Journal of Cancer Biomoleculars and Therapeutics

ETERNO PRESS

Review

A Comprehensive Review on Elastin-Like Polypeptides (ELPs): Characterizations, Synthesis, Purification and Application as Nanoparticles in Drug Delivery of Cancer

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Article history

Received: 20 July 2025
Revised: 22 August 2025
Accepted: 26 August 2025
Published online: 7 January 2026

Keywords

Cancer
Elastin-like polypeptides (ELPs)
Inverse transition temperature (ITT)
Chemotherapeutic drugs
ELP nanoparticles

Abstract

Cancer is the most challenging disease with high rates of prevalence and mortality in the world. Traditional therapies of cancer include surgery, radiation therapy, and chemotherapy are often unsuccessful in some patients with tumor recurrence. Recently, application of nanoparticles and nanomaterials have developed based on targeted therapy to decrease side effects and non-specificity of current treatments. Elastin like-polypeptides (ELPs) are synthetic and temperature-sensitive biopolymers in the form of repeating polypeptides that attract attention of researchers to use in biomedical applications due to exhibiting some outstanding features such as inverse transition temperature (ITT), high biocompatibility, biodegradability, self-assembly and stability. This review deals with characterizations of ELP, de novo synthesis and purification. In addition, it highlights some applications of ELP in protein purification, biomedical application, tissue engineering, vaccine carriers as well as usage as a drug delivery system. Moreover, it summarizes application of anti-cancer chemotherapeutic drugs, small molecules, peptides and proteins conjugated-ELP as drug delivery in treatment of cancer with introducing soluble ELP Unimers, injectable ELP nanoparticles and ELP block copolymer nanoparticles. These promising approaches could open a way to introduce novel strategies to combat various types of cancer.

Abbreviations

CPP, Cell-penetrating peptide; CD, Circular Dichroism; CLP, Collagen-like polypeptide; CCMV, Cowpea mosaic virus; DAAO, Di-amino acid oxidase; DSC, Differential scanning calorimetry; DTX, Docetaxel; ELPs, Elastin-like polypeptides; EPR, Enhanced permeability and retention; FKBP12, FK506-binding protein 12; GBM, Glioblastoma; GLP-1, Glucagon-like peptides; GRP78, Glucose-regulated protein 78; His, Histidine; iTEPs, Immune system-resistant ELPs; IFN, Interferon; Inverse transition cycling; ITT, Inverse transition temperature; LCST, Lower critical solution temperature; PTX, Paclitaxel; RDL, Recursive directional ligation; sTNFRII, Soluble tumor necrosis factor receptor type II; SOD, Superoxide dismutase; TMZ, Temozolomide; TRAIL, Tumor necrosis factor-related apoptosis-inducing ligand

1. Introduction

Cancer is a significant global health challenge, characterized by a high mortality rate. In the United States, an estimated 1,958,310 new cancer cases were diagnosed in 2023. This resulted in an estimated 609,820 fatalities attributed to the disease [1]. Although established cancer therapies such as surgical intervention, hormonal treatment, radiation therapy, and chemotherapy demonstrate efficacy in certain patients, these approaches often result in tumor recurrence and several side effects [2]. To address these challenges, novel strategies of treatment have been developed based on personalized medicine such as immunotherapy and targeted therapy utilizing specific molecules and carriers to target cancer cells specifically [3-5]. Also, researchers are investigating diverse nanomaterials as vehicles for drug delivery, including nanopolymers like polyethylenimine, PLGA, chitosans, collagen, and gelatin [6,7]. One of the most important biological agents with outstanding properties that make it possible to be manipulated and engineered to be implicated in efficient drug delivery and treatment of cancer is elastin. Elastin is an extracellular matrix protein found in connective tissues in all vertebrates. It provides elasticity to large blood vessels, tendons and muscles, lungs, and skin. Elastin is derived from the hydrophobic domain of tropoelastin (60-72 kDa), which is a precursor of elastin. Elastin-like polypeptides (ELPs) are synthetic biopolymers in the form of repeating polypeptides, containing several copies of the pentapeptide motif Val-Pro-Gly-Xaa-Gly (VPGXG), where Xaa can be any amino acid except proline. The presence of proline in this position causes ELP to lose its inverse transition temperature (ITT) property [8]. ITT is a phenomenon where ELPs, undergo a phase transition from a disordered, soluble state to a more ordered, folded state as the temperature increases. Outstanding charactrizations of ELP make it attractive for researchers to apply it as a nanocarrier and

biomedical vehicle in tissue engineering, biomedical and therapeutic applications. This review highlights the specific characterizations of ELP, its synthesis, purification and applications as a nanoparticle in several investigational, biomedical and therapeutic fields. Also, it summarizes application of ELP and its modifications as drug delivery system in conjugation with small molecules, therapeutics agent, peptides and proteins in treatment of several types of cancer. These promising approaches could open a way to introduce novel strategies to combat various types of cancer.

2. Methodology

A literature search was conducted to identify relevant articles on ELPs, synthesis, purification and their characterization as well as their application in cancer drug delivery. The search was performed across three major scientific databases: PubMed, ScienceDirect, and Google Scholar. The search was limited to publications from 2010 to 2025. A combination of keywords and terms were used to formulate the search queries. These terms were grouped into three main categories: **ELP-related terms:** elastin-like polypeptides, recombinant polymers, inverse temperature transition, peptide-based carriers. **Nanoparticle/delivery-related terms:** nanoparticles, nanocarriers, drug delivery, nanomedicine, self-assembly. **Cancer-related terms:** cancer, tumor, oncology, anticancer agents, chemotherapy, targeted delivery. Original research articles, review articles and book chapters published in peer-reviewed journals were included in the literature search and Studies published before 2010 were excluded from the study.

