RSC Advances

Supplementary Information

Formation of Lipid and Polymer Based Gold Nanohybrids Using a Nanoreactor Approach

Dominik Witzigmann, Sandro Sieber, Fabiola Porta, Philip Grossen, Andrej Bieri, Natalja Strelnikova, Thomas Pfohl, Cristina Prescianotto-Baschong, Jörg Huwyler *

** Author for correspondence:*
Prof. Dr. Jörg Huwyler
Phone: +41 (0)61 267 15 13
E-mail: joerg.huwyler@unibas.ch

1. Supplementary Information Experimental Section

1.1 Gold nanoparticle (AuNP) synthesis

The AuNP synthesis was optimized using a 2^3 full factorial design of experiment (DoE) [Stavex 5.2, Aicos Technologies, Basel, Switzerland]. Variables are listed in Table S1. All glass equipment was cleaned with aqua regia to prevent the formation of gold seeds. The best condition (smallest AuNPs and mild reaction condition) from our screening was used for further AuNHyb preparations (1.0 mM $HAuCl_4$ / 4.1 mM citrate; 1:4 molar ratio).

Table S1: AuNP synthesis screening using Turkevich method. Variations of citrate or tetrachloroaurate concentration, pH, and temperature.

Sample	c ($HAuCl_4$) [mM]	c (Citrate) [mM]	c (NaOH) [mM]	T [°C]	Size [nm]
Factorial Design (Mild Conditions)					
A	0.5	2.0	-	70	20.2
B	1.0	2.0	-	70	24.3
C	0.5	4.1	-	70	43.1
D	1.0	4.1	-	70	16.6
E	0.5	2.0	-	85	20.8
F	1.0	2.0	-	85	19.8
G	0.5	4.1	-	85	17.0
H	1.0	4.1	-	85	17.8
Other Approaches [1–4]					
I	0.5	2.0	-	boiling	18.7
K	1.0	4.1	-	boiling	19.3
L	2.5	5.1	5.0	70	26.6
M	2.5	5.1	6.6	85	19.6

1.2 Microfluidics device design and fabrication

For the master fabrication, SU8 (Microchem, Newton, USA) spin coated silicon wafers (Si-Mat, Kaufering, Germany) were used. To produce a master with a staggered herringbone micromixer, multi-layer photolithography was carried out using appropriate photomasks (JD Pho-tools, Oldham, UK) and a MJB4 mask aligner (SUSS MicroTec AG, Garching, Germany).
The first layer was made of SU8-3025 and the second one of SU8-3050 negative photoresists. PDMS and cross-linker (Sylgard 184, Dow Corning GmbH, Wiesbaden, Germany) were mixed in the mass ratio of 10:1, degassed, and poured on the masters followed by overnight curing. Cured PDMS stamp was peeled off from the wafer.
To prepare a 3D channel device, two sides (bottom side and top side) of polystyrene foil/NOA 81 were prepared as follows. A drop of NOA 81 was pipetted onto the structure of the PDMS stamp. Then a piece of polystyrene foil was placed on the NOA 81 and pressed down until the polystyrene foil touches elevated channel on the PDMS stamp that the channels were only laterally defined by NOA 81. NOA 81 was cured under UV-light for 3 min (366 nm, 2 x 8 W, Camag, Muttenz, Switzerland). The second side of the device was prepared the same way. Additionally, 0.75 mm holes were punched for tubing connection (Harris Unicore). Both polystyrene foil/NOA 81 sides were aligned, gently pressed, and cured under UV-light for 10 min.

1.3 Flow visualization

Deionized water, ethanol, and a fluorescein solution were used to visualize the flow patterns of the microfluidics device (Figure 1D). Images were taken using an Olympus IX81 inverted microscope (Olympus, Tokyo, Japan) equipped with fluorescence illumination (X-Cite Series 120 Q).

1.4 Computational Fluid Dynamics Simulation

For the numerical computational fluid dynamics (CFD) simulations we used the CFD module of the software COMSOL 4.3a (COMSOL Inc, Burlington, USA). The microfluidics device was modeled in 3D using 792,779 finite elements solving the incompressible Navier-Stokes-equation and advection-diffusion equation. The stationary flow field was calculated and in a second step the material transport of the diluted species were calculated using the beforehand-obtained stationary flow fields. We assumed a Newtonian fluid having the properties of water at room temperature.[5] Flow rates and inflow concentrations were specified at the seven inlets and the following diffusion coefficients were assumed: $D_{\text{lipids}} = 5 \cdot 10^{-11}$ m^2 s^{-1}, $D_{\text{citrate}} = 8.9 \cdot 10^{-10}$ m^2 s^{-1} and $D_{\text{AuCL4}} = 5.6 \cdot 10^{-10}$ m^2 s^{-1}.

