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Article *in* Angewandte Chemie International Edition · June 2009

DOI: 10.1002/anie.200901486 · Source: PubMed

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Hydrogen-Bond-Selective Phase Transfer of Nanoparticles across Liquid/Gel Interfaces**

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An important defense mechanism for organisms are the various interfaces between fluids and cellular layers, which act as biological barriers to prevent foreign substances from reaching targets in a larger mass fraction. However, this mechanism also suppresses the efficiency of targeting and imaging in vivo.[1–3] Despite extensive studies of biomedical use,[4–6] the use of colloidal nanoparticles (NPs) for crossing biological barriers has been little addressed. Mimicking the interfacial transport of molecules with NPs should offer new insights into imaging translocation pathways within extravascular spaces and delivering drugs across biological barriers. Thermodynamically, colloidal NPs prefer to attach to interfaces,[7–10] but they will not cross an interface readily because their surface wettability is not easy to change and they have the solvation energies much higher than small molecules. Procedures to transfer hydrophobic NPs from the oil phase to the water phase have been reported, namely, phase transfer and ligand exchange with the aid of amphiphilic molecules,[11] but these methods are obviously not practical for crossing biological barriers. We have recently succeeded in transferring NPs from a salt-water phase to an oil phase by relying on the stimuli response of the polymer brushes anchored on them.[12] Once transferred into the oil phase, however, further environmental change drove the NPs to attach to the water/oil interface but not to cross it. On the other hand, the water/oil interface is not a proper model for biological barriers composed of different cellular layers. Herein we demonstrate a hydrogen-bond-selective method to direct hydrophobic NPs, coated with a mixture of polylactide (PLA) and poly(ethylene glycol) (PEG) brushes, to transfer from the organic to the aqueous phase during PLA degradation across not only water/oil but also liquid/gel interfaces: that is, the water/organogel and the oil/hydrogel interface.

PLA is widely used for drug delivery and tissue engineering because of its good biocompatibility and biodegradability.[13,14] To anchor PLA brushes on NPs, we used bis(2-hydroxyethyl) disulfide as initiator to synthesize PLA disulfide by ring-opening metathesis polymerization (ROMP; Figure 1a). The resulting PLA disulfide, bearing two single PLA chains, has a mass M_n of about 4000 Da, with each single PLA chain being about 2000 Da (see the Supporting Infor-