3. Features of ELP

3.1 Inverse Transition Temperature (ITT)

ELPs have some specific properties for biomedical applications [9]. ELPs are temperature-sensitive biopolymers that exhibit ITT in response to temperature changes. They resemble tropoelastin and have a notable charactrization that allows them to remain soluble at a lower critical solution temperature (LCST), however conversely, they form aggregates or protein masses at temperatures above that critical temperature [8,9]. LCST is the temperature below which a polymer solution, is completely soluble and above which it undergoes a phase transition, becoming insoluble. The temperature related to ITT can be precisely adjusted between 0 °C and 100 °C and optimized for specific applications such as drug delivery and tissue engineering and other therapeutic and scientific applications [10]. This phase transition behavior of ELPs depends on many variables, including molecular weight, concentration, Xaa subunits, and the hydrophobicity of these subunits. The distribution of Xaa subunits along the polypeptide chain and the attachment of these subunits to ionizable side chains in ELPs can alter the ITT of this biopolymer. The more hydrophobicity of the Xaa amino acids, the lower the ITT and vice versa. The precise position and nature of each amino acid are also important for the ITT behavior of ELPs [11].

3.2 High Biocompatibility, Biodegradability and Self-Assembly

ELPs are very safe and functional without activating the immune system because they are derived from human tropoelastin. Due to their high biocompatibility, biodegradability, and non-immunological features, ELPs are highly suitable for *in vivo* applications [12]. The molecular weight of ELPs is larger than the kidney threshold, allowing them to remain in the bloodstream for an extended period, thus giving them a long half-life. Another important feature of ELPs is that when they are integrated or attached to other proteins, they are capable of retaining their ITT characteristic, and the proteins attached to ELP can also be purified using the ITT feature of ELP [8].

Another feature of ELPs is their biodegradability through proteolytic reactions, which reduces their harmful side effects. These polypeptides are hydrolyzed by elastolytic enzymes such as elastase, endopeptidases, and collagenases. This important characteristic of ELPs regulates their accumulation at the target site and also their clearance from the body [13]. ELPs enter cells through the process of pinocytosis and are located in the lysosomal organelle, where they are eradicated within 2-24 hours after proteolytic degradation by elastolytic enzymes [8].

Another characteristic of ELPs is their self-assembly, which is reversible and occurs due to temperature changes caused by the presence of specific hydrophobic domains [14]. ELPs can form crosslinks that is irreversible and is due to the presence of the amino acid subunits glutamine and lysine [15].

3.3 Stability of ELP

The secondary structure of ELPs is determined by FTIR technique and circular dichroism (CD) spectroscopy, and enthalpy changes are measured by differential scanning calorimetry (DSC) [16]. Hydrogen bonds are an important factor in stabilizing ELPs. ELPs are stable for a long time and under various conditions because the side chains of the amino acid subunits glycine, valine, and proline consist of either a simple hydrogen atom or an aliphatic group [17]. To achieve greater stability, a collagen-like polypeptide (CLP) is attached to an ELP, and this CLP-ELP label is used for the construction of recombinant fusion proteins. For further stabilization, CLP-ELP is also connected to superoxide dismutase (SOD) and diamino acid oxidase (DAAO). In this case, the effect of H_2O_2 on the secondary structures of the recombinant protein is significantly reduced. This method also improves the stability of recombinant proteins against denaturation by urea.

ELP fusion proteins have gained significant attention due to their long half-life and prolonged yield. Studies have also shown that attaching ELP sequences to small therapeutic proteins increases their half-life. For example, it has been demonstrated that the half-life of the 12-KD (TNF)-α VHH (single-domain) antibody increases when it is attached to ELP. The study has also shown that labeling ELP with a histidine (His) tag, significantly

improves the stability of the recombinant proteins without affecting the activity and secondary structure of the protein (e.g. beta-glucosidase enzyme) [8].

3.4 Synthesis of ELP

ELPs can be expressed as unstructured recombinant proteins in E. coli. Due to their highly repetitive sequences, ELP genes cannot be assembled using the usual assembly methods that depend on hybridization. Additionally, due to the high GC content in ELP genes, PCR yields weak amplification, resulting in the production of scattered gene products. Therefore, alternative approaches have been developed to address this issue [18].

The initial method used for ELP synthesis was Concatemerization, in which ELP monomer genes with overlapping sticky ends are joined together to create an oligomer within a vector. This method is simple and quick; however, plasmid-mediated joining can lead to uncontrolled oligomerization [19]. To address this problem, Meyer and Chilikoti employed a technique called Recursive directional ligation (RDL), where the ELP gene is introduced into a linear vector, and only one oligomer is added at each step, ultimately producing a gene of uniform and defined length. However, linearizing the vector with a restriction enzyme can lead to self-ligation, reducing oligomer entry and decreasing gene synthesis efficiency [20]. This technique was further developed by McDaniel and colleagues, who introduced a second cutting site in two plasmids encoding ELP to prevent self-ligation (Figure 1). Using this method, a functional plasmid with the joining of two ELP coding halves is produced [18].

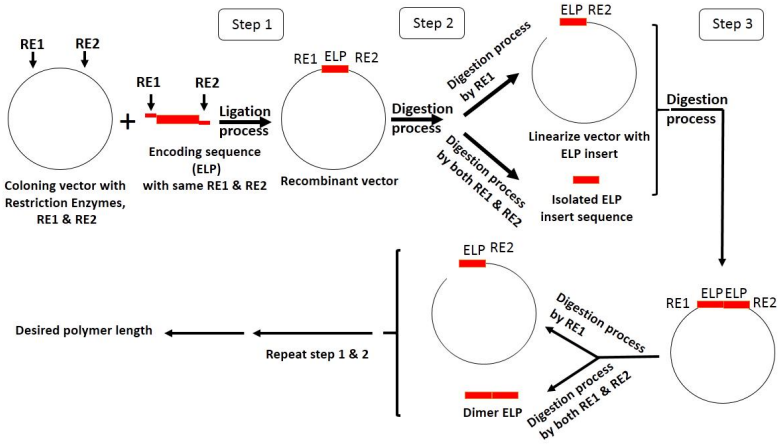

Figure 1. Method of Recombinant DNA ligation for ELP synthesis.