1.5 Preparation of fluorescent lipid based gold nanohybrids (AuNHybs)

To broaden the application spectrum of AuNHybs for bioimaging purposes, we introduced an additional fluorescence marker, namely Rho-PE (Avanti Polar-Lipids, Alabaster, USA), into lipid based nanocarriers. Rho-PE was post inserted into preformed 75mol% POPC, 25mol% POPG liposomes as described elsewhere.[6] The modified liposomes possessed specific absorption properties based on the encapsulated AuNPs and an additional fluorescence based on the post-insertion of Rho-PE. The non-Rho-PE liposomes showed neither an absorption peak between 260 nm and 750 nm, nor a fluorescence after excitation at 560 nm. In contrast, the Rho-PE containing liposomes possessed a strong fluorescence with a maximum at 596 nm after they were excited near their absorption maximum at 560 nm. Compared with lipid-AuNHybs, the Rho-PE containing AuNHybs showed an additional absorption peak and were fluorescent after they were excited at 560 nm.

2. Supplementary Information Figures

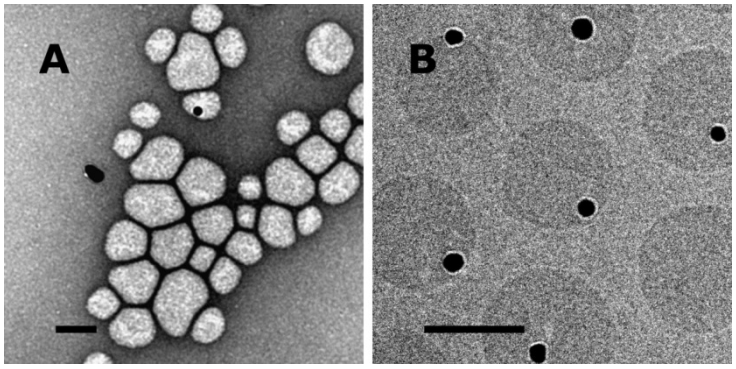

Figure S1. Electron microscopy analysis of PEG-PCL-gold nanohybrids (AuNHybs). Representative transmission electron microscopy (TEM) and Cryo-TEM images of solid polymeric nanoparticles are shown. (A) Preparation of PEG-PCL-AuNHybs using preformed AuNPs results in low encapsulation efficiency. (B) Cryo-TEM analysis of PEG-PCL-AuNHybs prepared via nanoreactor approach. Scale bars indicate 50 nm.

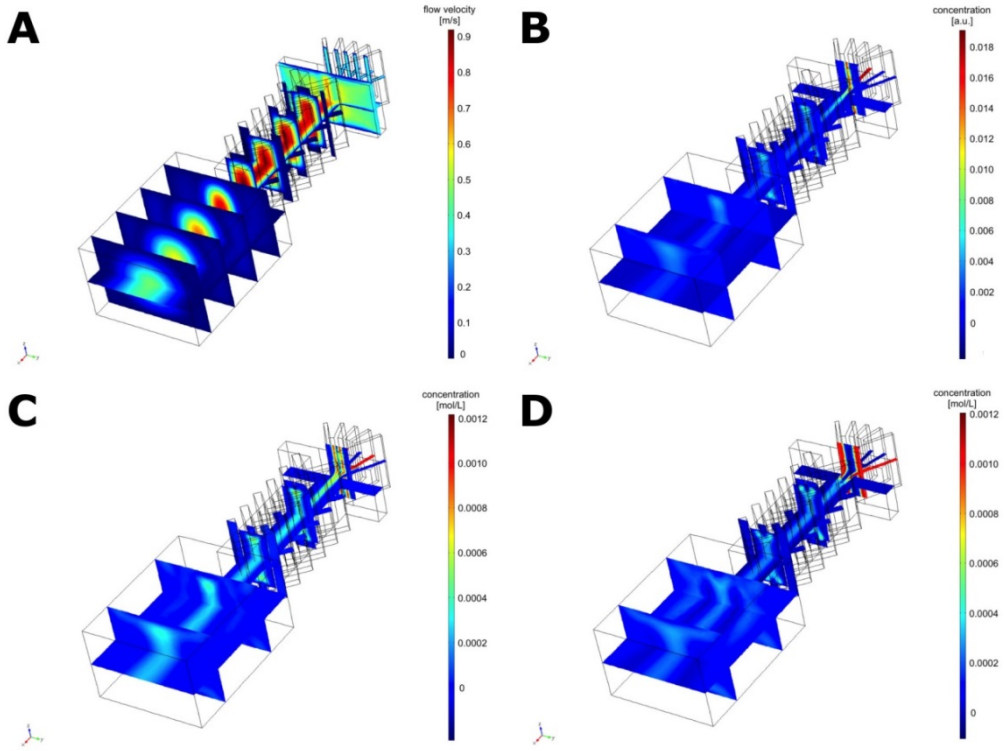

Figure S2. Computational fluid dynamics (CFD) simulations. (A) CFD simulation of the flow rate (m/s) of all seven inlet channels converging to a single micromixer is shown. Simulation of concentration gradients of (B) ethanol (in a.u.), (C) tetrachloroaurate (in mol/L), and (D) citrate (in mol/L) solution are presented.

