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A Novel Targeting Drug Delivery System Based on Self-Assembled Peptide Hydrogel

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ABSTRACT

In the last two decades, 5-fluorouracil (5-FU) is widely used in clinical practice to inhibit the fibroblasts to proliferate and improve the success rate of glaucoma-filtering surgery, but 5-FU has many toxic effects to normal ocular tissues. The self-assembled peptide hydrogels may serve as a new class of biomaterials for applications including tissue engineering and drug delivery. How to deliver 5-FU quickly and precisely to the target sites of ocular tissue by a self-assembled peptide hydrogel remains unexplored. RGD (arginine-glycine-aspartic acid) sequence is cell attachment site in extracellular matrix (ECM). Thus, If the self-assembled peptide hydrogel containing the RGD sequence that act as a specific attachment site for the proliferated fibroblasts adhesion could be designed, after integrated 5-FU, a novel targeting drug delivery system will be put into practice in the future.

Keywords*: *Drug Delivery System*, *Self-Assembly*, *Filtering Surgery

1. Introduction

The success rate of glaucoma-filtering surgery unfortunately has been limited by postoperative scarring [1]. Scar formation results from infiltration of fibroblasts into damaged areas, proliferation of those fibroblasts, and synthesis of ECM glycoproteins. For treatment of scar formation, 5-FU was widely reported as adjunct to improve surgical results by inhibiting the postoperative proliferation of fibroblasts of the filtering site [2-4]. Yet, It was reported toxic effects of 5-FU included toxicity on the conjunctival and corneal epithelium, wound dehiscence, and wound leaks [2], some of which are vision threatening [3]. If a drug delivery system could be designed to specifically target the proliferated fibroblasts after the filtering surgery, not only the success rate of surgery will be significantly improved, but also the possible toxic effects of 5-FU to the surrounding normal ocular tissues will be avoided eventually.

A family of peptides has been developed whose ability to self-assemble into supramolecular hydrogel material is directly linked to their intramolecularly folded state. These peptides adopt random coil conformations in aqueous solution and are freely soluble until intramolecular folding is triggered by the addition of a stimulus. Upon folding, the peptides adopt a conformation conducive to self-assembly. Assembly ultimately leads to the formation of a structurally hydrogel without the need for incorporation of covalent crosslinks [5]. This self-assembled peptide hydrogels may serve as a new class of biomaterials for applications including tissue engineering and drug delivery system [6-9]. Given that the degradation products consist of the drug and amino acid, this drug delivery system has an advantage over polymer-based drug delivery system that generate polymer fragments with heterogeneous chain lengths upon degradation that may present complex toxicity profiles [10].

The RGD sequence was discovered as cell attachment site in ECM some 20 years ago [11], and the receptors for these RGD sequence were identified and organized in the integrin family. The integrin family is one of the most important cell adhesion molecules which are found on the cell surface that act as receptors for cell-to-cell and cell- ECM adhesion [12-14].

Whether can we design a novel targeting drug delivery

system based on self-assembled peptide hydrogel which contains the RGD peptide sequence that act as a specific receptor for the proliferated fibroblasts adhesion?

We hypothesized that a novel peptide containing a bioactive RGD sequence was designed and prepared. When dissolving the peptide in distilled water, a supramolecular hydrogel with nanofibers was formed through the self-assembly of the peptide. In addition, this self-assembled peptide hydrogel could integrate 5-FU into its nanofibers during the process of self-assembly of the peptide. The proliferated fibroblasts can be attached in the peptide hydrogel through the recognition of the RGD sequence, and 5-FU is delivered to the fibroblasts by this model of targeting of drugs (**Figure 1**).

In the past decade, hydrogels formed from self-assembled proteins or peptides have attracted considerable attention. Unlike the conventional polymeric hydrogels that are made of covalently crosslinked polymers, protein- or peptide- based hydrogel is composed of peptide molecules that self-assemble from aqueous solution into cylindrical nanofibers that display bioactive epitopes on their surfaces [15]. The bioactive epitopes could be recognized by some receptors on cell surface, allowing cell adhesion to self-assembled peptide hydrogel [14]. In addition, this self-assembled peptide hydrogel forms a network of nanofibers that are similar in scale to the natural ECM and therefore provides an "*in vivo*" environment for cell growth, migration, and differentiation [5]. Actually, much recent effort has been focused on the synthesis of short peptides to create a new generation of self-assembled materials for the use in biomedical applications. One of the well studied short peptides is the build block comprising of a specific peptide sequence

