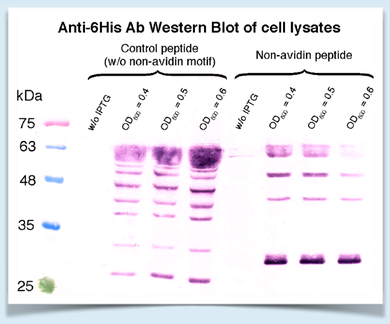


Figure 4: ELP peptide Western blot for semi-quantitative assessment of expression efficiency at different induction times

- We are currently making attempts to **optimise overexpression conditions** and achieve **maximum yield of the ELP peptides** (non-avidin and control peptide).
- Overexpression conditions so far (see western blot at **Figure 4**):
  - ➡ **NAV-ELP:** LB Broth-kanamycin, 37°C, 120rpm, 4h, +1mM IPTG at OD_{600}=0.4
  - ➡ **CTRL-ELP:** LB Broth-kanamycin, 37°C, 120rpm, 4h, +1mM IPTG at OD_{600}=0.6
- Further optimisation is needed to eliminate proteolysis (multiple bands in the western blot)

REFERENCES

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