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Controlled release and capture of aldehydes by dynamic imine chemistry in nanoemulsions: from delivery to detoxification

Fei Liu,^{1,2} Nicolas Anton,² Yosuke Niko,³ and Andrey Klymchenko^{1,*}

¹Université de Strasbourg, Laboratoire de Bioimagerie et Pathologies, UMR 7021 CNRS, 74 route du Rhin, 67401 Illkirch (France)

²INSERM UMR 1260, Regenerative Nanomedicine (RNM), CRBS, Université de Strasbourg, 67000 Strasbourg (France)

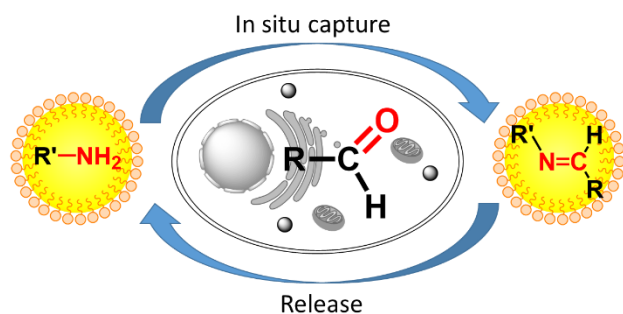
³Research and Education Faculty, Multidisciplinary Science Cluster, Interdisciplinary Science Unit, Kochi University, 2-5-1, Akebono-cho, Kochi-shi, Kochi, 780-8520 (Japan)

* Corresponding author: E-mail: andrey.klymchenko@unistra.fr

Abstract

Current biomedical applications of nanocarriers are focused on drug delivery, where encapsulated cargo is released in the target tissues under control of external stimuli. Here, we propose a very different approach, where the active toxic molecules are removed from biological tissues by the nanocarrier. It is based on the drug-sponge concept, where specific molecules are captured by the lipid nanoemulsion droplets due to dynamic covalent chemistry inside their oil core. To this end, we designed a highly lipophilic amine (LipoAmine) capable to react with a free cargo-aldehyde (fluorescent dye and 4-hydroxynonenal toxin) directly inside lipid NEs, yielding lipophilic imine conjugate well encapsulated in the oil core. The formation of imine bond was first validated using a push-pull pyrene aldehyde dye, which changes its emission color during the reaction. The conjugate formation was independently confirmed by mass spectrometry. As a result, LipoAmine-loaded nanoemulsions (NEs) spontaneously loaded cargo-aldehydes, yielding formulations stable against leakage at pH 7.4, which can further release the cargo at low pH range (4-6) in solutions and living cells. Using fluorescence microscopy, we showed that LipoAmine NEs can extract pyrene aldehyde dye from cells as well as from an epithelial tissue (chicken skin). Moreover, successful extraction from cells was also achieved for a highly toxic aliphatic aldehyde 4-hydroxynonenal, which allowed to obtain the proof of concept for detoxification of living cells. Taken together, these results show that the dynamic imine chemistry inside NEs can be used to develop detoxification platforms.

TOC graphic



Introduction

Traditional biological application of nanoparticles is drug delivery, where the concept of “magic bullet” ensures specific targeting and local release of drug at a diseased site in the body with a simultaneous reduction of toxic effects for non-targeted normal tissues.¹⁻³ Ideally, to meet this standard, smartly designed drug delivery systems should release the drug in response to the physiological stimuli in the body.⁴⁻⁶ One of the most popular stimuli in the field of controlled drug delivery is pH, since tumor microenvironment, inflammatory sites and bacterial infections tissues have slightly more acidic pH values than physiological media and healthy tissue.⁷⁻⁹ To this end, various pH-responsive drug delivery vehicles have been prepared from different pH-responsive chemical functionalities, including hydrazone¹⁰⁻¹² acetal,^{13,14} ortho ester,^{15,16} imine^{17,18} and vinyl ether.^{19,20}