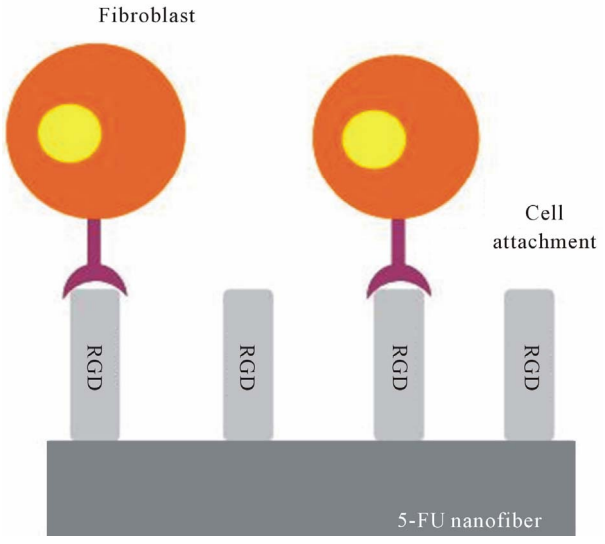

Figure 1. The novel model of targeting of drug based on peptide hydrogel with nanofibers.

and a hydrophobic aromatic tail such as the popular N-Fluorenyl-9-methoxycarbonyl (FMOC) group, which has an ability to self-assemble into hydrogel by taking advantage of π-π stacking interactions [16,17].

Based on this point, a novel peptide containing a bioactive RGD sequence and FMOC tail could be synthesized and the corresponding hydrogel formed subsequently. The specific experiments include the peptide synthesis and peptide hydrogels preparation.

The peptide was synthesized manually in 1.98 mmol scale on the 2-chlorotrityl chloride resin employing a standard FMOC solid phase peptide synthesis (SPPS) method. Before the reaction, the resin was washed with CH2Cl2 (three times) and DMF (three times) and then immersed in DMF for 30 min. After draining off DMF solution, a DMF solution of the mixture of FMOC protected amino acid (4 equiv relative to resin loading) and DiEA (6 equiv) was added to the resin and shaken for 2 h at room temperature. After removing the reaction solution, the resin was washed with DMF (three times). Subsequently, 20% piperdine/DMF (V/V) solution was introduced to the resin to remove the FMOC protected groups. After shaking for 30 min at room temperature, the reaction solution was drained off and the resin was washed with DMF (three times). The presence of free amino groups was indicated by a blue color in the Kaiser test. Thereafter, a DMF solution of the mixture of FMOC protected amino acid (4 equiv), HBTU (4 equiv), HOBt (4 equiv) and DiEA (6 equiv) was added. After shaking for 1.5 h at room temperature, the reaction solution was drained off and the resin was washed with DMF (three times). The absence of free amino groups was indicated by a yellow color in the Kaiser test. After repetition of the deprotection and acylation reaction, the resin was finally washed with DMF (three times) and CH2Cl2 (three times) and dried under vacuum for 24 h. Cleavage of the expected peptide and the removal the protected groups of side chains from the dried resin were performed using a mixture of TFA, deionized water, and TIS in the ratio of 95:2.5:2.5. After 2 h shaking at room temperature, the cleavage mixture and three subsequent TFA washing were collected. The combined solution was concentrated to a viscous solution by rotary evaporation. Cold ether was added to precipitate the product. After washing with cold ether (five times) to remove TFA residual, the precipitate was dissolved in distilled water and then freeze-dried under vacuum for 3 days. The obtained crude products were purified by high-pressure liquid chromatography (HPLC) with a C18 column and using a linear gradient of acetonitrile and DI water containing 0.1% TFA. IR: ~3470 cm amide A band, ~1657 cm^{-1} amide I band, ~1558 cm^{-1} amide II band; ESI-MS: 1009.4, found: 1008.4 (M-H)-.

After peptide synthesis, peptide was well dissolved in ultra purified water to form 1.5 wt% peptide solution and subsequently filtrated for the sterilization. After placing at room temperature for 30 min, a well defined peptide hydrogel appeared based on the self-assembly of the peptide molecules.

Simultaneously, it is possible to design this kind of novel peptide containing a bioactive RGD sequence to specifically bind the fibroblasts due to the expression of high level of α1 integrin in proliferated fibroblasts [18]. Because hydrogels have been widely applied as intelligent carriers in controlled drug delivery systems [19-21], 5-FU could be integrated into this self-assembled peptide hydrogel and released upon enzyme-mediated hydrogel degradation [10].

Currently, filtration surgery has been recognized as a standard therapy for glaucoma, which involves generating a filtration fistula to allow the escape of aqueous humor to reduce the intraocular pressure (IOP). The success rate of glaucoma filtration surgery is limited by the postoperative scarring formation. The scarring formation is usually attributed to the proliferation of fibroblasts at the surgical site after glaucoma filtering surgery, leading to the scleral flap fibrosis and eventual filtration failure. In the case of evaluation of effect of this novel targeting drug delivery system in filtering surgery of rabbit eye, we chose the clinical observation and pathology analysis that could test the decrease amount of intraocular pressure, the duration of bleb, the present of filtration fistula and the proliferated status of fibroblasts in surgical site.

After administrating this self-assembled peptide hydrogel containing 5-FU in filtering surgery intraoperatively, the success rate of surgery will be improved by a localized and targeting delivery of a very small yet efficient amount of the antifibroblastic agent 5-FU, offering potential benefits to decrease the toxic side effects in patients who are at high risk of failed filtering surgery.

2. Conflicts of Interest Statement

The authors have no conflict of interests.

3. Acknowledgements

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