Whole Cell Imprinting in Sol-gel Materials (Macromolecular Fingerprinting) for Rapid Bacterial Recognition in Water Samples via QCM Detection

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The sol-gel process is a room-temperature technique for synthesizing porous, glass-like materials and ceramics. The process accuracy at a relatively low temperature allows the doping of various inorganic, organic, and biomolecules during the formation of a glassy matrix. Entrapment of biomolecules within a sol-gel derived matrix had led to important utilization of such materials in biosensing devices for the rapid detection of investigated analytes.

Yoon, B et al. 2008
Carta, D et al. 2005
The attractiveness of sol-gel derived materials can be attributed to:

- Enhanced chemical, mechanical and thermal stability
- Optical transparency
- Tailored porosity
- Entrapment ability of sensing elements (i.e., from enzymes to whole cells)
- Ability to appear as thin film on substratum surface

In many cases, the entrapped biomolecules remain in their functional state over longer periods compared to their free forms.
The sol-gel reaction (1)

- The sol-gel process involves system transition from a liquid “sol” (mostly colloidal) into a solid “gel” phase.
- The starting materials used in the preparation of sol are usually metal alkoxides \([M(OR)_n]\), where M is a forming element: Si, Ti, Zr, Al, B; and R is an alkyl group.
- The most commonly used precursors in sol-gel process are organosilicates:

  - TMOS- Tetramethyl orthosilicate
  - TEOS- Tetraethyl orthosilicate
  - MTMS- Methyl Tetramethyl orthosilicate
The sol-gel reaction (2)

The sol-gel process occurs in a few-stage reaction:

1. Acid or base-catalyzed hydrolysis leads to the formation of silanol groups
   \[
   \equiv Si-OR + H_2O \xrightarrow{\text{Hydrolysis}} HO-Si\equiv + ROH \quad (i)
   \]

2. Condensation reactions produce siloxane bonds, resulting in the production of alcohol and water as by-products
   \[
   \equiv Si-OR + HO-Si\equiv \xrightarrow{\text{Alcohol condensation}} \equiv Si-O-Si\equiv + ROH \quad (ii)
   \]
   \[
   \equiv Si-OH + HO-Si\equiv \xrightarrow{\text{Water condensation}} \equiv Si-O-Si\equiv + HOH \quad (iii)
   \]

3. Through overall reaction of polycondensation, silanols react with siloxanes to form a rigid, porous network
   \[
   \left[\equiv Si-O-Si\equiv\right]_n \xrightarrow{\text{Polymerization}} \text{Silica polymeric matrix} \quad (iv)
   \]

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Chemical reactions in sol-gel synthesis (Gupta and Kumar, 2008)
Biomolecule immobilization into sol-gel matrix

- The biomolecule is usually added to the sol during the polymerization process.
- The sol continues to polymerize and becomes more viscous; and eventually gels, entrapping the biomolecule within its pores.

http://biomaterials.kaist.ac.kr
Molecularly Imprinted Sol-gel Materials

- The underlying principal of molecular imprinting is the assembly of a cross-linked polymer matrix around a template, which upon removal, yields micro or nano-cavities with a specific size, shape, and chemical functionality. The resulted imprinted film cavities can then “remember” (i.e., selectively bind) the target molecule.

- The molecular imprinting technique can be applied to different kinds of target molecules (from small organic molecules to even whole cells).

- Sol-gel process has become an attractive field for molecular imprinting method due to its ability to form thin films and vary in matrix porosity.
Detection of biomolecules (enzymes, cells, bacteria, and viruses) is becoming an increasingly important task in a variety of fields like food technology, medicine, health care, water supply.

Although molecular-imprinting technique is widely developed for imprinting of organic molecules, this approach has not been adequately investigated for biomaterial applications.

Dickert and Hayden (2001) presented the combination of mass sensitive transducer with a surface-imprinting technique (using polyurethane composition) for selective detection of different yeast genera.

Harvey (2006) used affinity augmented beads imprinted with *Bacillus thuringiensis* and *Bacillus anthracis* as a semi-selective matrix to capture their specific spores.