On the other hand, an emerging direction in bio-nanotechnology is nanomaterials for uptake/removal of toxic molecules and drugs from blood or biological tissues (detoxification).^{21,22} Detoxification by nanomaterials has been well established for decontamination of the environment,²³ while biological and biomedical applications of detoxification is an emerging direction.²⁴ Thus, NPs functionalized with enzymes with complementary functions alcohol oxidase and catalase could reduce blood alcohol levels in intoxicated mice.²⁵ Polymersome nanoreactor loaded with enzyme was used for *in vivo* detoxification of organophosphates.²⁶ To capture specifically a peptide toxin, NPs based on molecularly imprinted polymers were also proposed, capable of neutralizing the toxin even in a complex biological milieu.²⁷ In an alternative strategy, biodegradable polymer poly(lactic-co-glycolic acid) (PLGA) NPs coated with red blood cell membranes were proposed as “nanosponges” that absorb pore-forming toxins *in vivo*.^{28,29} In a similar approach, PLGA NPs were coated with biomembranes isolated from macrophages to obtain biomimetic microphage-like NPs capable to capture endotoxins and proinflammatory cytokines in order to combat sepsis.³⁰ The key challenges in this new field include ever-lasting question about long-term toxicity of nanomaterials^{31,32} and capacity of NPs to capture specific molecules within the multitude of other species present in biological fluids or tissues.²¹

An attractive approach to capture toxic molecules with minimal toxic effects is to use lipid nanoemulsions (NEs), which are nanodroplets composed of oil core stabilized by a surfactant.³³⁻³⁷ NEs are highly promising for biomedical applications because they are (1) inexpensive and easy to produce; (2) composed of excipients generally recognized as safe (GRAS) and/or FDA approved and (3) biodegradable.³³⁻³⁷ Therefore, they attracted attention as safe biomimetic nanocarriers for drug delivery³⁸ and bioimaging^{37,39,40} as well as for food and cosmetics applications.^{41,42} Importantly, despite their liquid core, NEs are highly stable in biological media like serum and *in vivo* in blood circulation.^{40,43} Their oil core could serve as reservoir for capturing low polar toxic molecules. Indeed, a number of studies have shown their application for treatment of acute poisoning, although efficacy of NEs as detoxifying agents remains limited.⁴⁴ A recent study designed nano-sponges based on cell-membrane-cloaked NEs for non-specific capture of low polar toxicants by the oil core, combined with specific capture of toxic molecules by the biomembrane shell.⁴⁵ Overall, accumulation inside nano-droplet oil core is efficient only for lipophilic molecules with low water solubility, whereas partially water soluble compounds undergo a fast leakage from droplets in presence of acceptor media.⁴⁶ Accordingly, a strategy to enhance partition of a molecule inside NEs is their functionalization with lipophilic groups.^{46,47} Therefore, we hypothesized

that efficient uptake inside NEs of the toxic molecules could be done through *in situ* reaction with a highly lipophilic molecule, with the objective to generate *in situ* a stable highly lipophilic conjugate.

In this respect, capturing aldehydes is of particular interest, because they are toxic molecules present in biological systems and their aldehyde group could undergo an *in situ* reaction inside an oil core. An example of biologically active aldehyde is 4-hydroxynonenal (4-HN), which is associated with numerous diseases, such as chronic inflammation, neurodegenerative diseases, adult respiratory distress syndrome, atherogenesis, diabetes and different types of cancer.⁴⁸ To realize an *in situ* reaction with an aldehyde, one could exploit dynamic covalent chemistry, which deals with dynamic bonds that form and disrupt reversibly under environmental control.⁴⁹⁻⁵¹ Dynamic covalent chemistry was successfully applied for designing intelligent materials with self-healing and adaptive properties.⁵²⁻⁵⁴ Dynamic covalent bonds are particularly attractive for the field of drug delivery, because these bonds can be broken under environment stimuli providing controlled drug release.⁵⁵ The most popular dynamic covalent bonds include hydrazone,^{12,56,57} oxime,⁵⁸ imine,^{59,60} disulfide,⁶¹⁻⁶³ boronate ester⁶⁴⁻⁶⁶ etc. Recently, we have shown that lipophilic hydrazide LipoHD encapsulated inside NEs can form *in situ* dynamic hydrazone bonds with ketones, which ensure efficient capture of model cargo dye NRK and ketone drug doxorubicin.⁶⁷ This approach showed a unique possibility to remove molecules bearing ketone group from cells and tissues. However, hydrazine and its derivatives are relatively toxic,⁶⁸ which makes this approach incompatible with detoxification applications in living systems. In this respect, dynamic imine chemistry^{69,70} appears as attractive alternative to hydrazones, because it would exploit amino functional group ubiquitously present in nature.