In addition to the methods described above, other methods have also been introduced by researchers. Among them, Kaufman and Waberskrich described an effective synthetic method for the synthesis of ELPs, which is based on solid-phase peptide synthesis, using resin as a solid protector in the presence of HoBt/PyBob as activating agents. Therefore, in general, ELPs can be synthesized either by chemical methods through solid-phase peptide synthesis as synthetic polypeptides or by recombinant DNA methods genetically. Among these methods, recombinant DNA methods are recommended due to many advantages such as high specificity in sequence, molecular weight and low limitations [11].

3.5 Purification of ELP

The sensitivity of ELPs to temperature allows for cost-effective purification of ELPs without the use of chromatography. The purification of ELP is carried out by applying a method called inverse transition cycling (ITC), which was introduced by Meyer and Chilcott in 1999. ITC consists of three main stages, which are described below [18].

- ELP-expressing cells are lysed, and the lysate is centrifuged at a temperature below the ITT of ELP (the temperature at which ELP is soluble) to precipitate and ultimately remove cellular debris.

- The supernatant containing soluble ELP is collected and heated at a temperature above the ITT of ELP. The resulting suspension is then centrifuged to precipitate the aggregated ELP.

- The ELP pellet is dissolved in cold buffer along with centrifugation at a temperature below the ITT. Centrifugation at this stage is intended to remove any contaminants and protein impurities, ultimately resulting in pure ELP in solution without any protein contamination [18].

ELPs can also be used as a tag for the purification of other proteins fused with ELP [21]. ELP fusion proteins can be purified by applying the ITC method, and then the ELP can be removed by a proteolytic cleavage site or a self-splicing protein domain, or with a self-cleavage intein [22]. Chiloti and other researchers have developed several different methods for the cleavage and separation of ELP from the target protein or ELP fusion protein [18].

The first method, which is also the most successful, involves the connection of a peptide sequence between ELP and the target protein. This sequence can be recognized and cleaved by proteases, resulting in the

separation of ELP from the target protein [13]. The second method introduces an intein between the target protein and ELP, which self-cleaves upon the addition of a reducing agent such as dithiothreitol [23]. The third method introduces an enzyme recognition site for sortase A (the Leu-Pro-Glu-Thr-Gly motif), which is recognized by this enzyme in the presence of tripeptide glycine and cleaves between the glycine and threonine subunits, subsequently linking glycine to threonine. The sortase A cleavage method is suitable not only for cleaving ELP from the target protein or fusion protein but also for simultaneously attaching an external molecule such as a fluorophore, imaging factors, or polymerization initiators. These methods allow to produce a high-performance protein using laboratory equipment and software [18].

3.6 Determination of ELP Characteristics

After the expression and purification of ELP, its characteristics must be determined to ensure the presence of suitable biophysical properties and ITT behavior. Mass spectrometry / matrix-assisted laser desorption can be used to determine their molecular weight, ensuring that the construct maintains the same expression quality during the cutting and production process. High-performance liquid chromatography and gel electrophoresis should also be performed for their purification [24,25]. The characteristics of protein domains and active peptides that are attached and integrated into ELP should be determined using *in vitro* assays to confirm that their activity is preserved throughout the process. The application of tissue engineering methods may also assist in determining the strength and elasticity properties of ELP through mechanical testing [16]. Finally, the endotoxin levels of all ELPs and their integrated proteins must be determined to ensure they are within the limits set by the FDA. If the endotoxin level exceeds the limits established by the FDA, endotoxins are removed by polymyxin chromatography or ion exchange chromatography. Additionally, the biocompatibility of ELPs and their integrated proteins should also be assessed [18].

4. Applications of ELP

4.1 Protein Purification

ELPs are primarily used in protein purification. They are combined with other proteins and allow them to separate from other cellular components when placed at temperatures above the LCST. The purification of ELPs offers a simple method for purifying peptides or ELP-combined proteins without the use of chromatography. Recently, ELPs have been fused or linked with many proteins, and all these combined proteins play significant roles in various fields of life sciences [8,26].

4.2 Biomedical Applications

Another application of ELPs can be found in the biomedical industry [27]. Protein drugs, due to their specificity and high activity, have been recognized as one of the important treatments for various diseases. The very low biodegradation of ELPs (approximately 2.5WT% per day) indicates that ELP can have significant stability in therapeutic methods and increases the half-life of protein drugs. ELPs play an important role in the formation of temperature-sensitive particles in tumors using the body's temperature. Studies have shown that the decrease in ITT temperature of ELP is associated with tumor persistence [27]. Additionally, some proteins linked to ELP (specifically, the fusion proteins interferon (IFN)-ELP and RGD-tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-ELP) have long-lasting effects and significant potential for cancer treatment. Furthermore, this system can also be used for the treatment of vascular injuries and Sjögren's syndrome (a type of eye disease) [8].

4.3 Tissue Engineering

ELPs are utilized in tissue engineering through modulating some variables such as the amino acid sequences, chain length, polymer structure, as well as the quantity and positioning of linkages at the gene and protein levels. After the injection of ELP into the damaged tissue, a solid matrix forms under specific conditions. The ITT property of ELP facilitates this state for the formation of masses and aggregates of ELP [8,28,29].

