SUPPLEMENTARY INFORMATION

Hyaluronan degrading silica nanoparticles for skin cancer therapy

Scodeller, Pa; Catalano, PNb; Salguero, Nb; Duran, Hb; Wolosiuk, Aa; Soler-Illia, GJAAb

a Gerencia de Química, Comisión Nacional de Energía Atómica (CNEA), Av. Gral. Paz 1499, (B1650KNA) San Martín, Argentina
b Laboratorio de Aplicaciones Biológicas, Departamento de Micro y Nanotecnología, Comisión Nacional de Energía Atómica (CNEA), Av. Gral. Paz 1499, (B1650KNA) San Martin, Argentina
email: pscoedeller@sanfordburnham.org

INDEX

Section 1 - TEM of core-shell particles..............................................................
Section 2 - DLS of core-shell particles..............................................................
Section 3 - Estimation of amount of protein on NP...........................................
Section 4 - SEM characterization of core-shell particles and tumor loaded particles......
Section 5 - Enzymatic assay for Hyal and Hyal carrying particles............................
Section 6 - Further evidence of hyaluronic acid degradation by Hyal carrying particles....

SECTION 1 - TEM OF CORE-SHELL PARTICLES

SiNP

S1. TEM image of SiNP
**SiNP/PAH/Hyal**

Shell thickness: 
~ 6 nm

S2. TEM image of SiNP/PAH/HYAL.

**SECTION 2 - DLS OF CORE-SHELL NANOPARTICLES**

**SiNP**

<table>
<thead>
<tr>
<th>STOPPED</th>
<th>A CR (avg):</th>
<th>43.8 kcps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run:</td>
<td>M. Base:</td>
<td>5.4548e+05</td>
</tr>
<tr>
<td>First Delay: 5.0 μsec</td>
<td>C. Base:</td>
<td>5.4516e+05</td>
</tr>
<tr>
<td>Last Delay: 81.92 msec</td>
<td>Base diff:</td>
<td>0.056%</td>
</tr>
<tr>
<td>Elapsed Time: 00:01:00</td>
<td>Eff Dia:</td>
<td>253.3 nm</td>
</tr>
<tr>
<td>Angle: 90.0</td>
<td>Poly:</td>
<td>0.005</td>
</tr>
<tr>
<td>Temp: 24.8 deg C (GTE)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S3. DLS results for SiNP.

**SiNP/PAH**
S4. DLS results for SiNP/PAH.

*SiNP/PAH/Hyal*
SS. DLS results for SiNP/PAH/Hyal in PBS.

SECTION 3 - ESTIMATION OF AMOUNT OF PROTEIN ON PARTICLES

Taking the difference between 0.1mg.mL\(^{-1}\) SiNP/PAH/Hyal and SiNP/PAH spectra:

We get a peak height at 280nm of: 0.02
- And from UV-Vis spectrum of weighed Hyal in water, we know that: 
  1mg.mL\(^{-1}\) protein@mQ \(\rightarrow\) \(A_{279nm}\) = 0.67 a.u
- So 0.02 a.u corresponds to 30\(\mu\)g.mL\(^{-1}\)
- But the dispersions of SiNP used in the treatments are 0.5mg.mL\(^{-1}\) so the protein concentration in them is:
  - 150 \(\mu\)g.mL\(^{-1}\) of protein
  - Since the volumes used were 100uL, the total protein content in those sample was:
  - 15\(\mu\)g

SECTION 4 - SEM CHARACTERIZATION OF CORE-SHELL PARTICLES AND TUMOR LOADED PARTICLES

\textit{SiNp/PAH/HYAL}
S7. SEM image of SNp/PAH/HYAL dried on a carbon substrate from a diluted dispersion in water, and further metallized. The yellow line highlights the diameter of a typical particle of 256nm.

_Nanoparticle injected tumor_
S8. Typical SEM image from a 21 days postinjection A375 tumor, excised two hours after a sole injection of 0.1mL of $2 \times 10^9$ NP.mL$^{-1}$ dispersion of SiNp/PAH/Hyal. The yellow line highlights the diameter of a typical particle of 264nm.

SECTION 5 - ENZYMATIC ASSAY FOR HYAL AND HYAL CARRYING PARTICLES
S9. Schematic diagram illustrating the typical enzymatic assay for Hyal.

S10. Schematic diagram of the procedure used to establish enzymatic assay of SiNp/PAH/Hyal.

**SECTION 6- FURTHER EVIDENCE OF HYALURONIC ACID DEGRADATION BY HYAL CARRYING PARTICLES**

Absorbance at 230nm is a further evidence of HA degradation, since:

\[
\text{HA} + \text{HYAL} \rightarrow \text{HA fragments}
\]

Absorbance of HA, before and after incubation with SiNp/PAH/Hyal is shown in Fig. S11 A:
S11. A: UV-Vis spectra of HA before (dotted line), and after incubation with SiNp/PAH/Hyal and post removal of the particles by centrifugation (solid line). B: Difference between the spectra of panel A.

The difference in these two spectra is shown in Fig. S11B and the peak at 229nm is another evidence of HA degradation by the Hyal derivatized particles.

\[\text{Absorbance} / \text{a.u.} \]
\[\text{Wavelength} / \text{nm} \]

---