In the present work, we explored a possibility to capture aldehyde molecules by *in situ* formation of dynamic imine bonds with highly lipophilic amine inside oil core of NEs. To this end, we synthesized lipophilic amine LipoAmine and studied its reactivity with different aldehydes inside NEs. A push-pull pyrene dye (PA) reported by us⁷¹ was selected as a model aldehyde, because of its high fluorescence brightness suitable for cell imaging and presence of the aldehyde group in the fluorophore. In this push-pull dye, the modification of the aldehyde group with an imine should alter electronic configuration of the dye and thus its fluorescence properties, which makes the imine formation detectable by fluorescence.⁷² We found that the dynamic imine bond between PA and LipoAmine can be formed *in situ* inside NEs, which leads to significant change in the emission color of the dye. The stability of this bond can be altered by low pH, leading to controlled release of the dye into the recipient medium and live cells. Even more importantly, we showed that LipoAmine-loaded NEs can effectively capture PA aldehyde from cells and tissues. Moreover, LipoAmine NEs were able to extract a highly toxic aldehyde 4HN, increasing cell survival, which provided a proof of concept of cell detoxication by NEs with help of dynamic covalent chemistry.

Results and discussion

Design and synthesis. In our design, a highly lipophilic amine encapsulated inside oil core of NEs is expected to form dynamic imine bonds with an aldehyde, which would produce lipophilic conjugate and thus effective aldehyde capture (Fig. 1A). To this end, we synthesized a lipophilic amine LipoAmine,

bearing a primary amino group and two octadecyl alkyl chains (Fig. 1B). As model aldehyde, we selected a previously reported push-pull pyrene dye PA.⁷¹ Its electron acceptor aldehyde group is expected to drastically change optical properties of the dye on formation of dynamic imine bond with amines (Fig. 1), which was previously observed in cells.⁷² The spectroscopic effect would allow us to monitor formation of the dynamic covalent bond *in situ*. On the other hand, due to the pH sensitivity of the imine bond, the formed conjugate is supposed to be cleaved under low pH, thus releasing the native cargo.

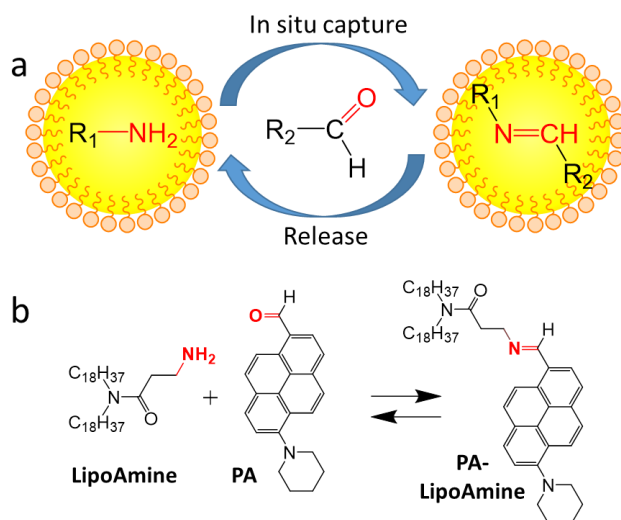


Fig. 1. (a) Scheme of *in situ* dynamic imine bond formation inside NEs for *in situ* capture and release of aldehydes. (b) Reversible chemical reaction between LipoAmine and PA aldehyde.