4.4 Vaccine Carriers

Studies have shown the application of immune system-resistant ELPs (iTEPs) as vaccine carriers. The motifs selected to create iTEP repeats are derived from homologous peptide sequences of mouse or human elastins. iTEPs also possess ITT property [27,30].

4.5 Drug Delivery

One of the most important applications of ELPs in biological sciences and medicine is their use in drug delivery. ELPs are recognized as a system for drug delivery due to their unique biophysical properties. Studies have shown that ELPs provide a very easy system for both local and systemic drug delivery. ELPs can be covalently linked to a therapeutic peptide or protein and can also be specifically attached to small molecules using subunits placed along the ELP chain through covalent bonding [31,32]. The application of linking ELPs to drugs through the creation of covalent crosslinks has also been used for the delivery of small antibiotic molecules such as vancomycin and cefazolin, which typically provide localized delivery of antibiotics in orthopedic surgeries [33].

For drug delivery applications, ELPs can be engineered to assemble themselves into nanoparticles under physiological conditions. The ELP sequence and its molecular weight can be optimized for lower cytotoxicity, greater stability, high permeability of tumor blood vessels, better solubility and retention effect that is referred to a phenomenon where nanoparticles and macromolecules accumulate in tumors for a prolonged period [18]. Unlike many synthetic polymers, one of the advantages of ELPs as drug carriers is the non-toxicity of their degradation products. Additionally, ELP

degradation, largely carried out by serum elastase and collagenase, occurs beyond the timeframe relevant to its drug delivery [31].

Electrospraying has also been observed as an effective, innovative method for creating stimulus-responsive drug nanoparticles that have potential applications for drug delivery. Additionally, self-assembling nanoparticles are capable of encapsulating significant amounts of drugs and can serve as a system for controlled drug release [34].

One of the other applications of ELP drug delivery is the direct targeting of tumor cells by chemotherapeutic agents to prevent the side effects of chemotherapeutic drugs on healthy body cells [10]. To deliver drugs to tumors, the ITT temperature of ELP is adjusted between 37 and 42°C. As the temperature rises above the ITT, ELPs form insoluble aggregates, which then enter the cells through pinocytosis. Mild heat increases blood flow and enhances the permeability of blood vessels to drugs at the tumor sites. Additionally, heat affects the biological function of cancer cells by inhibiting DNA synthesis and hindering DNA repair mechanisms [11]. The enhanced permeability and retention (EPR) effect is attributed to the blood vessels in tumor tissue, where they have discontinuous and irregular endothelial tissue, which can lead to increased permeability of tumor blood vessels to macromolecules. The high permeability of tumor blood vessels occurs due to weak and abnormal angiogenesis, creating pores of sizes ranging from 200 nanometers to 2 microns in the vessel walls. Compared to healthy blood vessels, the EPR effect allows macromolecules to enter the tumor. In contrast, the tight junctions present between endothelial cells in healthy tissue prevent the intercellular transfer of drug carriers and drugs to healthy tissues [35]. The synthesis of ELPs with precise molecular weight using genetic engineering techniques, pharmacokinetics, biocompatibility, and controlled degradation has made ELPs a popular drug delivery system for the delivery of systemic anticancer drugs [11].

ELPs also have advantages for delivering drugs to areas of the body that are not easily accessible by blood flow. Such applications include the treatment of degenerative joint diseases like osteoarthritis [31]. Neuronal inflammation is another therapeutic target where localized therapy with anti-inflammatory drugs is used to improve these conditions, thereby preventing the side effects of systemic therapy. Since TNFα is one of the key factors in neuronal inflammation, its inactivation is a very important therapeutic goal. However, drugs that target TNFα often come with dangerous side effects, such as immune system suppression. Therefore, localized delivery of drugs like soluble tumor necrosis factor receptor type II (sTNFRII) is very beneficial, as these drugs bind to TNFα and inactivate it [36]. Compared to free sTNFRII, ELP bound to sTNFRII has a longer presence at the inflammation site and a lower presence in the bloodstream, which may provide better therapeutic outcomes for ELP-integrated drugs compared to free drugs. Another drug that inhibits TNFα is a small molecule called curcumin, which is covalently linked to ELP. Curcumin is attached to the glutamate subunits in ELP via a degradable carbamate linker. Compared to free drugs, the activity of the drugs in binding to ELP particles is significantly preserved [36].

It has been shown that subcutaneous injection of ELP particles can also be used to control certain diseases such as diabetes. The conjugation of peptide drugs such as glucagon-like peptides (GLP-1) and recombinant ELP can form injectable ELP particles in ITT temperature of ELP below the body temperature and it is effective in treatment of type 2 diabetes [37].

4.5.1 Application of Anti-Cancer Chemotherapeutic Drugs and Small Molecule Conjugated to ELP in the Drug Delivery to Cancer Cells

Table 1. Application of anti-cancer chemotherapeutic drugs and small molecule conjugated to ELP in the drug delivery to cancer cells.

