Short Communication Journal of Biotechnology & Biomaterials 2020
Vol.10 No.6

Biomaterials 2019: Engineered Caf1 protein polymers form tuneable bioactive hydrogel scaffolds

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Here, we describe a new animal-free biomaterial with potential uses in 3D-tissue culture and regenerative medicine due to its low cost, high stability and definable bioactivity. Capsular antigen fraction1 (Caf1) is a protein from the plague bacterium Yersinia pestis that is secreted via the chaperone-usher pathway and protects the pathogen from phagocytosis by forming a non-stick protective layer around the cell. The 15.5kDa monomer has an Ig-like fold and resembles the extracellular matrix protein fibronectin. The subunits polymerise via donor-strand complementation, forming a highly stable non-covalent polymer. In this work, recombinant Caf1 polymers produced via batch fermentation using Escherichia coli were secreted by the bacterium into a flocculent layer above the cell pellet, and could be easily extracted and purified in large quantities. We demonstrate the polymers robust thermostability by circular dichroism and SDS-PAGE, and observe their large size using electron microscopy and SEC-MALS. Additionally, we have selectively reversed the natural non-stick behaviour of the WT polymer by introducing an integrin binding sequence, RGDS, into loop 5 that can promote U2OS cell adhesion. Additional bioactive peptides motifs from osteopontin, bone morphogenic protein2, collagen and laminin were then introduced at different positions within Caf1. Finally, PEG based chemical cross linkers were used to form stable 3D hydrogels with designed porosities and tuneable stiffness, ideal for use in cell culture and drug delivery applications. The combination of these new motifs into tuneable Caf1 hydrogels will help to expand the functionality of this exciting new biomaterial for use in a variety of biomedical applications.

The basis for Caf1's high thermal stability is the tight, non-covalent interaction between subunits, where small hydrophobic residues on the N-terminal β-strand of one subunit slot into pockets in the body of the next subunit. Mutation to larger hydrophobic residues reduced the thermostability of Caf1 periplasmic oligomers. Our objective was to create a Caf1 polymer with lower stability to allow us to differentiate it from the wild-type protein. We selected the A5I mutation as the most promising candidate from the previous study, as it caused a drop in Caf1's unusual thermostability without affecting its ability to oligomerise. Here, we show that the A5I mutation permits the production of high molecular weight (> 500 kDa) Caf1 polymers, but with a lower thermal stability. This mutation was of particular utility in this study, but the production of Caf1 polymers of reduced stability could be of further use, for example in enhancing the biodegradability of implanted cell scaffolds.

It had been observed that producing different Caf1 subunits on two separate plasmids results in the simultaneous production of the two types of Caf1 in polymeric form. It was not known whether these were mosaic heteropolymers, containing a random mixture of both types of subunit, or two separate types of polymers due to a possible sorting mechanism within the cell. Here, we have demonstrated that these subunits are assembled together in a random mosaic heteropolymer, as evidenced by the differential breakdown products at 70 °C between the Caf1A5I homopolymer, the Caf1A5I:His mosaic polymer and a mixture of the two separate Caf1A5I and Caf1WT:His polymers, as well as the observation of Caf1His:Cys mosaic polymers by transmission electron microscopy.

The yields of the mosaic polymers produced here were lower than those of the single subunit polymers (~ 22 mg/L vs. ~ 200 mg/L. This could be due to the inclusion of the Caf1His subunit, which could not be expressed on its own and may be more challenging for the cells to express and incorporate into polymers than the wild-type subunit. Further optimisation of the production process may help to improve these yields.

The dissociation constant (Kd) of the Caf1 subunit: subunit interaction has been estimated to be at least 10^{-14} M, which would place it amongst some of the tightest known interactions known. Moreover, the interaction between FimG and FimF (homologous pilin subunits from E. coli) has a half-life of 3×109 years, providing it with an "infinite stability against dissociation". Therefore, it is unlikely that the Caf1 subunits, once formed into a polymer, would be able to dissociate spontaneously, eliminating the possibility of subunit exchange within the polymer. Indeed, even the subunits on the ends of the polymer associate via the same donor strand complementation mechanism and so are likely to remain stably incorporated. Therefore, polymer growth can only take place through the addition of subunits to the growing ends of a polymer chain. The ability to produce, mosaic polymers with defined content increases the utility of Caf1 as a biomaterial.

This work is partly presented at 4th Annual Conference and Expo on Biomaterials, February 25-26, 2019 London, UK