***In situ* formation of PA-LipoAmine conjugate.** We explored NEs as a nano-reactor for the *in situ* imine formation. To prepare NEs loaded with LipoAmine (LipoAmine NEs), LipoAmine was dissolved in oil (Labrafac WL) at 5.7 wt%, further mixed with surfactant (Kolliphor ELP®) and finally mixed with water to trigger nano-emulsification.³⁴ Blank NEs without LipoAmine were prepared as a control. Incubation of PA dye with LipoAmine-loaded NEs produced blue shifts in both excitation (~25 nm, Fig. 2a) and emission (~50 nm, Fig. 2b,c) spectra. Thus, the *in situ* reaction can be monitored by fluorescence spectroscopy. The emission spectrum blue shifted gradually over time and stabilized after 4h of incubation at 40 °C (Fig. S1), indicating the completion of the reaction. The blue shift was accompanied by the increase in the fluorescence intensity. However, when excited at 420 nm, the blue shifted band was less intense, because at 420 nm, the excitation of the newly formed conjugate is less efficient than at 380 nm, in line with the corresponding excitation spectra (Fig. 2a).

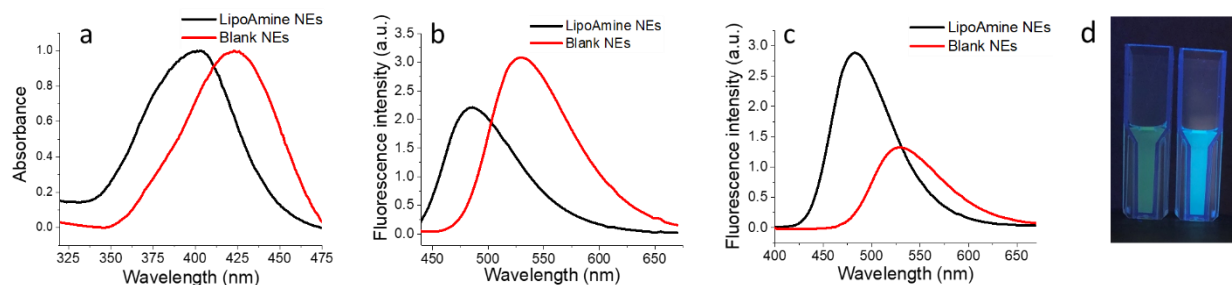


Fig. 2. Spectroscopic study of LipoAmine NEs mixed with PA versus blank NEs (without LipoAmine) mixed with PA, after 4 h incubation at rt. Normalized excitation spectra with emission wavelength at 490 nm (a) and emission spectra with excitation wavelength at 420 nm (b) and 380 nm (c). (d) Change in the emission color of NEs without LipoAmine (left) and with (right) mixed with PA, incubated for 4 h at rt and photographed under UV lamp (excitation wavelength at 365 nm).

In order to verify the formation of PA-LipoAmine conjugate, the extraction by dichloromethane of the reaction mixture was performed (with or without reduction by NaBH_4), followed by a thin layer chromatography (TLC) (Fig. 3a). According to TLC, compound isolated from the reaction mixture (yellow spot, sample 1) without NaBH_4 showed the same R_f value as its control without LipoAmine (yellow spot, sample 2). However, in the presence of NaBH_4 , which was expected to reduce imine bond to a stable amine, a novel blue spot (sample 3) was observed, characterized by higher R_f compared to its control without LipoAmine (sample 4, Fig. 3b). One should note that change in the color of PA in the control samples with NaBH_4 is probably linked to a reduction of the PA aldehyde into an alcohol. The observed much higher R_f in the presence of LipoAmine supports the formation of highly lipophilic conjugate. This blue spot (circled in red, Fig. 3b) was then isolated from TLC and studied by mass spectrometry. We found that this new spot corresponded to the molecular weight of reduced PA-LipoAmine conjugate (calculated molecular weight: 889.78 g/mol; observed 889.73 g/mol). These experiments suggested that PA-LipoAmine conjugate was formed *in situ* inside the oil core, although without NaBH_4 treatment it was hydrolyzed during the extraction process due to the instability of imine bond, yielding starting material PA dye. After the reduction of this imine by NaBH_4 into a stable amine, it was possible to identify the conjugate.

