QCM-D as an useful tool for the combined immobilization of cell adhesion peptide and growth factor on biomaterial surfaces.

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Statement of Purpose:

Polyethylene glycol (PEG) is an interesting material to create non-fouling surfaces [1] that enable immobilization of biomolecules such as peptides and growth factors to promote specific cell interactions. Our team has previously developed a new method for oriented tethering of growth factors using strong electrostatic interaction between two coils (K and E-coils), one fixed on the biomaterial surface and the second linked to the growth factor [2]. We now aim to study the possible synergy between coiled-coil EGF and an integrin binding peptide (KQAGDV) on PEG surfaces to enhance VSMC growth in pro-apoptotic conditions. Since KQAGDV is an adhesive peptide for VSMC and EGF is a signaling molecule in regulating VSMC survival and proliferation, there is a great potential for their cooperative binding on PEG surfaces to enhance VSMC behavior[3]. However, controlling the surface grafting densities for combined tethering is an important criterion to achieve the objective. Herein, we describe a real time follow-up method for the optimization of grafting densities using Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D).

Methods:

Primary amine-rich plasma coatings (called LP) deposited by low-pressure plasma [4] was used to introduce high concentration of primary amines (7.5%) on the surface. A star shaped 4-arm PEG-NHS was chosen in this study, with four long chains ended with N- hydroxy succinamide (NHS) functional groups that can easily react with primary amines. Gold plated sensor surfaces were coated with LP followed by covalent chemical coupling of 5% (w/v) star PEG [1], followed by grafting of a heterobifunctional linker using carbodiimide chemistry (EDC). This linker enables to graft cysteine tagged Kcoil and/or KQAGDV peptide via disulfide bonding. The sensor surfaces were inserted into the QCM-D system equipped with 4 parallel flow modules to follow the serial immobilization of peptide, Kcoil and Ecoil EGF *in situ*. For combined immobilization, KQAGDV peptide at various concentrations (12.6, 4.2, 1.4 and 0.46 µM) was allowed to immobilize first, and after reaching a stable baseline**,** Kcoil was injected (1µM). Unreacted groups on EMCH linker were blocked with cysteine. Kcoil and cysteine only grafted surfaces were used as positive and negative controls, respectively. For all experiments, stable PBS baseline was achieved prior to the injection of biomolecule solutions and rinsing steps were performed using PBS.

Results:

The best linker among PDPH and EMCH was chosen by evaluating the Kcoil grafting densities using QCM-D and EMCH showed better results than PDPH. The ability of the QCM-D to detect coil-coil interactions was confirmed by injecting Kcoil followed by Ecoil-EGF on EMCH grafted PEG surface: both the steps increased the frequency shift. Since the unreacted sites were blocked with cysteine prior to Ecoil EGF injection, it is obvious that the last increase in frequency is only due to the coil-coil interactions. QCM-D also allowed to observe the impact of KQAGDV peptide concentration on its grafting density on the surface: excepted for the 0.46µM concentration, the grafted mass was increased as peptide concentration increased. Subsequent Kcoil grafting varied depending on peptide concentration, and finally, as expected, Ecoil EGF immobilization was decreased as peptide concentration increases as shown in **figure1**.

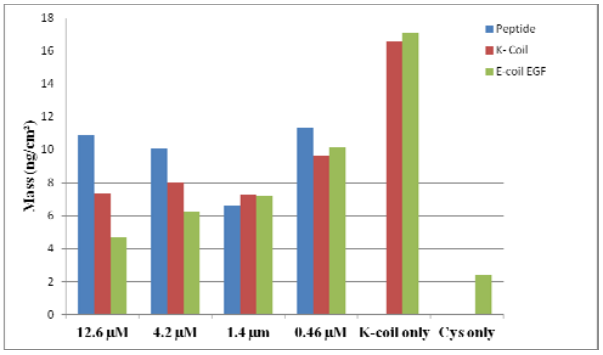

Fig.1 *Estimated mass of KQAGDV peptide, Kcoil and Ecoil EGF on the surface when using various concentrations of peptide during the initial grafting step. Kcoil only (without peptide injection) and Cys only (without peptide and kcoi linjection) are positive and negative controls, respectively. QCM-D data were analyzed using the Sauerbrey equation.*

Conclusions:

Our findings suggest that QCM-D is an interesting method to evaluate and optimize the surface grafting densities of small peptides like KQAGDV and coils, and it allows controlling the grafting densities for combined tethering. The synergistic effects of peptide and growth factor in modulating cell function is under evaluation.

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References:

1.Thalla *et al*. Journal of Biomedicine and Biotechnology. 2012.

2.Boucher C, Ruiz J-C. Biomaterials. 2010;31:7021-31.

3.Dong C-L, Li S-Y. Biomaterials. 2012;33:2593-9.

4.Ruiz J-C, St-Georges-Robillard A. Plasma Processes and Polymers. 2010;7:737-53.