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Design and synthesis of elastin-like polypeptides for an ideal nerve conduit in peripheral nerve regeneration

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ABSTRACT

The study involves design and synthesis of three different elastin like polypeptide (ELP) gene monomers namely ELP1, ELP2 and ELP3 that encode for ELP proteins. The formed ELPs were assessed as an ideal nerve conduit for peripheral nerve regeneration. ELP1 was constructed with a small elongated pentapeptide carrying VPGVG sequence to mimic the natural polypeptide ELP. The ELP2 was designed by the incorporation of 4-penta peptide chains to improve the biocompatibility and mechanical strength. Thus, the third position in unique VPGVG was replaced with alanine to VPAVG and in a similar way modified to VPGKG, VPGEG and VPGIG with the substitution of lysine, glutamic acid and isoleucine. In ELP3, fibronectin C5 domain endowed with REDV sequence was introduced to improve the cell attachment. The ELP1, ELP2 and ELP3 proteins expressed by *Escherichia coli* were purified by inverse transition cycling (ITC). The purified ELPs were confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting. The Schwann cell (SC) morphology and cell adhesion were assessed by fabrication of ELP membrane cross-linked with glutaraldehyde. The Schwann cell proliferation was measured by WST-1 assay. Immunofluorostaining of Schwann cells was accomplished with SC specific phenotypic marker, S100.

1. Introduction

Elastin is a key extracellular matrix protein found mainly in large arteries, lung, ligament, tendon, skin, and elastic cartilage. It supports the elasticity and resilience of many vertebrate tissues [1]. An extracellular matrix (ECM) analog with a well-defined molecular architecture comprising different functional domains was designed and synthesized by genetic engineering approach [2]. The bioengineered elastin like protein in spider silk has displayed equal mechanical properties to that of native silk [3]. Elastin-like polypeptides (ELPs) are genetically engineered polypentapeptide biopolymers with structural homology to mammalian elastin [4]. ELPs are considered to be non-immunogenic and have not induced any antibody production in animal and human applications. ELPs are repetitive artificial polypeptides derived from recurring amino acid sequences found in the hydrophobic domain of tropoelastin. The most commonly used ELPs consist of repeats of the motif (VPGXG)*n* where X, the guest residue, is any amino acid other than proline, and *n* represents the number of pentapeptide repeats in the ELP [5]. The hydrophobic domains of the elastin protein are rich in valine (V), proline (P), alanine (A) and glycine (G) and are often present in tetrapeptide, pentapeptide and hexapeptide tandem repeats, VPGG, VPGVG and VAPGVG, respectively [1,6]. It was reported that the precise and rapid synthesis of genes encoding a polypeptide of desired sequence and length is therefore a key requirement for producing genetically encoded, repetitive polypeptides for specific applications.

ELPs are a class of stimuli responsive thermal sensitive peptide polymers that undergo thermally triggered phase separation. The ELP polymers possess lower critical solution temperature (LCST), where a critical transition temperature (T_t) of the ELP is a soluble unimer in aqueous solution and above its T_t, the ELP undergoes a phase transition and aggregates into an insoluble coacervate [7]. When the ELPs are subjected to temperature increase higher than T_t, it undergoes hydrophobic collapse accompanied by an increase in secondary/tertiary structure formation much like folded proteins [8]. The LCST of ELPs can also be controlled by varying the length of the ELP or its amino acid composition. This tunable property can support the use of ELPs as biologically inspired polymers that can respond to thermal and other environmental cues. The purification of recombinant ELPs from cell contaminants was accomplished by inverse thermal cycling (ITC) that eliminates the expensive purification methods like chromatography. These advantages of ITC techniques include low cost of purification, technological simplicity (requiring only a laboratory centrifuge or filter that is readily available in most molecular biology and biochemistry laboratories), ease of multiplexing, and high yield [9].

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Peripheral nerve regeneration is a complicated and long-term medical challenge that requires appropriate nerve conduits or channels for bridging nerve injury gaps and restoring nerve functions. Injuries to the peripheral nervous system can result in substantial functional loss and decreased quality of life because of permanently impaired sensory and motor functions and secondary problems, such as neuropathic pain [10]. Several endogenous neural mechanisms can be engaged to retrieve the functional deficits caused by nerve injuries. However, clinical and experimental evidences suggest that these mechanisms failed to provide a satisfactory functional recovery [11,12]. A common treatment option for peripheral nerve injury (PNI) is end-to-end suturing, but this type of repair is limited to short-distance gaps. The use of autografts in repairing PNI is now considered as the gold standard, although it has many drawbacks [13]. Alternative to autografts, the use of different artificial grafts also called as conduits or guidance channels has been tested to bridge the nerve gaps. A large number of natural and synthetic materials such as collagen, laminin and polyglycolic acid (PGA) have been proposed as nerve conduits [14]. One of the main limitations of collagen-based biomaterials is poor mechanical property and rapid degradation by extra-cellular collagenases *in vivo*. Similarly, the synthetic degradable polymer such as PGA also suffered from rapid degradation and relatively weak mechanical strength. In contrast, poly(L-lactic acid) (PLLA) is considerably more stable than PGA but the slower degradation of PLLA limits its application [15]. Thus far, none of the materials are considered as ideal conduit or can successfully bridge the long gaps. Numerous applications of ELPs in biotechnology and medicine have been proposed [16,17]. Elastin-like polypeptides (ELPs) are a class of stimulus-responsive biopolymers and are well suited for *in vivo* applications, such as drug delivery and tissue engineering due to its tunable physicochemical properties and biocompatible nature. ELPs could be a potential material for the design of functional scaffolds in tissue engineering. Since, ELPs are derived from an ECM protein it will be expected to provide an ECM-like environment and cues for the regeneration of cells and tissues. In addition, ELPs possess good biocompatibility with mechanical and viscoelastic properties similar to natural elastin [18]. The objective of this study is to design and synthesis genetically modified functional ELPs named as ELP1, ELP2 and ELP3. The recombinant ELPs expressed in *Escherichia coli* were purified by a relatively simple technique called as "inverse transition cycling" (ITC). The ITC purified ELPs were shown to be a promising biomaterial for PNI as a nerve guidance conduit. The purification of ELPs was assessed by SDS-PAGE and immuno-blotting. The biocompatibility and cell adhesion results indicated that the recombinant ELPs are not toxic to the Schwann cell growth and proliferation and improved cell adhesion were observed with ELPs.