Therapeutic drug	Conjugation	Function	Tumor reduction	*In vitro*/ *in vivo* status	Type of Cancer	Reference
Doxorubicin	CPP-ELP-Dox	Inhibition of proliferation	50%	*In vitro*/ *in vivo*	Breast cancer	[38]
Doxorubicin	CPP-ELP-Dox	Inhibition of proliferation	-	*In vitro*	Glioma	[39]
Doxorubicin	CCMV-ELP-Dox	Inhibition of tumor growth	~97.5% and ~69%	*In vitro*/ *in vivo*	Melanoma and colorectal cancer	[40]
Rapamycin	Rapamycin-ELP	Inhibition of proliferation	~83%	*In vitro*/ *in vivo*	Breast cancer	[41]
Rapamycin	Rapamycin- csGRP78 targeting domain-ELP	Enhanced cellular uptake and reduction in mTOR activity	-	*In vitro*/ *in vivo*	Breast cancer	[42]
Paclitaxel	Paclitaxel-ELPs engineered with cysteine residues	Inhibition of proliferation	~85% and ~96%	*In vitro*/ *in vivo*	Breast and prostate cancer	[43]
Paclitaxel	PTX -iRGD- ELP	Promoting cell death	-	*In vitro*	Lung cancer	[44]
Paclitaxel	AP1-ELP-PTX	Inducing cell cycle arrest and apoptosis	-	*In vitro*	Ovarian cancer	[45]
Docetaxel	RGD peptides-ELP-Docetaxel	Inhibition of proliferation	-	*In vitro*	Breast cancer	[46]
Gemcitabine	ELP-gemcitabine	Downregulating the NT5C3 gene	-	*In vitro*	Ovarian cancer	[47]
DNA aptamers	CPP-ELP-DNA aptamer	Inhibition of proliferation	-	*In vitro*	Doxorubicin-resistant breast cancer	[48]
miRNA-34a	ELP- miRNA-34a	Inhibition tumor growth	38%	*In vitro*/ *in vivo*	Lung carcinoma	[49]

There are some evidence demonstrating the drug delivery application of ELP in a various types of cancer cells. Table 1 summarizes application of anti-cancer chemotherapeutic drugs in conjugation with ELP in drug delivery to cancer cells.

Chemotherapeutic agents are being modified through conjugation with ELPs, enabling localized delivery and reducing systemic toxicity [10]. Researchers attached the anti-cancer drug Dox to a cell-penetrating peptide (CPP) and an ELP. Fluorescence microscopy studies in breast cancer cell line (MCF7) have demonstrated that the CPP-ELP-Dox complex delivers Dox to the cell nucleus. Under heat conditions, tumor inhibition by CPP-ELP-Dox is 2 times greater than treatment with an equal dose of free Dox [38]. Another study has used Dox attached to ELP along with CPP in a drug delivery system for brain tumors. This study shows that the CPP peptide facilitates the transport of the drug delivery system across the blood-brain barrier into the cells. Additionally, CPP-ELP-Dox inhibits the growth of glioma cells [39]. A novel nanomedicine platform has utilized the cowpea mosaic virus (CCMV) for improved chemotherapy delivery. The researchers engineered CCMV nanoparticles functionalized with ELPs, enabling them to encapsulate the Dox. These nanoparticles have exhibited uniform size, facilitated efficient tumor cell uptake and intracellular drug release, leading to enhanced anti-tumor efficacy in murine models of melanoma and colorectal cancer. Furthermore, the ELP-functionalized nanoparticles activated an immune response within the tumor microenvironment, contributing to further tumor growth inhibition. This approach addresses limitations of conventional chemotherapy by offering targeted drug delivery with reduced systemic toxicity, highlighting its potential as a promising therapeutic strategy against cancer [40].

In a method called ELP brachytherapy, an ELP is chemically linked to a radionuclide (I131) to increase the survival time of the radionuclide after injection and also to protect I131 from degradation by dehalogenase. It has been shown that after the infusion of ELP-I131 into the tumor, I131 remains in the tumor for a long time. This type of ELP significantly increases the survival and retention of the drug in the tumor compared to soluble ELP. Doubling the length of the ELP chain also greatly increases the survival and retention of I131-ELP compared to ELP with a shorter chain length and lower molecular weight. Increasing the injection concentration also significantly increases the drug's survival in the tumor compared to injecting I131-ELP at a lower concentration [50]. Rapamycin, a chemical drug in cancer treatment, has several disadvantages, such as cellular toxicity, low bioavailability, and fast clearance, which limit its application. Shi and colleagues have used an encapsulation strategy for Rapamycin with ELP. The study results have shown that ELP carriers deliver more Rapamycin to the breast tumor [41]. Furthermore, by fusing ELPs with the FK506-binding protein 12 (FKBP12) receptor, researchers have achieved selective delivery of Rapamycin to breast cancer cells via macropinocytosis [51]. Similarly, ELP-based nanocarriers containing Rapamycin-binding domains effectively target glucose-regulated protein 78 (GRP78), leading to enhanced cellular uptake and a significant reduction in mTOR activity [42].

Researchers have successfully conjugated paclitaxel (PTX) to ELPs engineered with cysteine residues. The resulting nanoparticles exhibited significantly enhanced cellular uptake compared to the commercially available formulation Abraxane, leading to near-complete tumor suppression in murine models of breast and prostate cancer [43]. Moreover, a protein nanoparticles based on genetically engineered ELP was designed for targeted drug delivery to lung cancer cells. The nanoparticles are functionalized with the iRGD peptide, which facilitates both targeting and internalization into tumor tissues via integrin and neuropilin-1 interactions. It has shown the successful ability of iRGD-modified ELP to effectively deliver the PTX to lung cancer cells, leading to cell death [44]. A functionalized ELP with the AP1 peptide for targeted delivery of PTX to ovarian cancer cells has been designed. Researchers successfully conjugated PTX to ELPs and demonstrated that AP1-functionalized ELPs exhibited enhanced binding to IL-4 receptor-expressing ovarian cancer cells compared to non-functionalized ELPs. AP1-ELP-PTX displayed increased cytotoxicity against ovarian cancer cell lines, inducing greater cell cycle arrest and apoptosis. Furthermore, 3D spheroid models confirmed the superior cytotoxic efficacy of AP1-ELP-PTX [45]. Furthermore, ELP nanoparticles have been utilized to encapsulate docetaxel (DTX), a chemotherapy drug commonly used to treat breast cancer. The incorporation of RGD peptides on the nanoparticle surface has facilitated targeted delivery and enhanced cytotoxicity towards breast cancer cells [46].