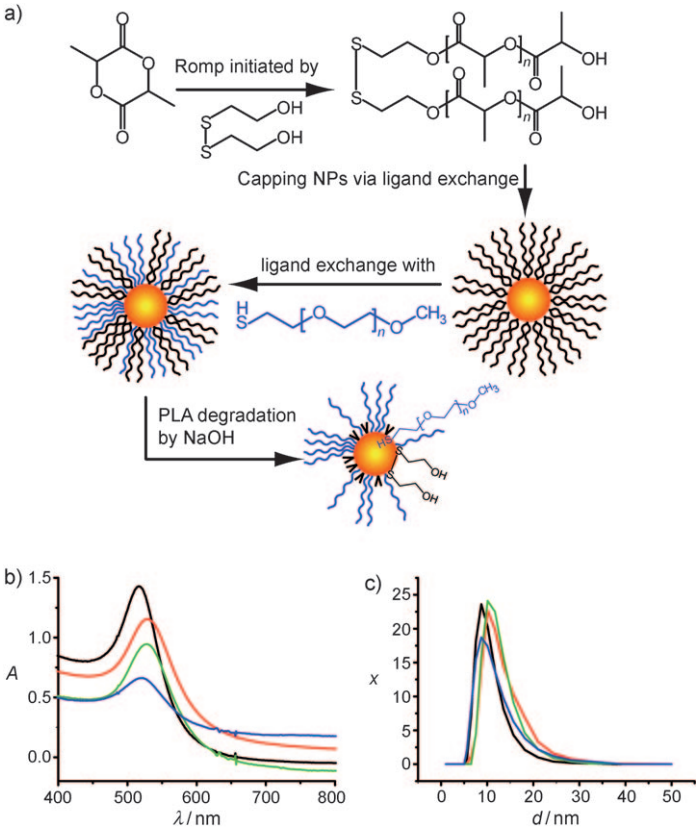

Figure 1. a) Synthesis of PLA disulfide by ROMP initiated with bis(2-hydroxyethyl) disulfide and coating of colloidal NPs with a mixture of PLA disulfide and PEG thiol by ligand exchange. b) Absorption spectra of Au@citrate NPs in water (——), Au@PLA NPs in toluene (——), Au@PLA/PEG NPs in toluene (——), and Au@PEG NPs in water obtained after PLA degradation (——). c) Size distribution profiles obtained by DLS for Au@Citrate NPs in water (——), Au@PLA NPs in toluene (——), Au@PLA/PEG NPs in toluene (——), and Au@PEG NPs in water obtained after PLA degradation (——). x = number fraction, d = diameter.

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[**] This project is supported by the Max Planck Society. D.W. is supported in part by a DFG grant (WA 1704/4-1) and an EU-STREP grant (BONSAI, LSHB-CT-2006-037639). T.L.N. thanks DEST for support through ISI Grant CG110036. We are grateful to Prof. H. Möhwald for valuable discussion.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200901486.

mation). The PLA disulfide brushes were anchored on 10 nm AuNPs that were obtained by citrate reduction via ligand exchange in THF (see the Supporting Information). The success of the ligand exchange was confirmed by FTIR spectroscopy (Supporting Information, Figure S4). The resulting Au@PLA NPs were fairly soluble in low-polarity organic solvents, such as toluene and chloroform (Supporting Information, Figure S5a). Dynamic light scattering (DLS) indicated that the Au@PLA NPs were 12 nm in diameter, which corresponds to a PLA shell of 1 nm in thickness (Figure 1c). Thermogravimetric analysis indicated a grafting density of 510 single PLA chains per AuNP (Supporting Information, Figure S6), indicating a dense brush configuration of the PLA chains on the NPs (1.7 chains nm^{-2}). The surface plasmon absorption band of the AuNPs red-shifted from 518 nm in water ($n = 1.33$) to 525 nm in THF ($n = 1.407$) and to 528 nm in toluene ($n = 1.496$; Figure 1b). The larger shift that is observed on going from water to THF arises mainly from the dense PLA coating rather than the solvent change, as suggested in a previous study.[15]

To accelerate PLA degradation, all experiments were conducted at 40 °C. When a dispersion of Au@PLA NPs in toluene was brought into contact with 0.1 M NaOH to degrade the PLA coating at the interface, the color of the colloidal NPs in the organic dispersion changed from red to blue and eventually to colorless, and the NP precipitates were visible at the interface (Supporting Information, Figure S5b). During degradation, the PLA disulfide brushes on the AuNPs were eventually transformed back to bis(2-hydroxyethyl) disulfides, which increased the NP surface hydrophilicity and thus caused the adsorption of NPs at the water/toluene interface.[9,10] However, there was no transfer of AuNPs across the interface, even when citrate was added in the aqueous phase to enhance hydration of the disulfide alcohol coating on the NPs by hydrogen bonding (Supporting Information, Figure S5b).

To increase the surface hydrophibicity of the AuNPs after degradation of the PLA coating, we replaced a part of PLA disulfide brushes with PEG thiol brushes by ligand exchange. The PEG thiol brushes had a mass M_n of 2000 Da, which is similar to that of a single PLA chain of the PLA disulfide brushes. The Au@PLA/PEG NPs thus obtained were similar to Au@PLA NPs in terms of surface plasmon absorption (Figure 1b) and hydrodynamic size (Figure 1c). The coexistence of PLA and PEG brushes on AuNPs was determined by FTIR spectroscopy (Supporting Information, Figure S4), and the molar ratio of PEG to PLA was determined to be 2.3 by ^{1}H NMR spectroscopy (Supporting Information, Figure S7). Au@PLA/PEG NPs were only soluble in organic solvents. Upon contact with 0.1 M NaOH solution, the toluene dispersion of Au@PLA/PEG NPs became colorless owing to PLA degradation; at the same time, the alkaline solution turned red in the presence of 0.05 M citrate, suggesting the transfer of AuNPs to the aqueous phase across the interface (Supporting Information, Figure S5e). The transferred AuNPs were colloidally stable into the aqueous NaOH/citrate solution as the PEG brushes remained on the NP surfaces (Figure 1b,c; Supporting Information, Figure S8). However, the AuNPs accumulated at the interface in the absence of citrate in the aqueous phase, suggesting that the PEG coating cannot readily be hydrated by water at the water/oil interface without the help of hydrogen bonding with citrate (Supporting Information, Figure S5d). Poly(acrylic acid) and poly(L-lysine) in the aqueous alkali phase also promoted the transfer of Au@PLA/PEG NPs across the water/oil interface after PLA degradation (Supporting Information, Figures S5f,g). This result supports the proposal that the driving force for AuNPs to cross the water/oil interface is the hydrogen bonding of their PEG brushes in the aqueous phase after degradation of their PLA brushes. As shown in Figure 2, the

