Nanodiamond as promising material for bone tissue engineering

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Nanodiamond is a perspective material for advanced biomedical applications, e.g. controlled drug and gene delivery, novel imaging techniques, microchips, nanorobots or biosensors.

Their mechanical resistance also makes them suitable for coating of implantable materials for bone tissue surgery.

Therefore, in this study, we investigated the adhesion, growth and differentiation of human osteoblast-like MG 63 cells in cultures on nanocrystalline diamond (NCD) films.
Material and Methods I

- Deposited onto silicon substrates (diameter 1.6 cm) by a micro-wave CVD method in the ellipsoidal cavity reactor at the Institute of Physics, Acad. Sci CR.
- Dr. Alexander Kromka
- Samples treated for 5 min. in oxygen plasma
- Water drop contact angle about 30°
- Layers were stable in strong acids (HNO₃) at 200°C, mechanically resistant
Microwave Plasma CVD Process

AIXTRON - P6
- ellipsoidal reactor
- microwave frq. 2.45 GHz
- maximum power 6 kW
- substrate size 2” or 3”
- rotating substrate stage
- stable plasma at high pressure
- fully PC controlled

(Inst. of Physics, Acad. Sci. CR, Dr. A. Kromka)

Process parameters:
- Pressure: 30 mbar
- Gas mixture: $\text{H}_2$, $\text{CH}_4$; 1% $\text{CH}_4$ in $\text{H}_2$
- Substrate temperature: 860°C
- Crystal size: < 100 nm (nanocrystalline); $rms = \text{less than 10 nm}$
- Process monitoring: 2 pyrometers (temperature), emission spectra (chemistry)
AFM – Nanostructured NCD Layer

Scan: 1 x 1 mm

$rms = 8.2$ nm

(Performed in the Inst. of Physics, Acad. Sci. CR, Dr. A. Kromka)
AFM – Micro-nanostructured NCD

Scan: 50 x 50 mm
$rms = 301 \text{ nm}$

Detailed scan: 1 x 1 mm
$rms = 7.6 \text{ nm}$
Nanostructured NCD

Micro-nanostructured NCD

Raman spectroscopy

Nanostructured NCD

Micro-nanostructured NCD

Intensity [a. u.]

Wavenumber [cm$^{-1}$]

1140 cm$^{-1}$ nanocrystalline

1333 cm$^{-1}$ diamond

1350 and 1600 cm$^{-1}$ D and G band (sp$^2$)

E, F

C, D

[Scale bar: 20 μm]
Material and Methods

4 sets of samples:
- Nanostructured Si substrate
- Microstructured Si substrate
- Nanostructured diamond ($rms = 8.2\ nm$)
- Hierarchically micro- and nanostructured diamond ($rms$ of 301 nm and 7.6 nm)

**Cells:**
MG 63 osteoblast-like cells (European Collection of Cell Cultures, Salisbury, UK) 6000 cells/cm² in DMEM+10%FCS

**Cell number and viability:** was measured using micrographs after staining with LIVE/DEAD Kit 1, 3, and 5 days after seeding.

**Immunocytochemical staining** of β-actin and talin (cytoskeletal proteins)

**Statistics:** ANOVA, Student’s-Newmann-Keuls method
MG 63 cells on day 1 after seeding

Stained with LIVE/DEAD kit (calcein and ethidium homodimer-1; Invitrogen)

Obj. 4x, Olympus IX 50

Mean ± S.E.M. from 3 samples.

ANOVA, Student-Newman-Keuls Method.

Statistical significance: I, II, III, IV, V, VI: p≤0.05 in comparison with the sample of the same number.
MG 63 cells on day 3 after seeding

Stained with LIVE/DEAD kit (calcein and ethidium homodimer-1; Invitrogen)

Obj. 4x, Olympus IX 50

Mean ± S.E.M. from 3 samples.
ANOVA, Student-Newman-Keuls Method.
Statistical significance: I, II, III, IV, V, VI: p≤0.05 in comparison with the sample of the same number.
MG 63 cells on day 5 after seeding

Stained with LIVE/DEAD kit (calcein and ethidium homodimer-1; Invitrogen)

Obj. 10x, Olympus IX 50

Mean ± S.E.M. from 3 samples.

ANOVA, Student-Newman-Keuls Method.

Statistical significance: I, II, III, IV, V, VI: p≤0.05 in comparison with the sample of the same number.
Immunofluorescence staining of β-actin, day 3 after seeding

Glass Nano_NCD micro-nanoNCD

Area of MG 63 cells

<table>
<thead>
<tr>
<th></th>
<th>I. glass-control</th>
<th>II. nanoNCD</th>
<th>III. micro-nanoNCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>cell area µm²</td>
<td>2000</td>
<td>3000</td>
<td>4000</td>
</tr>
</tbody>
</table>

Olympus IX 50, obj. 20x
Immunofluorescence staining of talin (focal adhesion protein) and osteocalcin (marker of osteogenic cell differentiation), day 3 after seeding.

Olympus IX 50, obj. 100x
Conclusion

• The number as well as the viability of MG 63 cells on both nanodiamond films was similar to the control sample.

• The number of cells on day 3 after seeding was significantly higher than on the control polystyrene dish and silicon substrates.

• The viability of cells on both nanodiamond films was more than 98% during all detected intervals. In contrary, the cell viability on silicon substrates had strongly decreasing tendency. On day five the viability on silicon substrates was 0%.

• The cell adhesion was effective on both nanodiamond films, especially on micro-nano NCD, where the cell area was significantly larger in comparison with the control and nano NCD.

• According our results these nanodiamond films seem to be suitable for bone tissue engineering, on the other hand, silicon substrates seemed to be highly cytotoxic.
Thank you very much for your attention!

Please, see also posters UND 19 and UND 25

Enjoy your time in Algarve!
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