2. Experimental

2.1. Monomer gene design and ELP expression

The study involves the design and synthesis of three gene monomers namely ELP1, ELP2 and ELP3 encoding for ELP polymers. The basic structure of ELPs (VPGVG) is a repeating sequence (N = 10–12) found in the mammalian elastic protein. In a similar way, the ELP1 was constructed to mimic the natural polypeptide, ELP (hydrophobic). The ELP2 was designed to improve the biocompatibility and mechanical strength by the incorporation of 4-penta peptide chains. Alanine would replace the third position in VPGVG as VPAVG to improve the biocompatibility. Similarly, lysine, glutamic acid and isoleucine would take the X position in VPGVG and changed as VPGKG, VPGEG and VPGIG, respectively, for mechanical enhancement. The ELP3 was designed to exhibit improved cell adhesion, hence it was constructed with the fibronectin CS5 domains. The monomer sequence used to synthesize ELP1, ELP2 and ELP3 was listed in Table 1.

The ELP1, ELP2 and ELP3 genes were placed in a T7 bacteriophage promoter of the pGS-21a expression vector (Novagen, Madison, WI). It was then transformed into the host BLR (DE3) pLysS (Novagen). The resulting modified pGS-21a expression vectors encoding for lysine-based ELP polymers were individually transformed into the *E. coli*, BLR (DE3) pLysS (Novagen, Madison, WI) for expression. The starter cultures (100 mL flasks containing 10 mL terrific broth medium supplemented with 10 μL ampicillin (100 mg/mL)) were inoculated with 100 μL of transformed cells (ELP1, ELP2 and ELP3), from glycerol (20%) stocks, stored at −80 °C and incubated overnight at 37 °C with shaking at 225 rpm [23]. To remove β-lactamase from the media, the confluent starter culture was pelleted by centrifugation (3000 g for 10 min at 4 °C) and resuspended in 10 mL of fresh terrific broth (TB) medium. The expression cultures (2 L flask containing 500 mL of TB medium with 100 mg/mL ampicillin) were inoculated with 5 mL of the resuspended starter culture and incubated at 37 °C with shaking at 225 rpm. Growth was monitored by the optical density (OD) at 600 nm (typically about 3–4 h post inoculation as optimized) and the protein expression was induced at OD 600 (~0.6–0.8) by the addition of isopropyl β-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. The cells were then harvested 4 h after induction by centrifugation at 3000 *g* for 15 min at 4 °C and resuspended in 15–20 mL of cold, low ionic strength buffer (PBS at 4 °C). PMSF (100 mM) and proteinase inhibitor cocktail were added to the cells before they are subjected to sonication. Brief pulses of sonic disruption at 4 °C (frequency: 5 s on/5 s off at maximum power) (Hielscher Ultrasound technology, UP200S, Sonotrode S3/micro tip3) were used to lyse the cells until the resuspended liquid was no longer viscous and became clear. After sonication, the cell lysate was centrifuged at 16,000 *g* for 15 min at 4 °C to remove insoluble cellular debris, retaining the supernatant. Soluble nucleic acids were precipitated by the addition of polyethylenimine (0.5%, w/v) and removed by centrifugation at 20,000 *g* for 15 min at 4 °C.

2.2. ELP purification by inverse transition cycling (ITC)

The expressed ELP was purified by ITC, a process of selective aggregation by heating the cell lysate (30–45 °C) and adding NaCl (0.5–2 M). The method uses inexpensive reagents such as NaCl to trigger the inverse phase transition and the separation of fusion proteins was done by centrifugation. The purification of ELP was done with two cycles of ITC and the method was briefed below. The soluble lysate of ELP was precipitated by adding NaCl (1 M) in water bath at 37–42 °C. The aggregated ELP protein was separated from solution by centrifugation at 16,000 *g* for 15 min at 30–45 °C, which resulted in the formation of a translucent pellet at the bottom of the tube. The supernatant, containing soluble contaminants from the lysed *E. coli* cells, was discarded and the pellet containing the ELP was resolubilized in cold, low ionic strength buffer by repeated gentle agitation. Once fully suspended in solution, the first round of inverse transition cycling was completed

Table 1
The monomer sequence used to synthesize functional elastin-like polypeptide (ELPs).

ELPs	Monomer gene sequence
ELP1	VPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVG
ELP2	VPGKGVPGKGVPGIGVPGIGVPGKGVPGKGVPGKGVPGKGVPGEGVPGKGVPGKGVPAVGVPAVGVPAVG
ELP3	VPGKGVPGKGVPGIGVPGIGVPGKGVPGK*GEEIQIGHIPREDVDYHLYP*VPGKGVPGKGVPGEGVPGKGVPGKGVPAVGVPAVGVPAVG