Development of Combinatorial Peptide Screening to Identify Novel Nonfouling Sequences

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Statement of Purpose: Nonfouling properties are relevant to many biomedical applications including drug delivery, surface coatings, and biosensing. Natural materials such as peptides can offer several advantages as biomaterials: they are biocompatible, well-defined, and offer nearly infinite sequence combinations to explore. Recently, we have identified several low fouling peptide sequences including lysine/glutamic acid (KE) and asparagine (N) through rational and biomimetic design[1,2]. However, rational design only allows access to a limited number of sequences for testing. In order to probe for novel low fouling sequences we have developed a new combinatorial screening method that can test thousands of sequences simultaneously. Conventional combinatorial screening methods such as phage, yeast, bacterial, or resin display seek to identify targets with high specific binding. In this work, we want to identify low binding sequences and therefore traditional platforms cannot be used due to background interference. To overcome these limitations we developed a novel protein adsorption screening technique utilizing a controlled pore glass substrate coated with peptides[3]. A combinatorial library was created on this new substrate and screened for protein adsorption using fluorescently tagged fibrinogen. Low fouling sequences were identified and peptide sequences were recovered via partial Edman degradation.

Methods: Controlled pore glass substrates were modified with γ-glycidoxypropyltrimethoxysilane, followed by modification with tetraethyleneglycol diamine. Standard Fmoc-solid phase peptide synthesis was used to form an eight amino acid length library. Amino acids possessing hydrophilic, hydrophobic, cationic, and anionic properties were used to create the library including serine, threonine, asparagine, glycine, alanine, valine, leucine, phenylalanine, arginine, histidine, lysine, aspartic acid, and glutamic acid. Beads were deprotected after synthesis and screened with fluorescently labeled fibrinogen protein. After rinsing, beads with low binding were identified using confocal microscopy. Finally, peptide sequences were determined using partial Edman degradation and matrix-assisted laser desorption ionization time of flight mass spectroscopy (MALDI-TOF).

Results: Traditional polymer resins were identified as unsuitable for nonspecific screening due to background protein binding. A novel glass platform was synthesized to allow for effective formation and screening of sequences. A diverse library of beads was created with varying degrees of fouling (Figure 1a). Control peptide sequences possessing known low fouling and high fouling properties were synthesized and screened as controls to validate the proposed screening method (Figure 1b). The sequence KEKEKEKE-linker-bead was used as a low fouling control and the sequence FFFFFFFF-linker-bead was used as a high fouling control.

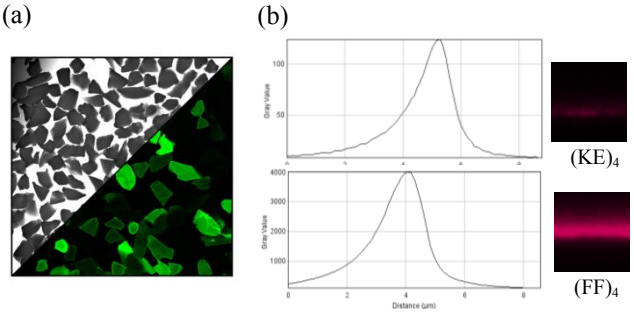

Fig. 1: (a) Bright field image of glass substrate used for combinatorial synthesis (top). Fluorescence screening of combinatorial peptide library to tagged fibrinogen (bottom). (b) Confocal microscopy analysis of fluorescence screening to known sequences KEKEKEKE (low fouling) and FFFFFFFF (high fouling). Intensity of fluorescence is analyzed via integration of the signal through the bead.

After platform validation, a combinatorial library of peptides was created and low fouling sequences were screened using fluorescent fibrinogen. Several sequences with lower fouling than the KE sequence were identified. Partial Edman degradation and MALDI-TOF were applied to recover peptide sequences.

Conclusions: A new combinatorial screening method was developed to screen for low binding target sequences. Several nonfouling sequences containing lower binding levels than previously known low fouling sequences were identified. Further screening of sequences can provide novel insights into characteristics needed to resist nonspecific protein adsorption.

References: (1) White A. D. *Chem. Sci.* **2012** *3*, 3488-3494 (2) Nowinski A. K. *J. Am. Chem. Soc.* **2012**, *134*, 6000-6005 (3) Keefe A. J. *Biomaterials* **2013**, *34*, 1871-1877