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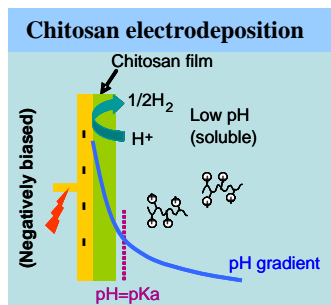
Abstract

This paper reports the signal-guided sequential assembly of labile nano-bio-components onto specific assembly sites inside a completely packaged microfluidic environment. We demonstrate (a) programmable assembly of biologically active green fluorescence protein (GFP) and (b) the sequential DNA hybridization on a chitosan scaffold at specific assembly sites in microchannels. The biomolecule assemblies in microfluidics are spatially and temporally controlled, which provide a flexible scaffold for biomolecular sensing, synthesis, and metabolic engineering applications.

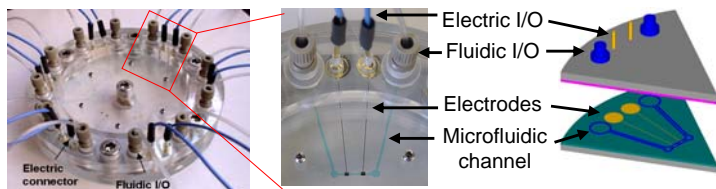
Method

Nano-bio-components assembly

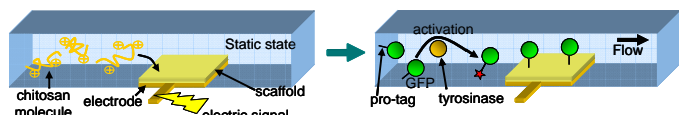
- Biofunctionalization in prefabricated microfluidic devices
- Spatially and temporally controlled chitosan electrodeposition
- Sequential assembly of nano-bio-components onto chitosan scaffold



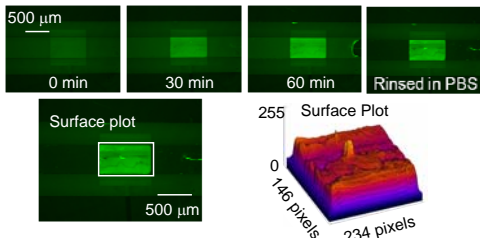
Prefabricated microfluidic device



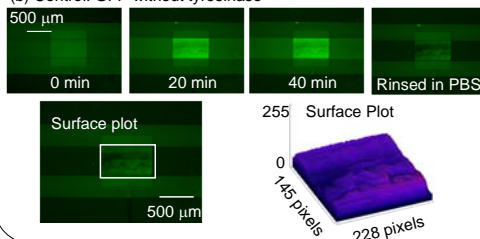
Programmable Protein Assembly



(a) Experiment: GFP with tyrosinase



(b) Control: GFP without tyrosinase



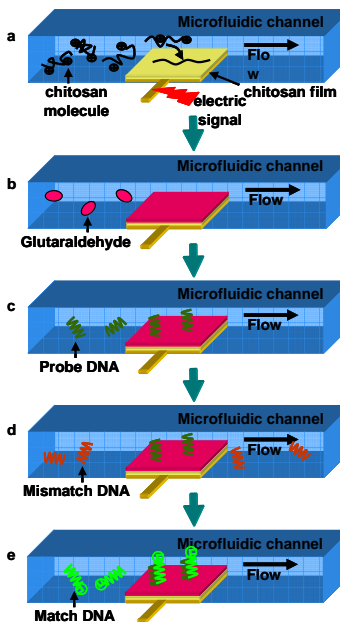
Experimental process

- Electric signal guides chitosan assembly (spatial and temporal).
- Tyrosinase activates the pro-tag on GFP for covalent binding onto chitosan.

Results:

- Experiment: GFP covalently bonds to spatially selective chitosan scaffold, thus withstands PBS rinsing.
- Control: GFP is non-specifically associates with chitosan, thus is easily rinsed away.

Sequential DNA Hybridization



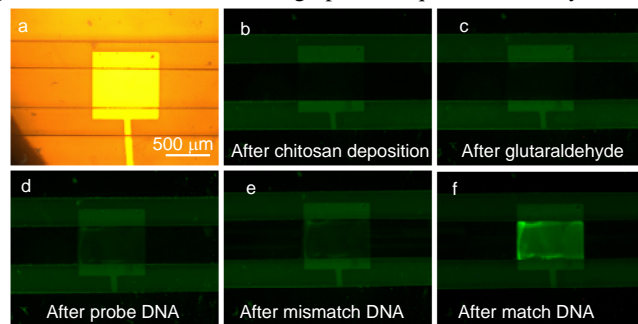
Experimental process:

- First, chitosan solution was introduced into a microchannel and electrodeposited on a selected electrode.
- Second, glutaraldehyde was introduced to activate the electrodeposited chitosan.
- Next, probe single string DNA (ssDNA) was introduced to be assembled onto the scaffold.
- Then a mismatched ssDNA is introduced into the microchannel
- Finally, a matched target ssDNA is introduced to hybridize onto the probe ssDNA.

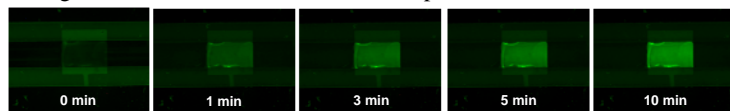
Results:

- Matched ssDNA complementarily hybridized to probe ssDNA on chitosan scaffold
- The hybridization saturated within 15 minutes

Bright field and fluorescent micrographs of sequential DNA hybridization



During introduction of match ssDNA over probe ssDNA on chitosan



Conclusions:

- Simple, robust and versatile biofunctionalization strategy
- Sequential assembly of labile proteins and DNA in microfluidic devices
- Spatial and temporal programmability of biomolecule assembly

Applications:

- The signal-guided programmability is attractive for multi-site/multi-step bioreactions in metabolic engineering and other bioMEMS applications.

Acknowledgements

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