In vitro biocompatibility of deposited chitosan films as a platform for living cells in BioMEMS systems

ITC-irst, Italy

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Park J.J. et al. AS+NS+PS+SE+TF+TuA3
Nano-Bio and Self-Assembly II
Outline

- Motivation
- Physico-chemical material properties
- Cell adhesive properties and surface morphology of electrodeposited or air-dried chitosan films
- Surface chemical composition of deposited films
- Conclusions
- Future work
Motivation

- Naturally occurring chitosan is an amine-rich polysaccharide derived by deacetylation of chitin
- Chitosan represent an increasingly important biopolymer for its unique physico-chemical properties
- Electrodeposition of chitosan and conjugation of nucleic acids and proteins established in joint work aimed at bioMEMS applications
- Cell attachment and growth would be highly attractive as part of the portfolio of capabilities we could exploit in bioMEMS
Electrodeposited chitosan films as a platform for living cells in BioMEMS systems

Chitosan is a linear polysaccharide of $\beta-(1\rightarrow4)$-linked D-glucosamine and N-acetyl-D-gluocosamine.

Deposition of chitosan on gold cathode in response to an applied voltage.

pH dependent electrostatic behavior
pH dependent solubility
Spatial selectively

DC Power Supply

Optical Image
Fluorescence Image
Magnification 20×
Physico-chemical characteristics of the utilized chitosan samples

- Chitosan available from a number of suppliers in various grades of purity, molecular weight and degree of deacetylation (DD: proportion of D-glucosamine units with respect to the total number of units)

<table>
<thead>
<tr>
<th>DD</th>
<th>CHIT A (Sigma)</th>
<th>CHIT B (Aldrich)</th>
<th>CHIT C (Novamatrix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company spec.</td>
<td>&gt;80%</td>
<td>75-85%</td>
<td>86%</td>
</tr>
<tr>
<td>XPS</td>
<td>64%</td>
<td>62%</td>
<td>79%</td>
</tr>
<tr>
<td>FTIR</td>
<td>49%</td>
<td>63%</td>
<td>43%</td>
</tr>
<tr>
<td>Chitosan titration</td>
<td>52%</td>
<td>49%</td>
<td>75%</td>
</tr>
</tbody>
</table>

- DD is a structural parameter which influences physicochemical and biological properties
- DD values of chitosan appeared to be highly associated with the analytical methods employed
Cell adhesion and proliferation tests

- Human osteoblasts (MG-63), mouse fibroblasts (NIH-3T3) and mouse monocytes (RAW 264.3) were seeded at the density of $4 \times 10^4$ and $1.5 \times 10^4$ cells/cm$^2$ respectively in cell culture medium supplemented with 10% of FBS.

- Incubation at 37°C in air containing 5% CO$_2$ for 24 hours (adhesion) and up to 10 days (proliferation).

- Cell attachment, spreading and proliferation were analyzed by optical microscopic observations.

Surface morphological analysis by AFM on neutralized chitosan samples

Controls on cell culture polystyrene
Human osteoblast (MG-63) adhesion on electrodeposited chitosan film. CHIT B, fixed voltage 2V for 2 min.

Reflected light bright field

AFM analysis (2x2 \( \mu \text{m}^2 \) scans)

\[
\begin{array}{c}
\text{Roughness= 9.8nm} \\
\text{Roughness= 8.0nm}
\end{array}
\]
Human osteoblasts (MG-63) adhesion on electrodeposited chitosan film. CHIT B, fixed current 0.3mA for 2 min.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Adhesion</th>
<th>Roughness</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH 1M</td>
<td></td>
<td>5.9 nm</td>
</tr>
<tr>
<td>NH₄OH 0.47 M</td>
<td></td>
<td>3.6 nm</td>
</tr>
</tbody>
</table>

AFM analysis (2x2 μm² scans)

Roughness= 3.6 nm

Roughness= 5.9 nm
Human osteoblast (MG-63) adhesion on electrodeposited chitosan film. CHIT B, fixed current 0.3mA for 5 min.