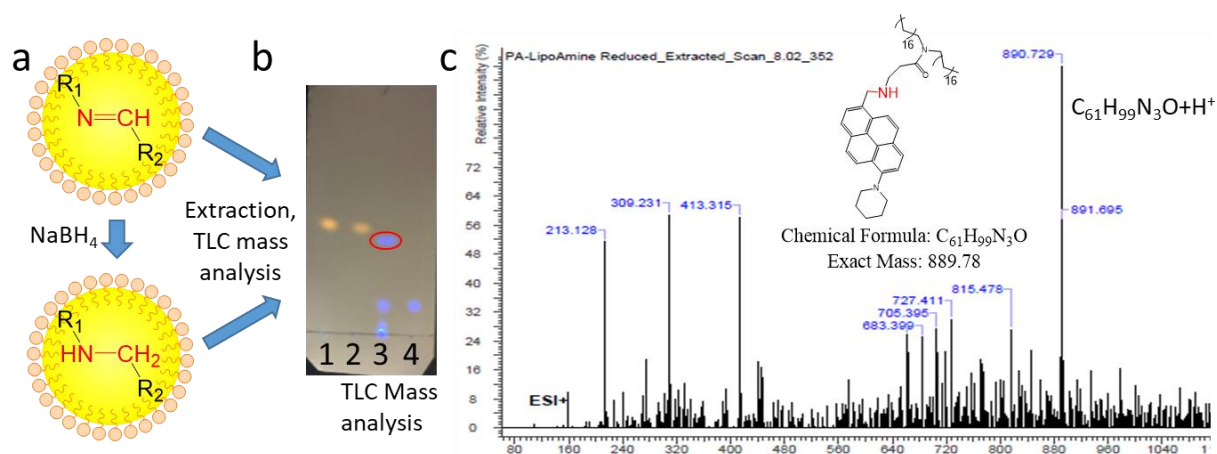


Fig. 3. (a) Scheme of PA-LipoAmine NEs reduction by NaBH₄ and extraction (by dichloromethane) for TLC mass spectrometry analysis. (b) Photographed TLC plate (under excitation of UV lamp at 365 nm) of the extraction from (1) PA + LipoAmine NEs, (2) PA + blank NEs, (3) PA + LipoAmine NEs after the reduction and (4) PA + NEs after the reduction. (c) Chemical structure and theoretical molecular weight of reduced PA-LipoAmine and TLC mass spectrometry of the new TLC spot (red mark) from the reduced reaction mixture of PA + LipoAmine NEs.

Size characterization of NEs. We compared sizes and polydispersity of different nanoemulsion droplets formulated in this study. As shown in Table S1, the presence of PA-LipoAmine or LipoAmine inside NEs did not have a significant impact on the size of NEs, with the sizes 69.0 and 70.3 nm, respectively, close to blank NEs (73 nm). Some increase in the size was observed for control sample with PA added to blank NEs (without LipoAmine). Overall, the absence of the effect of LipoAmine on NEs size and polydispersity suggests that the formulation process is not affected and LipoAmine does not interfere with interfacial properties. This highly lipophilic molecule, owing to two long octadecyl chains, is probably well encapsulated inside NEs oil core without affecting the NEs formulation process and its colloidal stability.