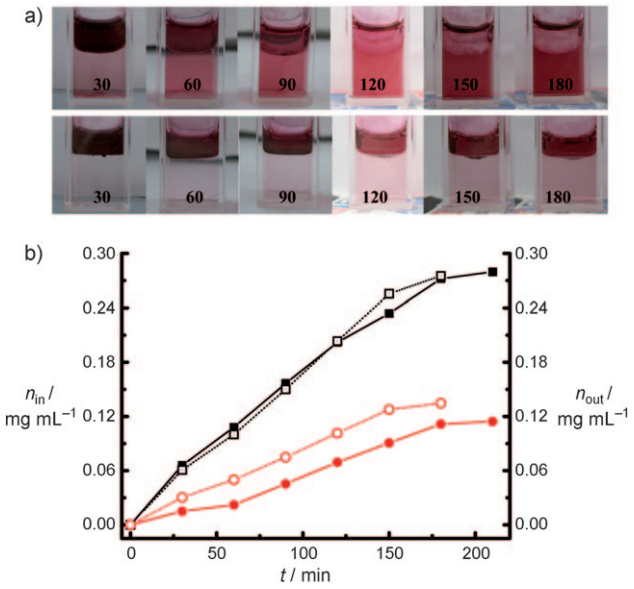

Figure 2. a) Time series (in minutes) of optical images of Au@PLA/PEG NPs crossing the water/oil interface. The toluene dispersion of the NPs (the upper phase) was brought in contact with an aqueous solution NaOH with 0.05 M citrate (the lower phase). The NaOH concentration was 0.1 M (upper panel) and 0.01 M (lower panel). b) Plots of the fraction n_{in} of Au@PLA/PEG NPs transferred into the aqueous phase (■,●) and n_{out} transferred out of the toluene phase (□,○) versus time during PLA degradation. Two NaOH concentrations, 0.1 M (□,■) and 0.01 M (○,●), were used for degradation of the PLA brushes on the NPs at 40 °C. The concentration of the Au@PLA/PEG NPs in toluene was 0.3 mg mL^{-1}.

kinetics of AuNP transport out of the organic phase is comparable to that of the NP appearance in the aqueous phase, suggesting that the interfacial crossing of NPs is an exceedingly fast process. The NP transfer rate across the water/toluene interface increased with the concentration of NaOH: 84.3 μg $mL^{-1}h^{-1}$ for 0.1 M, and 36.1 μg $mL^{-1}h^{-1}$ for 0.01 M.

We also studied the translocation of NPs coated with PLA and PEG brushes at the liquid/gel interfaces. Both hydrogel and organogel were derived from agarose. Owing to the difficulty of precisely measuring the absorption of AuNPs in the gel phase, luminescent 7 nm CdSe@ZnS quantum dots (QDs) stabilized by octadecylamine were used,[16] and they were visualized by confocal laser scanning microscopy (CLSM). Owing to the multilayer ZnS coating, the fluores-

cence of CdSe@ZnS QDs is robust and changes insignificantly in the different media and after ligand exchange, so that the relative concentration of QDs transferred into and out of the gel phase can be analyzed directly by the fluorescence intensity. After the replacement of their original ligands by pyridine,[17] QDs coated with PLA disulfide and PEG thiol brushes, CdSe@ZnS@PLA/PEG, were obtained by ligand exchange (see the Supporting Information). Agarose hydrogel spheres of hundreds of micrometers in size, swollen by 0.1 M NaOH aqueous solution containing 0.05 M citrate, were placed in a toluene dispersion of CdSe@ZnS@PLA/PEG QDs. The QDs slowly penetrated into the hydrogel spheres, which was evidenced by increase of the fluorescence intensity in the hydrogel spheres with the time (Figure 3a). This result