Studies have demonstrated the successful conjugation of hydrophobic cancer drugs with ELPs. This approach enhances drug efficacy and minimizes off-target effects. Furthermore, ELP conjugates have been explored for targeted delivery of therapeutic radionuclides and gene silencing agents. For example, ELP-gemcitabine conjugates have shown promising results in downregulating the NT5C3 gene, implicated in ovarian cancer progression [47]. Other studies have explored the conjugation of ELPs with targeting moieties such as DNA aptamers and CPPs for improved drug delivery specificity. These modifications resulted in increased cytotoxicity against doxorubicin-resistant breast cancer cells [48]. In addition, a multifunctional novel delivery system for miRNA-34a, a potential cancer treatment agent, using ELP nanoparticles (Tat-A86) has been applied for overcoming the limitations of direct miRNA delivery to lung cancer cells. The miRNA/Tat-A86 formulation significantly inhibits tumor growth compared to controls [49].

In conclusion, the utilization of ELPs for chemotherapeutic delivery has shown remarkable potential in preclinical studies. These biocompatible and versatile polymers enhance drug cellular uptake and cytotoxicity against tumors, highlighting their promise as an effective platform for cancer therapy. While preclinical studies have yielded encouraging results for ELP-based drug delivery systems, further investigation

into their potential adverse effects is crucial before clinical translation.

4.5.2 Application of Anti-Cancer Therapeutic Peptides/Proteins Conjugated to ELP in the Drug Delivery to Cancer Cells

Therapeutic peptides have garnered significant interest in recent decades due to their high selectivity in treating solid tumors. However, the presence of peptidases and proteases within the body limits the application of these peptides in cancer treatment [52]. Research has shown promising results with peptide-ELP conjugates. Table 2 presents summery of some anti-cancer therapeutic peptides/proteins conjugated to ELPs in the drug delivery to cancer cells. A cell cycle inhibitory peptide (p21) fused with ELPs has demonstrated enhanced cytotoxicity against pancreatic cancer cells by inducing cell cycle arrest. Also, these conjugated ELPs have exhibited synergistic antitumor effects in a xenograft model of pancreatic cancer in combination with the chemotherapeutic drug gemcitabine [53]. In addition, a pro-apoptotic peptide loaded onto ELPs ((KLAKLAK)2-ELP) effectively targeted breast cancer and melanoma, leading to tumor suppression in xenograft models [54]. Fluorescent ELPs nanocarriers have been engineered to deliver a specific peptide (PCK 3145) to EGFR-overexpressing tumors, triggering apoptosis and enhancing anti-tumor efficacy [55]. An ELP conjugate carrying a Notch inhibitory peptide (dnMAML) and paclitaxel has shown enhanced anti-tumor activity against glioblastoma cells, inducing apoptosis and cell cycle arrest [56].

Table 2. Application of anti-cancer therapeutic peptides/proteins conjugated to ELP in the drug delivery to cancer cells.

Therapeutics peptides/proteins	Conjugation	Function	Tumor reduction	*In vitro* /*in vivo*	Type of Cancer	Reference
p21- gemcitabine	p21- gemcitabine-ELP	Inhibition of proliferation, Cell cycle arrest	81%	*In vitro/ in vivo*	Pancreatic cancer	[53]
Pro-apoptotic peptide (KLAKLAK)2	(KLAKLAK)2-ELP	Tumor suppression	~80% and ~83%	*In vitro/ in vivo*	Breast and Melanoma	[54]
Anti-tumor Peptide (PCK 3145)	Peptide (PCK 3145)-ELP	Inducing apoptosis and enhancing antitumor efficacy	14 fold	*In vitro/ in vivo*	Colon adenocarcinoma	[55]
Notch inhibitory peptide (dnMAML)-paclitaxel	dnMAML-paclitaxel-ELP	Inducing apoptosis and cell cycle arrest	72%	*In vivo*	Glioblastoma	[56]
TRAIL	TRAIL-ELP	Inducing apoptosis	~2.75 fold	*In vitro/ in vivo*	Colon	[57]
IFN-α	IFN-α-ELP	Inhibition of proliferation	95%		Ovarian carcinoma	[58]
IFN-α-temozolomide	IFN-α-temozolomide-ELP	Sustained drug release and inhibiting Glioblastoma recurrence	-	*In vitro/ in vivo*	Glioblastoma	[59]

Huang and colleagues have demonstrated that ELP fusion with TRAIL significantly increases apoptosis in colon cancer cells. Moreover, a single intraperitoneal injection of RGD-targeted ELP-TRAIL effectively inhibits tumor growth in a xenograft model [57]. Further studies have shown the efficacy of ELPs for delivering IFN-α in cancer treatment. ELP-fused IFN-α exhibits superior activity retention and prolonged half-life compared to PEGylated IFN-α and IFN-HAS, leading to enhanced anti-tumor activity in ovarian carcinoma xenografts [58]. In glioblastoma (GBM), ELP fusion with IFN-α created a depot for sustained drug release within the tumor resection cavity, significantly improving pharmacokinetics and biodistribution. This approach, when combined with temozolomide (TMZ) chemotherapy, has demonstrated remarkable efficacy in inhibiting GBM recurrence [59].

These findings highlight the unique advantages of ELPs as delivery vehicles for therapeutic peptides and proteins. By prolonging half-life and enhancing tumor distribution, ELP fusion strategies contribute to improved anti-tumor efficacy.

A variety of ELP-based materials and compounds have been developed as drug carriers for drug delivery purposes, which are mentioned below.