pH-controlled release of PA-LipoAmine. A fundamental aspect of using dynamic imine bonds is the reversibility of the loading process, which allows a controlled release of the native cargo. Based on the pH-sensitivity of imine bond,^{69,70} PA-LipoAmine loaded NEs could enable controlled release PA. As the formation of PA-LipoAmine conjugate changed the emission color of PA, pH-controlled release of PA associated with the imine bond disruption was monitored by fluorescence spectroscopy. A 10-fold excess of blank NEs were added, as an acceptor medium in the PA-LipoAmine NEs suspension. Control sample under neutral pH (7.4) did not show any spectral shift of the emission band, confirming the stability of the PA-LipoAmine inside NEs (Fig. 4a). Lower pH (for Fig. 4b,c) led to a red shift in the PA emission spectra, suggesting cleavage of the imine bond. Importantly, the release of the model dye already occurred at weakly acidic pH 6, (Fig. 4c), characteristic for some tumor microenvironment.⁷³ Then, the release accelerated at lower pH, equal to 4 (Fig. 4c), so that PA release was nearly completed after 15 min.

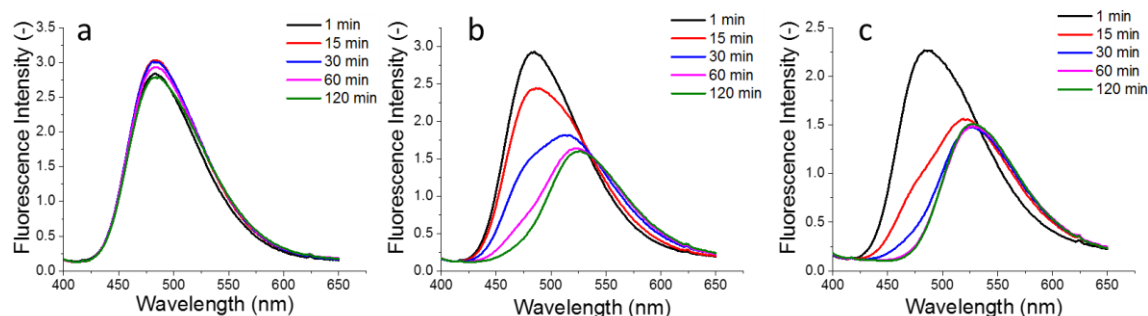


Fig. 4. pH-controlled release of PA from PA-LipoAmine NEs in the presence of 10-fold excess of blank NEs. Emission spectra under excitation wavelength of 380 nm at different pH: (a) pH 7.4, (b) pH 6 and (c) pH 4. The spectra were corrected from the blank NEs to remove of the effects of the light scattering.

In order to explore the potential of this functional NEs for controlled delivery in contact with living cells, experiments were conducted with PA-LipoAmine loaded NEs, incubated with HeLa cells, at neutral and low pH. The cellular PA fluorescence intensity could be used to detect PA released into cells. The intracellular fluorescence was readily detected after 5 min incubation with free PA under both neutral and acidic pH (Fig. 5), suggesting that PA can easily penetrate the cells. The incubation of cells with PA-LipoAmine NEs in neutral conditions showed relatively low fluorescence even after 30 min. Trace of signal could be related to slow NEs internalization, in line with previous data.⁷¹ Importantly, at pH 6, after 30 min incubation, a dramatic increase in the PA emission was observed inside the cells. This observation was confirmed by a quantitative analysis of the images (Fig. 5b). These results suggest that lipophilic NEs loaded with PA-LipoAmine conjugate inside oil core are stable at neutral pH, and release PA cargo at slightly lower pH.