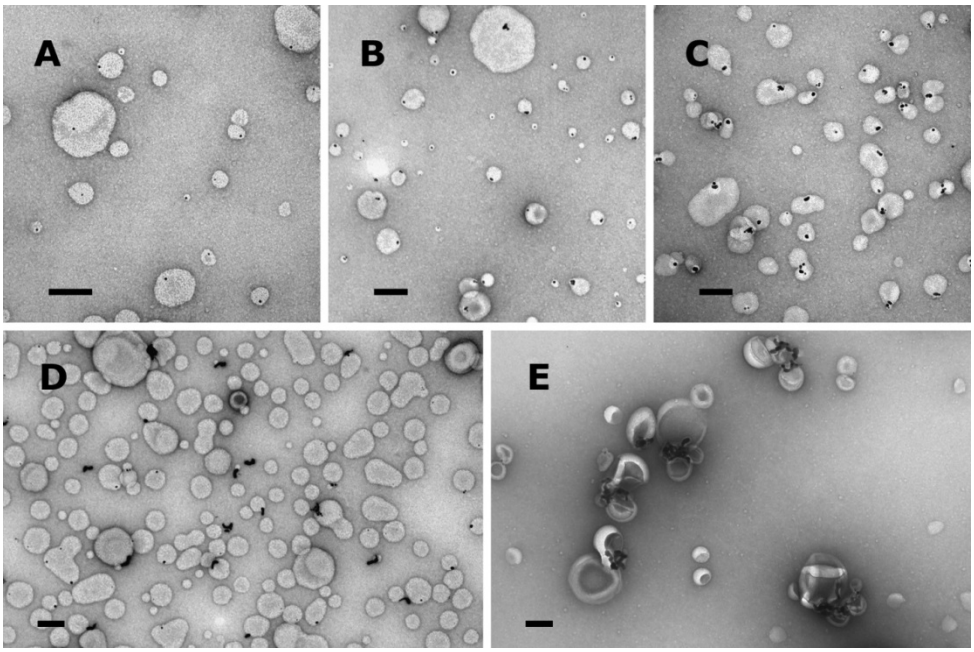

Figure S3. Preparation of gold nanohybrids (AuNHybs) using microfluidics. Representative transmission electron microscopy (TEM) images are shown. Increasing concentration of the reaction solutions (1x = 1 mM $HAuCl_4$ / 4.1 mM citrate) for the nanoreactor approach resulted in an increased number of AuNPs per nanocarrier. (A) 2x, (B) 4x, and (C) 8x reaction solutions were used. (D) Preparation of AuNHybs with one combined reaction solution in the same inlet resulted in AuNP formation during microfluidics procedure. (E) Direct use of AuNPs resulted in agglomerates and low encapsulation efficiency. Scale bars indicate 100 nm.

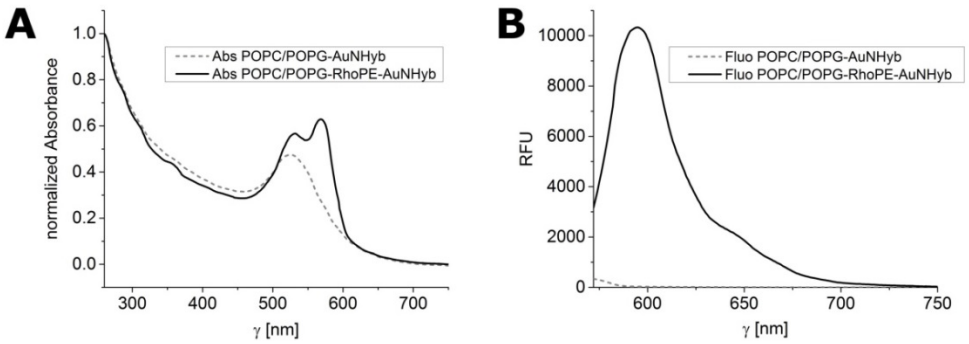

Figure S4. UV-Vis and fluorescence spectra of gold nanohybrids (AuNHybs) with and without fluorescent lipid (Rho-PE). (A) UV-Vis absorption from 260 nm to 750 nm (step size one nm) was measured. Spectra were normalized to an OD_{260} of 1.0. Fluorescently labelled AuNHybs showed two absorbance maxima, i.e. characteristic surface plasmon band and absorbance maximum of Rho-PE. (B) Fluorescence spectra of AuNHybs between 572 nm to 750 nm after excitation at 560 nm. POPC/POPG-RhoPE-AuNHybs showed strong fluorescence (RFU = relative fluorescence units), whereas POPC/POPG-AuNHybs were not fluorescent.

Supplementary Information References

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