4.6 Soluble ELP Unimers

The limitation of many small molecular drugs and peptide drugs is their short half-life. Specifically for peptides, despite their therapeutic potential, these small drug molecules are rapidly eliminated from the bloodstream, limiting their efficacy. Therefore, the use of high doses is necessary, which leads to dose-dependent side effects [60]. Small hydrophobic drugs also have low solubility, which limits their maximum dose and efficacy *in vivo*. Macromolecular carriers such as PEG are used to increase the half-life of drugs in circulation and target drug accumulation at the desired site. Conjugating these agents with ELPs, either through recombinant methods or chemical approaches, can provide a useful system for improving the delivery of small molecular drugs and peptide drugs [32]. In an aqueous solution, below ITT, the Soluble ELP unimers are in a disordered, soluble state. Its primary characteristic is its ability to undergo

the inverse phase transition, which is the basis for all other ELP forms.

4.7 Injectable ELP Nanoparticles

Nanoparticles formed by the self-assembly of ELPs or ELP-based copolymers above their ITT. These are discrete, spherical structures. In recent decades, nanoparticle formulation for improving drug delivery and distribution of therapeutic agents has been extensively studied in clinical research. These formulations, due to their larger size, can prevent drug clearance and increase drug efficacy. Additionally, nanoparticles can be designed to target drugs to a specific tissue and reduce toxicity. These features lead to more specific drug delivery and the application of higher doses of medication for more effective disease treatment [3,4,7]. Size, drug encapsulation efficiency, and biodegradability are significant challenges in designing a nanoparticle, and controlling these factors is difficult when using synthetic and artificial polymers. Therefore, to improve the design and production of nanoparticles, natural polymers have attracted more attention. ELPs are suitable materials for nanoparticles due to their ease of production, thermal responsiveness, and self-assembly capabilities in nanostructures [18].

The ITT temperature of ELP can be adjusted so that ELP is soluble at room temperature but forms masses or insoluble aggregates under the skin where the temperature is higher. In this case, the ELP aggregates are more diluted in the outer layers. Since the phase transition behavior of ELP is concentration-dependent, ELP slowly dissolves from the surface to its center to release the attached drug into the bloodstream. Additionally, in the bloodstream, the large size of ELP prevents the drug from being cleared by the kidneys [32].

4.8 ELP Block Copolymer Nanoparticles

Dual-responsive ELPs consist of two sections (blocks) that are hydrophobic and hydrophilic, which have different ITT temperatures and form micelles under specific temperature conditions when used as a drug delivery system [61]. A diblock copolymer might have a hydrophobic block with a low ITT and a hydrophilic block with a high ITT [62]. At temperatures between the ITT of the blocks or sections, the hydrophobic section dehydrates and aggregates to form the central core of the micelle, in contrast the hydrophilic section remains hydrated and forms the micelle crowns that are exposed to the surrounding environment [14]. The temperature at which micelles form is referred to as the critical micelle formation temperature. This temperature, the diameter of the micelle, and the number of ELP chains in each micelle are adjusted by changing the sequence and molecular weight, as well as by balancing the ratio of the ELP sections or blocks. This type of design is beneficial for encapsulating hydrophobic drugs in the central core of the micelle. Additionally, ligands and targeting molecules can be presented on the surface of the micelle [18].

The behavior and properties of ELPs are highly tunable by controlling their molecular design and environmental conditions. Table 3 provides a systematic comparison of the key parameters that dictate ELP behavior.

Table 3. Systematic organization of key parameters.

Parameter	Description	Effect on ITT and structure	Example
Amino Acid Sequence (Xaa)	The "guest" amino acid in the (VPGXG) repeat unit. This is the primary determinant of ELP properties	Hydrophobicity: More hydrophobic residues (e.g., isoleucine, leucine, valine) lower the ITT More polar or charged residues (e.g., serine, glutamic acid, lysine) increase the ITT	A VPGVG ELP will have a lower ITT than a VPGSG ELP of the same length because valine is more hydrophobic than serine
Molecular Weight (Mw) / Chain Length (n)	The total number of pentapeptide repeats in the polymer chain	Increasing the molecular weight or chain length lowers the ITT. Longer chains have more hydrophobic surface area, which facilitates aggregation at lower temperatures.	An ELP with 40 repeats, will have a higher ITT than one with 80 repeats
Polymer Concentration	The concentration of the ELP in solution	Increasing the polymer concentration lowers the ITT. At higher concentrations, polymer chains are more likely to interact and aggregate	A 1 mg/mL solution of an ELP will have a higher ITT than a 10 mg/mL solution.
Copolymer Architecture	The arrangement of different ELP blocks in a single polymer chain (e.g., diblock, triblock, alternating)	Dictates the final self-assembled structure. Blocks with different ITT values allow for the creation of complex nanostructures like micelles	A block copolymer of a low-ITT block and a high-ITT block will form a nanoparticle with a hydrophobic core and a hydrophilic shell above the lower ITT but below the higher ITT.
Environmental Conditions	External factors such as pH and salt concentration.	**pH:** For ELPs with charged guest residues, changes in pH can alter the charge state and solubility, thereby changing the T_t. **Salt:** The addition of chaotropic (e.g., thiocyanate) or kosmotropic (e.g., sulfate) salts can lower or raise the T_t, respectively, based on their effect on water structure and hydrophobic interactions.	The ITT of a poly-glutamic acid ELP will increase as the pH rises above its pKa due to the repulsion of negatively charged residues. The addition of a salt like sodium chloride can lower the ITT by "salting out" the polymer.

5. Comparison of ELP with Other Common Biomaterials

There are a variety of biomaterials that are applied for drug delivery because they can improve the efficacy and safety of therapeutic agents. They act as carriers or scaffolds to control a drug's release, protect it from degradation, and target specific areas of the body. Although there are some similarities between ELP and other biomaterials, ELPs differ significantly from other common biomaterials like PEG, liposomes, and nanoparticles in their origin, structure, and stimuli-responsive behavior [63].

