Bone-repair properties of biodegradable hydroxyapatite nano-rods superstructures

Noelia L. D’ Elía(a), Colleen Mathieu(b), Caroline D. Hoemann(b, c, d), Juan A. Laiuppa(e), Graciela E. Santillan(e), Paula V. Messina*(a).

(a) Department of Chemistry, Universidad Nacional del Sur, (8000) Bahía Blanca, Argentina. INQUISUR-CONICET. (b) Institute of Biomedical Engineering, École Polytechnique, Montréal, QC, Canada. (c) Groupe de Recherche en Sciences et Technologies Biomédicales (GRSTB), Canada. (d) Department of Chemical Engineering, École Polytechnique, Montréal, QC, Canada. (e) Department of Biology, Biochemistry and Pharmacy, Universidad Nacional del Sur, (8000) Bahía Blanca, Argentina.

* Author to whom correspondence should be addressed. Tel: +54 291 4595159. Fax: +54 291 4595160. Electronic mail: pmessina@uns.edu.ar.
Electronic Supplementary Information (ESI)

Single roughness depth ($R_{Z,i}$); the maximum roughness depth ($R_{Z,max}$) and the mean roughness depth ($R_{Z,mean}$) parameters. Examples of $R_{Z,i}$ are indicated in materials roughness profiles.

**Figure 1 ESI**: MI surface roughness profile.

![Figure 1](image1.png)

<table>
<thead>
<tr>
<th>$R_{Z,mean}$</th>
<th>SD</th>
<th>Minimum</th>
<th>Median</th>
<th>$R_{Z,max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>56.7</td>
<td>14.4</td>
<td>27.3</td>
<td>58.5</td>
<td>85</td>
</tr>
</tbody>
</table>

**Figure 2 ESI**: MII surface roughness profile.

![Figure 2](image2.png)

<table>
<thead>
<tr>
<th>$R_{Z,mean}$</th>
<th>SD</th>
<th>Minimum</th>
<th>Median</th>
<th>$R_{Z,max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>42.9</td>
<td>28.1</td>
<td>9.2</td>
<td>35.9</td>
<td>91.5</td>
</tr>
</tbody>
</table>
Electronic Supplementary Information (ESI)

**Figure 3 ESI:** MIII surface roughness profile.

![Figure 3 ESI](image)

<table>
<thead>
<tr>
<th>$R_z, \text{mean}$</th>
<th>SD</th>
<th>Minimum</th>
<th>Median</th>
<th>$R_z, \text{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>38.7</td>
<td>11.7</td>
<td>20.9</td>
<td>36.3</td>
<td>71.7</td>
</tr>
</tbody>
</table>

**Figure 4 ESI:** MIV surface roughness profile.

![Figure 4 ESI](image)

<table>
<thead>
<tr>
<th>$R_z, \text{mean}$</th>
<th>SD</th>
<th>Minimum</th>
<th>Median</th>
<th>$R_z, \text{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.8</td>
<td>21.6</td>
<td>7.4</td>
<td>37.9</td>
<td>90.7</td>
</tr>
</tbody>
</table>
Figure 5 ESI: Nano-hydroxyapatite superstructures degradation in physiological fluid conditions (phosphate buffer saline, pH = 7.4). At 36°C all materials exhibited a weight loss minor than 2.5%; while at 25°C no degradation can be recorded.
Figure 6 ESI: Optical microphotographs showing MSCs adhesion on the nano-HAp powder coatings (MI, MII, MIII and MIV) with different nanoparticles amounts. At high amount of nano-HAp (3100 µg/cm²) non-adherent cells are observed.
Figure 7 ESI: Optical microphotographs showing MSCs morphology after adhesion on Co-I; MI/Co-I coatings and non-coated glass slide used as positive control (C+). (1) Co-I (31 μg / cm$^2$); (2) HAp (MI) 7200 μg/cm$^2$. Slide A: A1, A2 cell adhesion on Co-I coating; A4, A5 example area with high mineral concentration and no visible adherent cells. Slide B: adherent cells on C+. Slide C: C1, C5 adherent cells on Co-I coatings; C2, C6 example area with high mineral concentration and no visible adherent cells; C3, C7 adherent cells on C+. 

Morphology of rabbit MSCs in a T75 Flask
20x images

Cell morphology in Slide A
10x images
24h post-seeding
1, 2: Col-1 (1)
4, 5: Col-1+HAp (MI) (2)
Cell morphology in Slide B
10x images
24h post-seeding
1→4: None (glass)

Cell morphology in Slide C
10x images
24h post-seeding
Controls:
1&5: Co I (1)
2&6: Co I/HA (2)
3&7: None (glass)
## Electronic Supplementary Information (ESI)

### MI / Co I Coating

<table>
<thead>
<tr>
<th>Glass (C+)</th>
<th>0/1</th>
<th>0.5/1</th>
<th>1/1</th>
<th>2/1</th>
</tr>
</thead>
</table>

**Optical microscopy**

- **20X Ph3**
- **20X Ph2**
- **20X Ph1**
Figure 8 ESI: Laser scanning confocal microphotographs showing MSCs α-SMA expression using large amounts of hydroxyapatite MI (7200 µg/cm²) / Co I (31 µg/cm²) coatings to test cytotoxicity.
**Figure 9 ESI**: Figure 10 (a) supplementary information. Immunofluorescence assay: confocal microphotographs 10×. The bar graph shows the number of nucleus counted by Image J software in each replicate slide. Non-coated glass slide was used as positive control (C+).

<table>
<thead>
<tr>
<th>MI/Co I Coating</th>
<th>Glass (C+)</th>
<th>0/1</th>
<th>0.5/1</th>
<th>1/1</th>
<th>2/1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D1</td>
<td>A1</td>
<td>A2</td>
<td>A3</td>
<td>A4</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D2</td>
<td>A5</td>
<td>A6</td>
<td>A7</td>
<td>A8</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D3</td>
<td>E1</td>
<td>E2</td>
<td>E3</td>
<td>E4</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of nuclei
Figure 10 ESI: Image analysis for α-SMA expression (monochromatic image, green laser 488nm), the mean green pixel intensity was determined from the mean brightness value of all green pixels analyzed per image. Supplementary information of methodology section 2.7.3: “MSC actin-based spreading on Collagen type I without and with nano-Hap /: Immunofluorescence confocal microscopy”.

Example#1
Grayscale image

Grayscale level histogram (Brightness value) of the image

Count: 1048576
Min: 0
Mean: 90.506
Max: 255
StdDev: 38.517
Mode: 85 (13509)

Example#2
Grayscale image

Grayscale level histogram (Brightness value) of the image

Count: 1048576
Min: 0
Mean: 81.236
Max: 255
StdDev: 55.142
Mode: 0 (105335)