Top-Down Synthesis of Versatile Polyaspartamide Linkers for Single-step Protein Conjugation to Materials

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Statement of Purpose: Materials used in various biological applications are often modified with proteins to regulate biomolecular and cellular adhesion. Conventional strategies of protein conjugation accompany monovalent bi-functional protein linkers, which present several limitations in molecular synthesis and protein conjugation. In this study, we present a new strategy of preparing multivalent polyaspartamide linkers in a simple top-down manner, and also demonstrate that the resulting polymer linkers allow us to readily conjugate proteins to both organic and inorganic materials.

The top-down synthesis of polyaspartamide linkers was performed by partially opening succinimidyl ring moieties of polysuccinimide (PSI) with the controlled number of nucleophiles reactive to photocross-linked hydrogel or gold-coated inorganic materials: (1) Poly(2-hydroxyethyl-co-2-methacryloxyethyl aspartamide) (PHMAA) presenting methacrylic functional groups was used to micro-pattern fibronectin on a hydrogel in order to regulate cell adhesion and growth area on a microscale. (2) Poly(2-hydroxyethyl-co-2-mercaptoethyl aspartamide) (PHMCA) presenting thiol functional groups was used to link fibronectin to a gold-coated silicon bio-microelectromechanical (bio-MEMS) probe designed to measure cell traction force.

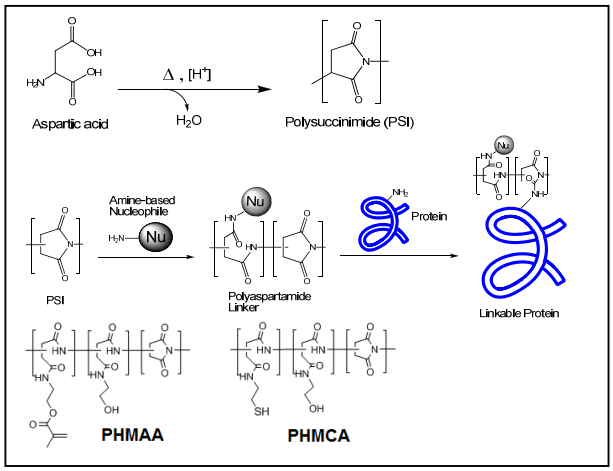

Figure 1. Synthesis of PSI and polyaspartamide linkers.

Methods: PSI was synthesized by acid-catalyzed polycondensation of aspartic acid (Fig. 1). PSI was then reacted with (1) amine-funcionalized methacrylate (2-aminoethyl methacrylate) to synthesize PHMAA or (2) amine-functionalized thiol (cysteamine) to synthesize PHMCA. Chemical structures of PSI, PHMAA and PHMCA were first characterized with NMR spectroscopy. These polyaspartamide linkers were conjugated to fibronectin (Fn), which was evaluated by trinitrobenzesulfonic acid (TNBS) assay which monitors the change in amine content. The amine content decreased over time due to the protein reaction with the linkers.

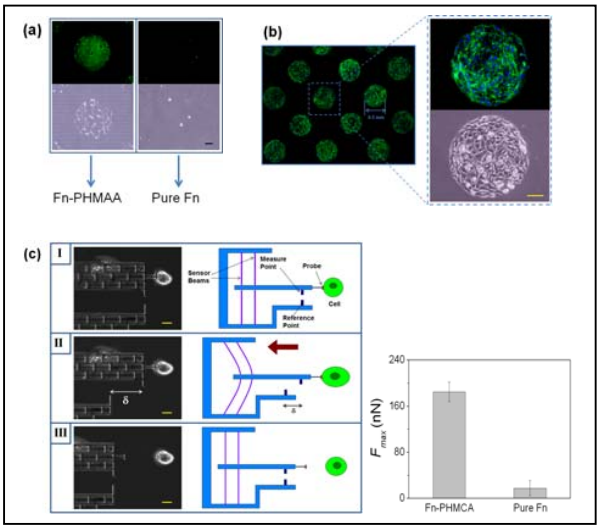

Figure 2. (a) Immunofluorescent labeling of Fn-PHMAA conjugated hydrogel. (b) Fibroblasts adhered to the Fn-conjugated area and proliferated over time. (c) Bio-MEMS device conjugated with Fn-PHMCA measured the traction force exerted by fibroblasts.

Results: First, polyacrylamide hydrogel was fabricated on a glass patterned with Fn-PHMAA via radical polymerization. Due to methacrylate groups on PHMAA, the Fn-PHMAA reacted with acrylamide monomers and conjugated to the hydrogel surface. Pure Fn, on the other hand, was not conjugated to the hydrogel. The presence of Fn on the surface was confirmed with immunofluorescent labeling (Fig. 2a). Fibroblasts were able to recognize the Fn and adhered to the Fn-patterned area, and proliferated over time, demonstrating that chemical linkage did not affect the protein activity (Fig. 2b).

Next, a gold-coated Bio-MEMS device was conjugated with Fn-PHMCA which contains thiol groups, since thiol is well known to react with gold (Fig. 2c). Fibroblasts recognized the Fn-conjugated probe and exerted a significant traction force on the device, whereas the cells did not exert force on the device coated with pure Fn, showing not enough Fn molecules were able to adsorb onto the device.

Conclusions: We demonstrate that polyaspartamide linkers developed in this study allows convenient and single-step conjugation of proteins to materials. The linkers also allow independent control of multivalencies of functional groups and proteins. Therefore, these polyaspartamide linkers present several attributes superior to conventional monovalent linkers. Furthermore, we expect the strategy of presenting various functional groups by single-step conjugation will make it possible to further utilize a wide array of conjugation chemistries necessary for tailor-made materials.

Reference: Cha C. Bioconjugate Chem. 2011; 22:2377.