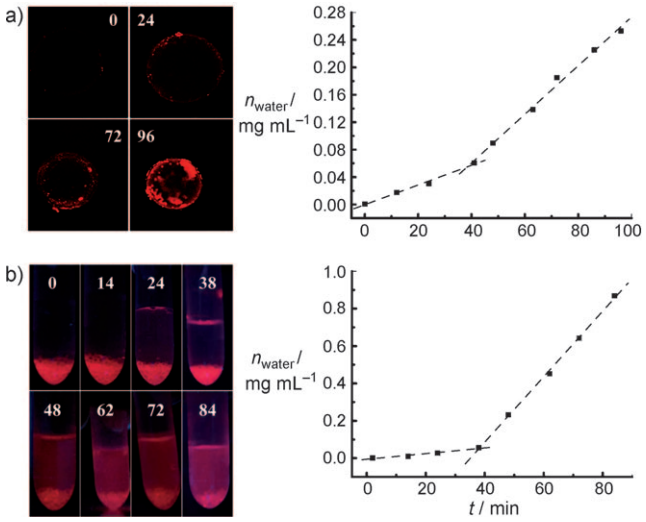

Figure 3. a) Transfer of CdSe@ZnS@PLA/PEG QDs across the hydrogel/oil interface at 40 °C. b) Transfer of CdSe@ZnS@PLA/PEG QDs across the water/organogel interface. Left panels: time series (in hours) of CLSM images (a) and optical images under a UV lamp (b). Right panels: Plot of the amount n of the QDs transferred into a) hydrogel (n_{hyd}) and b) water (n_{water}) versus time.

suggests that, as for the alkali water/oil interface, the alkali hydrogel/oil interface causes degradation of the PLA chains of the QDs, and the hydrogen bonding of the PEG chains with the citrate molecules absorbed in the hydrogel phase drives the QDs to cross the interface. As compared with the transfer across the water/toluene interface (84.3 $\mu g\,mL^{-1}\,h^{-1}$), the transfer across the hydrogel/toluene interface was rather slow; the transfer rate was 1.6 $\mu g\,mL^{-1}\,h^{-1}$. This difference is due to a slower PLA degradation at the hydrogel/oil interface as compared to the water/oil interface and in particular a slower QD diffusion into the hydrogel matrix. The QD transfer rate increased to 3.8 $\mu g\,mL^{-1}\,h^{-1}$ after 41 h incubation (Figure 3a), which is due to the fact that lactic acid and its oligomers, derived from the PLA degradation, can absorb on the surfaces of the hydrogel spheres and the QDs. This effect can lower the interfacial energy between the hydrogel and toluene phase and, at the same time, enhance the interfacial activity of the QDs, thus enabling more QDs to attach to the gel/toluene interface.

After using THF to replace the water, agarose gel spheres were incubated in the THF dispersions of CdSe@ZnS@PLA/PEG QDs, followed by re-dispersion in toluene or chloroform, thus yielding QD/agarose composite organogel spheres. When these composites were placed in 0.1 M NaOH solution containing 0.05 M citrate, the QD transfer from the organogel phase to the aqueous phase was slow for the first 38 h (Figure 3b). CLSM imaging indicated that immediately after placement into the aqueous phase, the QD-loaded agarose organogel spheres were swollen by water, leading to formation of an additional outmost hydrogel shell (Supporting Information, Figure S9a). Consequently, the QDs transferred from the organogel to the alkali water by the newly formed hydrogel layer. The transfer rate in the first 38 h was about 1.6 $\mu g\,mL^{-1}\,h^{-1}$, which was almost identical to the initial transfer rate (1.6 $\mu g\,mL^{-1}\,h^{-1}$) of the QDs from the oil phase to the hydrogel phase (Figure 3a). This result should further confirm that crossing the organic/aqueous interface, either organogel/water or toluene/hydrogel, is sufficiently fast, and the QD transfer from the organogel matrix to the aqueous surrounding is limited by the QD diffusion in the gel. After 38 h, the rate of QDs transferring from the organogel to the alkaline solution dramatically increased up to 18.5 $\mu g\,h^{-1}$ (Figure 3b), which is attributed to a burst release of the QDs that had accumulated in the newly formed hydrogel shells (Supporting Information, Figure S9b).

