

Peptide-Templated Deposition of Gold and CaMoO₄ Nanoparticles for Biosensor Applications

The use of peptide catalysts for the deposition of metallic and ceramic nanoparticles in templated patterns has the potential to produce sensor materials for both the conductors and sensor portions of devices deposited under benign conditions with lower chemical consumption. The catalytic activity of the peptides depends on acidity/basicity, sequence, peptide secondary structure, net charge, and type of amino acids [3]. Using peptides has several advantages over other deposition techniques, such as evaporation, sputtering and chemical synthesis of nanoparticles from solution. The technique of pre-patterning peptides for the templated catalytic deposition of materials is an inexpensive, affordable and cleanroom-free patterning process.

The selection of peptides for templated deposition is based on phage display technology to identify and isolate peptides with potential catalytic activity. Briefly, a library of bacteriophage viruses is exposed to the ceramic compound or metal powder you want to synthesize. The phage particles with higher affinity to the powder will bind to the powder. Unbound or loosely bound phage particles will be washed away. After elution of phage particles, *E. coli* bacteria are transfected with the eluted phage particles. Finally, DNA obtained from the *e-coli* cells is sequenced, and the peptide sequence is found from the DNA sequence [3]. Synthesizing of gold and calcium molybdate particles in solution has been done previously [1,2]. The morphology of the particles depends on the peptide sequence. In this work we deposited and patterned gold and calcium molybdate particles for potential use in biosensors. Functionalized gold nanoparticles can be used to detect different biomolecules using the enhanced Raman spectrum of the nanoparticles in different media. Calcium molybdate is a photoluminescence material, and its luminescence spectrum occurs in green light range. We are exploring the possibility that the spectrum shifts or photoluminescence quenching may occur upon binding of different biomolecules for sensor applications.

Patterning of gold nanoparticles

For this purpose we used bovine serum albumin (BSA) as our catalytic protein to deposit gold nanoparticles. A micro-transfer molding process was developed to produce micro-patterns of the peptides on a variety of substrates without the need for photolithography to define each pattern. Silicon wafers were patterned through a conventional photolithography process. A polydimethylsiloxane (PDMS) mold was made from the photolithographically patterned silicon wafers. The micro-transfer molding process used these PDMS molds for the pattern definition. Briefly, PPMA was spin coated on the PDMS mold and stamped on a thermally grown silicon dioxide substrate at 90 °C. BSA was immobilized on the surface physically and covalently, and the PPMA patterns were lifted off with acetone. For covalent bonding, the substrate was exposed to oxygen plasma for one minute to introduce -OH groups on the

surface and make the surface hydrophilic. Then mercaptopropyltriethoxysilane (MPTES) was deposited on the surface through a vapor deposition method. A bifunctional linker molecule (SMCC) with 2 functional groups, maleimide and succinimide, was bound to the silane-coated substrate through the maleimide end. The modified substrates, with the silane and the linker, were soaked in the protein solution. The amino terminus of the protein was reacted with the succinimide end of the linker to make a peptide bond. Finally, an aqueous solution of tetrachloroaurate was applied on the substrates. The protein acted as a reduction catalyst and the gold nanoparticles were deposited on the protein patterns. The results from the physically adsorbed and covalently bound protein indicate that the selectivity and density of nanoparticles are higher in the case of covalently bound peptides (Fig.1)

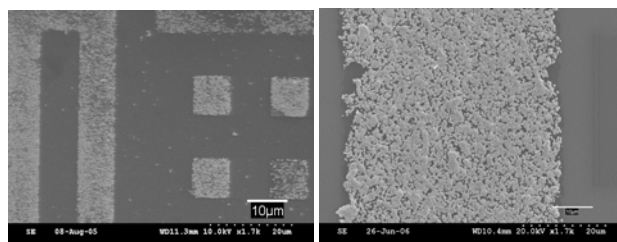


Figure 1. SEM image of gold nanoparticles on physically adsorbed (left) and covalently bound (right) BSA

We assume that in the physical adsorption, the proteins may be washed off during lift-off and spread on the surface. To confirm the conductivity of the patterns, Electron Beam Induced Current (EBIC) was used. The electron path is illuminated in EBIC, which is indicative of the conductivity of the pattern (Fig. 2).