Reflected light bright field

AFM analysis (2x2 μm² scans)

NaOH 1M

NH₄OH 0.47 M

Roughness = 32 nm

Roughness = 30 nm

100 μm

200 μm
Surface chemical analysis by Tof-SIMS on electrodeposited chitosan films.

Principal component analysis (PCA)

The surface chemical composition varies changing the deposition methods

- **fixed current, 2 minutes**
- **fixed current, 5 minutes**
- **fixed voltage**

- CHIT A
- CHIT B
CELL ADHESIVE CHITOSAN PROPERTIES
ELECTRODEPOSITED CHITOSAN FILMS

Adhesion on chitosan

MG-63

NIH-3T3 : no

RAW 264.3

Cell adhesion is strongly dependent on the cell type

NaOH 1M

NH₄ OH 0.47 M

CELL ADHESIVE CHITOSAN PROPERTIES
ELECTRODEPOSITED CHITOSAN FILMS

Adhesion on chitosan

MG-63

NIH-3T3 : no

RAW 264.3

Cell adhesion is strongly dependent on the cell type

NaOH 1M

NH₄ OH 0.47 M
The extent of osteoblasts cell adhesion is fairly low on electrodeposited films made of CHIT A and B with not significant differences between them.

The obtained results show that several factors have influence on cell adhesion besides the DD value.

The utilized neutralization reactions (NaOH 1M, NH₄OH 0.47M) result in a different cell surface affinity on both fixed voltage and current films.

A clear change of the cell adhesive behavior it's observed moving from fixed voltage to fixed current deposited films.

The fixed voltage samples (neutralized with NH₄OH) present a slightly better cell adhesion that probably could be correlated with the surface chemical composition.

Osteoblast cell adhesion doesn't depend on the surface morphology as observed with the AFM analysis.

The utilized deposition methods give chitosan films with different superficial chemical composition.

To clarify the influence of chemical and morphological properties of deposited chitosan films on cell adhesion, further data were collected using air-dried chitosan films.
Human osteoblast (MG-63) adhesion on air-dried chitosan films. NH$_4$OH 0.47 M neutralization

CHIT A  
Roughness= 7.3 nm

CHIT B  
Roughness= 3.1 nm

CHIT C  
Roughness= 19 nm

AFM analysis (2x2 $\mu$m$^2$ scans)
Surface chemical analysis by ToF-SIMS on air-dried chitosan films.

Principal component analysis (PCA)

The surface chemistry of CHIT C air-dried films is characterized for the presence of CHO fragments meanwhile the CHIT A and B show also nitrogen containing fragments as expected.
CELL ADHESIVE CHITOSAN PROPERTIES
AIR-DRIED CHITOSAN FILMS

### Proliferation

<table>
<thead>
<tr>
<th></th>
<th>CHIT A</th>
<th>CHIT B</th>
<th>CHIT C</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human osteoblasts</td>
<td>detached colonies</td>
<td>detached colonies</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>(MG-63)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse fibroblasts</td>
<td>detached colonies</td>
<td>detached colonies</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>(NIH-3T3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse monocytes</td>
<td>yes</td>
<td>Not determined</td>
<td>Not determined</td>
<td>yes</td>
</tr>
<tr>
<td>(RAW 264.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CONCLUSIONS

- Chitosan starting material is not well defined: the DD values vary considerably with the analytical methods employed
- Cell adhesion observed to limited extent on deposited films (air-dried better than electrodeposited)
- Several factors affect the chitosan cell adhesive properties:
  - the tested cell type
  - sources (purity)
  - amine site density (DD value)
  - surface morphology (fibers vs. globulars)
  - surface chemical composition
- These results suggest that chitosan with different surface properties can be deposited, modulating the in vitro level of cell attachment and spreading
• Covalent immobilisation of biomolecules (i.e. adhesive peptides) to improve the chitosan cell adhesion properties
• Definition of the correlation between the deposition method and the film nanostructures
• Analysis of the adsorbed protein layer mediating the cell adhesion
• Cell growth on patterned surfaces

Realisation of a cell-adhesive substrate

A two step chemical reaction procedure was developed in order to attach a RGD adhesion factors to an activated surface.