Because the hydroxy groups of the agarose gel network can form sufficiently strong hydrogen bonds with their PEG chains, CdSe@ZnS QDs should transfer from the organic phase into the citrate-free hydrogel phase. Therefore, we built up a triphasic system comprising a toluene dispersion of CdSe@ZnS@PLA/PEG QDs, a 0.1 M NaOH aqueous phase, and an agarose hydrogel swollen by 0.1 M NaOH aqueous solution; the toluene phase was sandwiched between the water and hydrogel phases (Figure 4). As mentioned above, the PLA chains on the QDs in toluene should degrade faster at the alkali/toluene interface than at the alkali swollen hydrogel/toluene interface. Surprisingly, the QDs transferred exclusively into the hydrogel rather than into water, despite their exceedingly slow diffusion into the former; the QDs penetrated into the hydrogel phase by about 200 μm after 2 days (Figure 4). This result underlines a high hydrogen-bonding selectivity of CdSe@ZnS QDs upon transfer from the organic phase to the aqueous phase across the interface.

In conclusion, we have successfully directed hydrophobic colloidal NPs coated with PLA and PEG to transfer from the organic to the aqueous phase across not only liquid/liquid but also gel/liquid interfaces during the PLA degradation. Crossing the interface for NPs is exceedingly fast, and the transfer kinetics is limited by the NP diffusion in the bulk phases and the NP attachment at the interfaces. The NP transfer to the aqueous phase is hydrogen bond selective; NPs transfer only into an aqueous phase that contains hydrogen-bond promoters, for example, citrate in the case of water and the hydroxy groups in the agarose, which can form strong hydrogen bonds with the PEG brushes coated on the NPs. Other interactions can also be used to direct the transfer of NPs across the

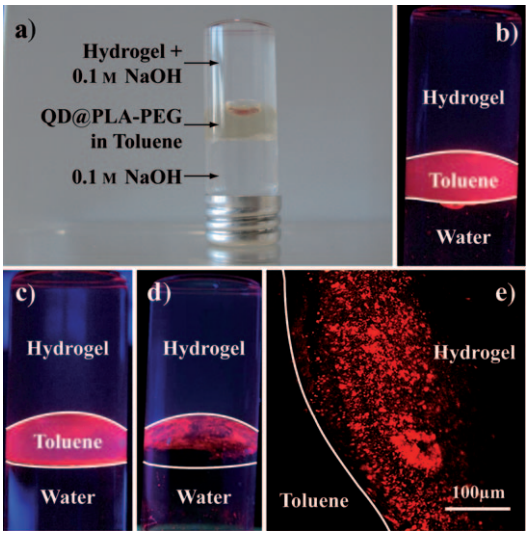

Figure 4. Transfer of CdSe@ZnS@PLA/PEG QDs from the organic phase to the aqueous phase in a triphasic system heated at 40°C. a,b) Optical images of the initial triphasic systems recorded under sunlight (a) and a UV lamp (b). c,d) UV optical images of the triphasic system (c) and the hydrogel phase (d) washed by toluene and water several times to remove the excess of QDs after 2 days of incubation at 40°C with gentle shaking. e) CLSM cross-section image of the hydrogel phase from (d), used to determine the QDs penetration depth. The water/toluene and the hydrogel/toluene interfaces are indicated by white lines.

interface, depending on their surface coating. Consequently, our study should not only shed light on molecular translocation across interfaces but also open new strategies for drug delivery across biological barriers, such as the stratum corneum epidermidis in the skin.[3]

Received: March 18, 2009
Published online: June 2, 2009

Keywords: biological barriers · gels · interfaces · nanoparticles · polymers

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