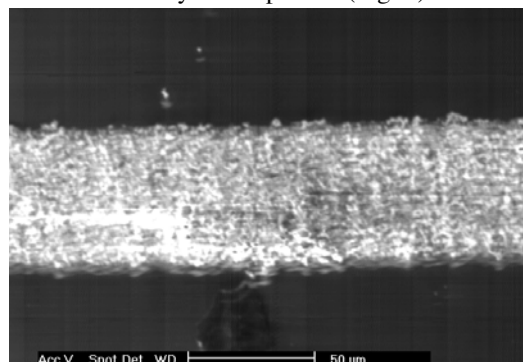


Figure 2. EBIC image of gold nanoparticles patterned from covalently bound BSA

Patterning of calcium molybdate particles

Calcium molybdate particles were patterned using a peptide with a YESIRIGVAPSQ sequence. A heterobifunctional PEG molecule (COOH-PEG-triethoxysilane) was used to keep the peptide off the surface and to prevent it from losing its bioactivity via interaction with the surface. First, thermally grown silicon dioxide wafers were patterned with photoresist via photolithography. The substrates were

modified with the heterobifunctional PEG. The substrate was exposed to oxygen plasma for one minute to make the surface hydrophilic. The PEG was then dissolved in toluene and the samples were soaked in the PEG/toluene solution for one hour. Then the samples were rinsed with toluene to remove unbound PEG molecules. SEM images prove binding of the PEG on the substrates (Fig. 3). The polymeric pattern was lifted off with acetone after patterning COOH-PEG-silane.

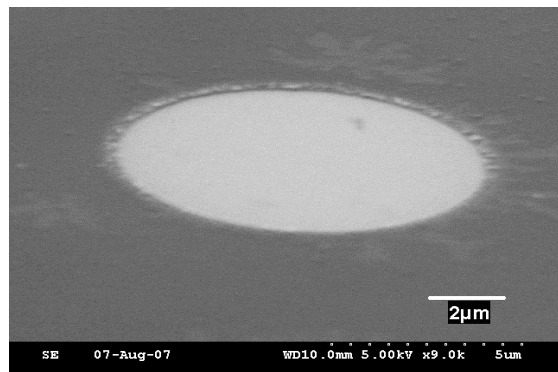


Figure 3. SEM image of COOH-PEG-silane on thermally grown silicon dioxide

An EDC/NHS solution in MES buffer at pH 6 was used to activate the COOH end of the bound PEG molecules on the surface. Then the samples were soaked into 5 mg/ml of the peptide solution in PBS buffer for 2 hours. The amino terminus of the peptide makes a peptide bond with the activated COOH group on the PEG, and therefore it binds covalently on the substrates. Figure 4 shows the peptide attachment on the surface.

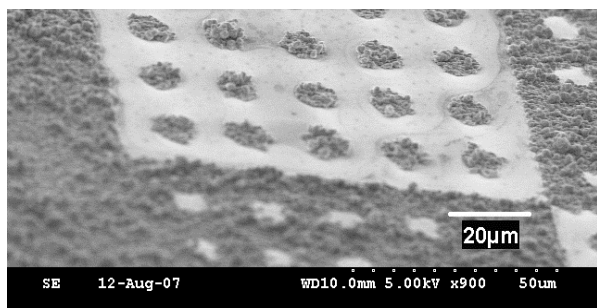
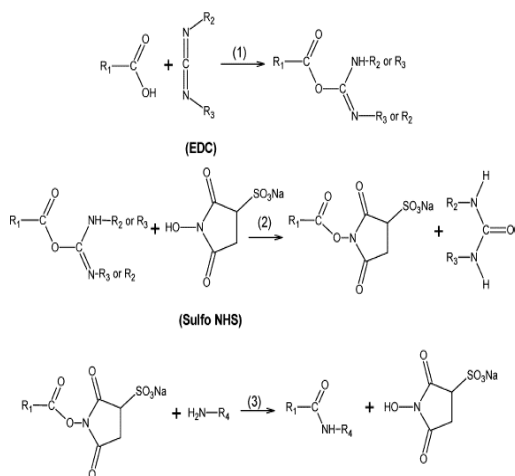


Figure 5. SEM image of deposited calcium molybdate particles on thermally grown silicon dioxide

In future work, the photoluminescence property of the particles will be tested, and focused ion beam (FIB) milling will be used to confirm the crystallinity and nucleation point of the particles. Additionally, the biosensing properties of these patterned particles will be tested.

References

1. Gul Ahmad et al, *Advanced Mat.*, 2006, 18, 1759
2. Slocik, J. M.; Stone, M. O.; Naik, R. R. *Small* **2005**, 1, 1048.
2. Richert et al., *Biomacromolecules*, Vol. 5, No. 2, 2005

Publications

3. H Borteh, N Ferrell, R Butler, and DJ Hansford, "Electroless deposition of gold nanoparticles over silicon-based substrates," *MRS Spring Meeting 2007*, **B8.17**, San Francisco, CA, 2007
4. Hassan M Borteh, Nicholas Ferrell, Randall Butler, Susan Olesik, and Derek J Hansford, Peptide-induced Deposition of Gold Nanoparticles over Silicon-based Substrates (Submitted)