Combined immobilization of KQAGDV peptide and EGF to modulate VMSC response: optimizing grafting densities using QCM-D

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Introduction:
Non-fouling surfaces such as PEG or dextran prevent nonspecific adsorption of proteins and cells. They can also be modified to promote specific cell growth when peptides and or growth factors are immobilized via functional groups. Our team has recently developed star PEG surfaces with low-fouling and non-thrombogenic properties [1] as well as a new method of oriented immobilization of growth factors through electrostatic interactions between K and Ecoils, where one coil is fixed on the biomaterial surface and other one linked to the growth factor [2]. Such oriented immobilization enables to avoid GF denaturation which leads to a loss of bioactivity. However, literature data suggest that GF immobilization on non-fouling surface may not be sufficient to promote initial good cell adhesion. On the other hand, integrin binding peptides have been shown to improve cell adhesion but decreases cell migration and proliferation[3]. We aim to study the possible synergy between coiled-coil EGF and an integrin binding peptide (KQAGDV) on PEG surfaces to enhance VSMC growth. 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 to favor VSMC coverage and growth on PEG modified surface [4]. We here report the use of Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) to control and optimize the surface grafting densities for combined tethering of peptide and growth factor.

Methods:
Primary amine-rich plasma coatings (called LP) deposited by low-pressure plasma [5] was used to introduce high concentration of primary amines (7.5%) on the surface. A star shaped 4-arm PEG-NHS with four long chains ended with N- hydroxy succinamide (NHS) functional groups was chosen in this study. Due to sterical constraints, one or two functional groups of star PEG react with the primary amines on LP surface and remaining are emanated outward surface and available for further immobilization of biomolecules. Gold plated sensor surfaces for QCM-D 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 K coil 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, K coil and E coil 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 K coil was injected. Cysteine solution was used to block unreacted groups on EMCH linker. Finally E coil EGF solution(160 nM) was injected to react with K coil through coiled-coil interactions. K coil 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 EMCH linker was chosen for sequential grafting based on superior Kcoil grafting densities compared with PDPH linker, as assessed by QCM-D. 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 steps lead to an increase in 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 showed significant changes in frequency shift when different concentrations of peptide, Kcoil and Ecoil-EGF were injected on EMCH-grafted PEG surfaces **(Fig1a)**. Increasing KQAGDV peptide concentration during grafting led to higher peptide grafted mass, excepted for 12.6 µM (as estimated from frequency shifts using the Sauerbray equation). Subsequent Kcoil grafting varied depending on peptide concentration and finally, as expected, Ecoil-EGF grafting density was decreased as peptide concentration increases as shown in **figure1b**.

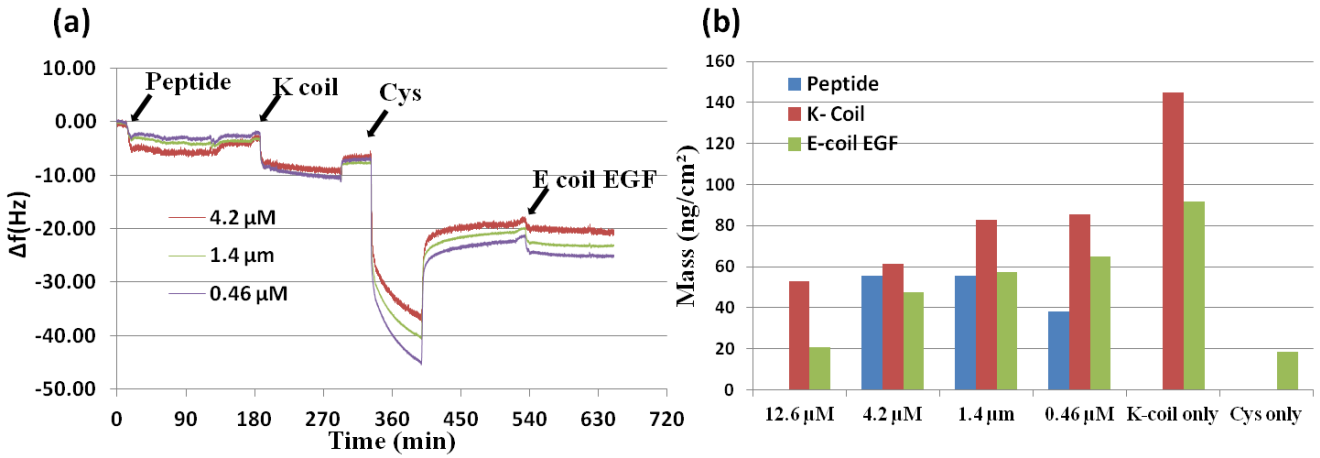

Fig.1 *Changes in frequency shift (a) and estimated mass (b) of KQAGDV peptide, K coil and E coil EGF on the surface when using various concentrations of peptide during the initial grafting step. K coil only (without peptide injection) and Cys only (without peptide and k coil injection) are positive and negative controls, respectively. QCM-D data were analyzed using the Sauerbrey equation.*

Conclusions:

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

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