*Cryptosporidium parvum*

*Sphaerotilus natans*

*Bacillus subtilis*
Quartz Crystal Microbalance (QCM)- based sensors

- Quartz Crystal Microbalance (QCM) is an electro-acoustic method suitable for mass analysis of adsorbed layers at the solid/water or solid/air interface

- QCM enables to measure the mass of thin films adhered to its surface in ng/cm²

- QCM immunosensor has been used for bacterial detection of: *Vibrio harveyi, Bacillus anthracis, Schistosoma japonicum*, and influenza virus

- Immobilized lectin QCM sensor was used for the recognition and discrimination of *Campylobacter jejuni* strains

- QCM provides one of the most promising sensor technology based on its low cost, rapid response, portability, nonhazardous label-free real-time procedure and high sensitivity, which is ideal for the sensitive online detection of analytes

http://www.q-sense.com/qcm-d-technology
The main goal of the present proposed study is to translate these preliminary findings to a more advanced phase involving QCM (quartz crystal microbalance) probes imprinted with pathogenic bacteria in order to detect in a real time fashion (through a flow system), primarily bacterial pathogens present in water and possibly other liquids.
Specific Objectives:

- Selection of the appropriate sol-gel matrix allowing easy template extraction and efficient integration in QCM sensing device
- Differentiating between various bacterial species based on morphological properties by QCM method application
- Impact test of change in: pH, ionic strength, temperature, etc., on QCM sensor sensitivity for bacterial recognition
- Determination of minimal initial bacterial concentration that can be detected by the system
- Evaluation of templates’ density in sol-gel imprinted film and their effect on QCM sensing
- Study of biological traces’ composition left by various bacterial species in the cavities throughout the MI in sol-gel process
Work Plan and Methods
Preparation of sol-gel thin films

Organic precursor (TEOS/MTMS) mixed with various amount of water/acid ratio

Overnight "Aging" at 4°C

Sol stock solution

Twice washed bacterial solution (0.85 % NaCl + 0.2 % Tween 80)

1:6

Mixed solution is immobilized by spreading on the clean substratum (glass slide or quartz crystal resonator)

Elution of immobilized bacteria

Overnight desiccation in room temperature

Bacterial Re-adsorption

250µl
# Model Bacteria

<table>
<thead>
<tr>
<th><strong>E. coli</strong></th>
<th><strong>Flavobacterium breve</strong></th>
<th><strong>Deinococcus radiodurans</strong></th>
<th><strong>Staphylococcus epidermidis</strong></th>
<th><strong>Pseudomonas aeruginosa</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram –</td>
<td>Gram –</td>
<td>Gram +</td>
<td>Gram +</td>
<td>Gram –</td>
</tr>
<tr>
<td>Indicator of fecal pollution and water contamination</td>
<td>Widely distributed in soil and freshwater habitats</td>
<td>Extremely resistant and it is often found in habitats rich in organic materials, such as soil, feces, meat, or sewage</td>
<td>Typically lives on the human skin and mucosa; responsible for causing the most common infections on catheters and implants</td>
<td>Strong tendency toward formation of biofilms on biological and engineered surfaces</td>
</tr>
</tbody>
</table>
Quartz Crystal Microbalance (QCM)

• Assessment of bacterial attachment is performed using QCM-922 (Princeton Applied Research) equipped with flow cell (092-QCA-FC)

• Attached bacteria on the QCM probes are detected through the change in frequency of a quartz crystal resonator

• Negative frequency shifts have been correlated to the mass of adsorbed bacteria
Microscopy

- CLSM (Leica TCS SP2) microscopy was used both for fluorescent stain experiments and bright-field scanning re-adsorption kinetics study.
- HR-SEM (HREM-DIMA) microscopy was used for template removal and specific re-adsorption visualization.