PEG is synthetic, while ELP is a protein-based and genetically-engineered biopolymer. Unlike PEG, this gives ELPs a level of sequence precision and natural biodegradability. On the other hand, while some PEG-based materials can be engineered to be stimuli-responsive, ELPs have an intrinsic and highly tunable temperature response that is a defining characteristic of their structure. Both materials are used to reduce protein adsorption. PEG does so passively due to its hydrophilic nature, while ELPs can be designed to resist protein catching or to actively bind to specific proteins based on their sequence [52,63].

Liposomes are lipid-based nanoparticles characterized by a spherical vesicle structure with a concentric lipid bilayer enclosing an aqueous core [64]. Liposomes are pre-formed vesicles used to encapsulate and protect a payload, in contrast ELPs can be used as building blocks that self-assemble into different nanostructures or hydrogels, trapping the payload within the matrix. While liposomes are made from natural lipids and are biodegradable, their degradation and release kinetics are often less controllable than the stimuli-responsive release possible with ELPs. Liposomes are typically prepared through processes like solvent dilution and extrusion, while ELPs are produced biologically, often in bacteria, which offers precise control over their molecular structure [31,52,65].

Nanoparticles are a broad category of materials with dimensions in the nanometer range. They can be made from various materials, including polymeric, inorganic, ceramic, viral, and lipid-based particles polymers. In the context of biomaterials, polymeric nanoparticles are often used for drug delivery. These can be solid spheres or have a core-shell structure [66]. While ELPs can be designed to self-assemble into nanoparticles, they are a specific type of protein-based nanoparticle. The term "nanoparticle" is a much broader category that includes a vast range of synthetic and natural materials. ELPs offer a distinct advantage in terms of site-specific functionalization due to their precise, genetically encoded sequence. This makes it easier to incorporate specific targeting ligands or bioactive peptides at defined locations. In contrast, while other polymeric nanoparticles can be functionalized, it is often a less precise chemical process [67]. While some polymeric nanoparticles can be made stimuli-responsive, ELPs are naturally and reversibly triggered by temperature changes, which can be an advantage for targeted delivery in the body [31].

Table 4 presents a comparison between ELP biopolymer with liposomes and nanoparticles as drug delivery systems [52].

Table 4. Comparing ELP with other common biomaterials (liposomes, Nanoparticles).

Type of drug delivery system	Material	Molecular weight	Size	Method of synthesis	Toxicity	Stability	Way to drug delivery
ELP	Protein-based biopolymer	10 -over 150 kDa	50-500 nm	Genetically-engineered and expressed by biological systems	Non-toxic	Stable	Temperature-responsive behavior and ITT
Liposome	Lipid-based biopolymer	-	20-over 1000 nm	Solvent dilution and extrusion	Non-toxic	Unstable	ERP effect
Nanoparticle	Polymeric, inorganic, ceramic, viral, and lipid-based particles polymers	-	1-100 nm	Chemical and biological synthesis	Potential toxicity	Stable	Targeted delivery

6. Challenges in Using ELPs

Despite the advantages of ELP and its promising potential as drug carriers rather than other common biopolymers, ELPs provide some challenges that limit their clinical applications. Concerns regarding *In vivo* stability immunogenicity and Large-Scale Production require further investigation. The most important concern for ELP-based therapy is the potential for immune responses. While ELPs are often considered non-immunogenic, their recombinant production and their attachment to drugs or targeting ligands can stimulate immune system [18].

Maintaining stability of ELPs in some physiological environment is a significant barrier. Once administered, ELP carriers are exposed to a variety of biological components, including proteases, which can degrade the polypeptide and lead to premature drug release. While ELPs have a prolonged circulation half-life, their ability to evade the body's natural clearance mechanisms is a key challenge that needs to be addressed for effective clinical use [16].

The transition from lab-scale to large-scale, cost-effective, and reproducible ELPs is a major bottleneck. ELPs are typically produced using recombinant bacterial expression systems, which can be challenging to scale up while maintaining product consistency and quality. The

purification process may not be economically viable for industrial production. Ensuring a high degree of purity and a reproducible manufacturing process according to Good Manufacturing Practice (GMP) standards is essential for regulatory approval [68].

7. Conclusion

ELPs are biopolymers exhibiting temperature-sensitive behavior due to their LCST. This property, coupled with the ability to attach bioactive groups, makes them ideal for applications such as tissue engineering and targeted drug release. Genetic engineering allows for the production of homogeneous ELPs in various structures, expanding their versatility. ELP-based drug delivery systems come in diverse forms: unimers, particles, nanoparticles, and two-component ELPs. Each structure offers distinct advantages, such as targeted delivery, prolonged half-life, and reduced toxicity. Recent research has focused on enhancing ELP properties through the incorporation of non-natural amino acids and post-translational modifications, leading to improved mechanical strength and binding sites. While bacterial expression systems are commonly used for ELP production, animal systems offer superior post-translational modification capabilities. ELPs also serve as efficient purification tags for recombinant proteins, simplifying downstream processing. 'Despite the advantages of ELPs and their promising potential as drug carriers, ELPs present some challenges that limit their clinical applications. Concerns regarding *in vivo* stability, immunogenicity, and large-scale production require further investigation. Nevertheless, ongoing clinical trials in areas like diabetes and heart disease demonstrate good tolerability and minimal immune responses, suggesting a bright future for ELPs in therapeutic applications.

Acknowledgments

Not applicable.

Disclosure of Potential Conflicts of Interest

The author has not any conflict of interest to disclose.

Availability of Data and Materials

Not applicable.

Ethical Compliance

The manuscript is a review that does not require ethics committee approval.

Funding Information

Not applicable.

Author Contribution

S.Y.: Conception, Providing the data and design, Manuscript writing.

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