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Protein polymer hydrogels by *in situ*, rapid and reversible self-gelation

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ABSTRACT

Protein-based biomaterials are an important class of materials for applications in biotechnology and medicine. The exquisite control of their composition, stereochemistry, and chain length offers unique opportunities to engineer biofunctionality, biocompatibility, and biodegradability into these materials. Here, we report the synthesis of a thermally responsive peptide polymer-based hydrogel composed of a recombinant elastin-like polypeptide (ELP) that rapidly forms a reversibly cross-linked hydrogel by the formation of intermolecular disulfide cross-links. To do so, we designed and synthesized ELPs that incorporate periodic cysteine residues (cELPs), and show that cELPs are thermally responsive protein polymers that display rapid gelation under physiologically relevant, mild oxidative conditions. Gelation of cELPs, at concentrations as low as 2.5 wt%, occurs in ~2.5 min upon addition a low concentration of hydrogen peroxide (0.3 wt%). We show the utility of these hydrogels for the sustained release of a model protein *in vitro*, and demonstrate the ability of this injectable biomaterial to pervade tumors to maximize tumor coverage and retention time upon intratumoral injection. cELPs represent a new class of injectable reversibly cross-linked hydrogels with properties intermediate between ELP coacervates and chemically cross-linked ELP hydrogels that will find useful applications in drug delivery and tissue engineering.

1. Introduction

Injectable hydrogels formed by *in situ* chemical polymerization or by sol-gel phase transition [1–5] are of increasing interest for drug delivery because they have the attractive feature of only requiring an injection to form a depot *in vivo*, which avoids the need for surgical implantation that is required for prefabricated sustained release implants [6–8]. An injectable biomaterial for the formation of an *in situ* depot should meet the following requirements: (1) the material should be soluble upon administration; (2) it should start to gel within minutes upon *in vivo* injection; (3) the gel should be non-cytotoxic and (4) bioresorbable; and (5) the degradation products should be non-toxic. In addition to these material requirements, the system should be able to (6) entrap a high enough concentration of a drug of interest and exhibit release kinetics that can be optimized at the material design level for the application of interest, which is dictated by the drug and its intended therapeutic function.

Recombinant peptide polymers provide an attractive route for the design of such materials as they are non-toxic, biodegradable, and bioresorbable. We are interested in the design of *in situ* depots using a class of recombinant peptide polymers called elastin-like polypeptides (ELPs). ELPs, a class of artificial peptide polymers inspired by the amino acid sequence of tropoelastin, are composed of oligomeric repeats of the pentapeptide sequence Val-Pro-Gly-Xaa-Gly — where Xaa is any amino acid except Pro. ELPs are attractive as injectable biomaterials because they undergo a soluble to insoluble phase transition when heated above a tunable transition temperature (T_t; also called lower critical solution temperature (LCST)).

To date, two approaches have been taken to form ELP-based depots for drug delivery. In the first approach, ELPs are designed to undergo their inverse phase transition under physiological conditions, so that a solution of ELP, upon injection *in vivo*, forms a viscous insoluble “coacervate”. We have shown that an ELP coacervate is retained for up to a week *in vivo* [9,10] and can entrap and release drugs and entrap cells for regenerative medicine applications [11,12]. While this approach is attractive due to its simplicity, ELP coacervates are not chemically cross-linked, and hence have poor structural stability and mechanical properties, features that may be necessary for some applications.

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