HR-Scanning electron microscope and CLSM images of *Deinococcus radiodurans*: (1) Eluted bacteria and its molecular fingerprints (cavities); (2) Re-adsorbed (imprinted) bacteria after exposure to bacterial
Results
## Results (1)

<table>
<thead>
<tr>
<th>Sol-gel composition</th>
<th>Imprinting procedure quality</th>
<th>Contact angle (°)</th>
<th>Porosity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 ml:2 ml: 0.5 ml (0.1M) TEOS: H₂O: HCl</td>
<td>Complete elution of bacteria</td>
<td>38 ± 1.35</td>
<td>Max</td>
</tr>
<tr>
<td>4 ml:2 ml: 0.5 ml (0.01M) TEOS: H₂O: HCl</td>
<td>Sol-gel coating is cracked, no bacterial elution</td>
<td>41.9 ± 2.96</td>
<td></td>
</tr>
<tr>
<td>4 ml:1ml: 1 ml (0. 1M) TEOS: H₂O: HCl</td>
<td>Complex branching polymer</td>
<td>40.4 ± 4.25</td>
<td></td>
</tr>
<tr>
<td>4 ml:1 ml: 1 ml (0. 01M) TEOS: H₂O: HCl</td>
<td>Complex branching polymer</td>
<td>43.2 ± 2.96</td>
<td></td>
</tr>
<tr>
<td>4 ml:2 ml: 0.5 ml (0. 01M) MTMS: H₂O: HCl</td>
<td>No elution of bacteria</td>
<td>85.1 ± 2.68</td>
<td></td>
</tr>
<tr>
<td>4 ml:2 ml: 0.5 ml (0. 1M) MTMS: H₂O: HCl</td>
<td>Partial elution of bacteria</td>
<td>93.4 ± 3.25</td>
<td>Min</td>
</tr>
</tbody>
</table>
Results (2)

Deinococcus radiodurans

Control sol-gel film

Eluted bacteria and its molecular fingerprints

Re-adsorbed (imprinted) bacteria after bacterial exposure

EDS
**Results (3)**

*Staphylococcus epidermidis*

Control sol-gel film

Eluted bacteria and its molecular fingerprints

Re-adsorbed (imprinted) bacteria after bacterial exposure

**EDS**
Results (3)

*Flavobacterium breve*

Control sol-gel film

Eluted bacteria and its molecular fingerprints

Re-adsorbed (imprinted) bacteria after bacterial exposure

EDS
**Results (4)**

*E. coli CN*$_{13}$

Control sol-gel film

Eluted bacteria and its molecular fingerprints

Re-adsorbed (imprinted) bacteria after bacterial exposure

**EDS**
Results (5)

*Pseudomonas aeruginosa*

- Control sol-gel film
- Eluted bacteria and its molecular fingerprints
- Re-adsorbed (imprinted) bacteria after bacterial exposure

**EDS**
Results (6)

- **Staphylococcus epidermidis**
  - Fluorescence vs. Wavelength (nm)
  - Synchronous Fluorescence vs. Wavelength (nm)
  - Absorption vs. Wavelength (Cm)

- Footsteps, Control, Imprinted

- Polyethylene IR card
Residual bacterial surface components left in SG films were further electrophoretically analyzed for protein/glycoproteins presence against non-imprinted SG film and *D. radiodurans* whole cell lysate. The imprinted SG films revealed protein bands corresponding to whole cell lysate bands, but at lower intensity. As expected, no protein bands were obtained from non-imprinted SG films.
Results (8)

Re-adsorption Modeling
*Staphylococcus epidermidis*

After pretreatment with enzyme and bacterial re-adsorption after it

Proteinase K
Secondary elution and re-adsorption of St. epidermidis
Results (10)

Immunofluorescence Selectivity

(A) bacteria in the film, (B) imprinted film, (C) imprinted film after re-adsorption with *E. coli* sp., (D) imprinted film after re-adsorption with *S. typhimurium* and (E) after re-adsorption of mixed suspension of both bacteria types.
SEM Selectivity

*E. coli* CN₁₃ mixed with *Staphylococcus epidermidis*
Results (12)

Imprinted and non-imprinted quartz crystals using E.coli CN_{13} 10^3 CFU/ml

![Graph showing frequency changes over time with different conditions: DDW, NaCl, NaCl+, bacteria, NaCl. The graph includes four different lines representing Clean Manufactured Quartz Crystal Sensor, Non-Imprinted Quartz Sol-Gel Sensor, and Imprinted Quartz Sol-Gel Sensor. There is also an inset image showing a greenish texture.](image-url)
Results (13)

Real-Time Experiment through the Flow System
How amoebas see us!
Vă mulțumim pentru atenție