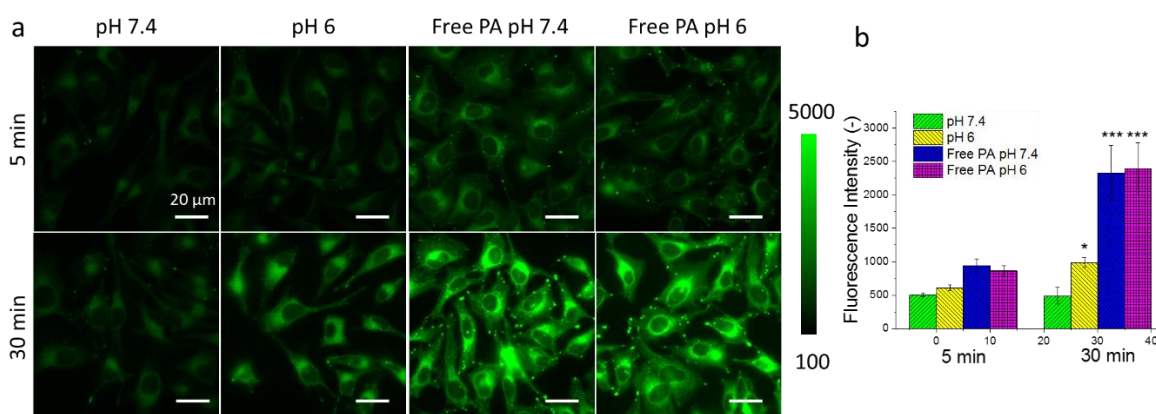


Fig. 5. pH-dependent release of PA from NEs in HeLa cells. (a) Fluorescence images of cells incubated for 5 and 30 min with PA-LipoAmine NEs at pH 7.4 and 6 and with the same concentration of free PA (at pH 6 and 7.4). Excitation was at 470 nm and the emission filter was 531/40 nm. (b) Mean fluorescence intensity of 10 cells. Statistical analysis for data at 30 min compared to the same sample after 5 min: $p < 0.1$ (*), $p < 0.001$ (***)).

***In situ* cellular uptake of PA by LipoAmine-loaded NEs.** The capacity of LipoAmine NEs to spontaneously accumulate PA opens a possibility to develop a system for capturing toxic aldehydes in biological environments. To explore this application, PA was used as a model of the aldehyde administered into HeLa cells. Then, they were incubated with LipoAmine NEs (blank NEs or PBS as controls) with an objective to perform PA extraction from the cells. After 30 min of incubation with LipoAmine NEs, drastic decrease in the fluorescence intensity of PA, by ~7-fold, was observed (Fig. 6). In contrast, only 1.4-fold drop in the fluorescence intensity was induced after incubation with blank NEs, and nearly no changes were observed for the control with PBS (Fig. 6). This tendency could also be observed in the videos (see Video S1) that showed a rapid fluorescence decrease after the incubation with LipoAmine NEs, compared to the control blank NEs and PBS. In addition, the presence of PA inside the cells induced a phototoxicity, which was higher for blank NEs and PBS compared to LipoAmine NEs, as identified by the shrinking of the cells, followed by formation of bubbles at the cell membrane in the first two cases (Fig. S2). This was the consequence of the prolonged excitation of PA in cells that generated highly toxic singlet oxygen, which was known to produce similar morphological changes in the stressed cells.⁷⁴ On the other hand, this phenomenon did not happen with LipoAmine NEs, likely due to the fast and efficient extraction of PA from the cells, thus providing first demonstration of the detoxification effect by these NEs.

Then, quantification of the PA extraction was done by fluorescence spectroscopy of the extraction media (LipoAmine NEs, blank NEs and PBS) beforehand incubated with HeLa cells (Fig. S3). Standard calibration curves of fluorescence intensity were established for PA alone in the presence of NEs. Due to different spectroscopic properties of PA-LipoAmine conjugate, calibration curves were also done for PA-LipoAmine in NEs. We found that the amounts of extracted PA from cells after 30 min were 220, 20 and 2.0 pMol, for LipoAmine NEs, blank NEs and PBS, respectively. The estimated total amount of PA uptaken by cells was 270 pmol, which was obtained from comparison of the fluorescence intensity in the extracellular medium before and after cell staining (see details in Fig. S3). Therefore, LipoAmine NEs, NEs and PBS media extracted 81%, 7% and 0.7 % of total PA from the cells, respectively. These results confirmed the microscopy data and the quantitative image analysis.

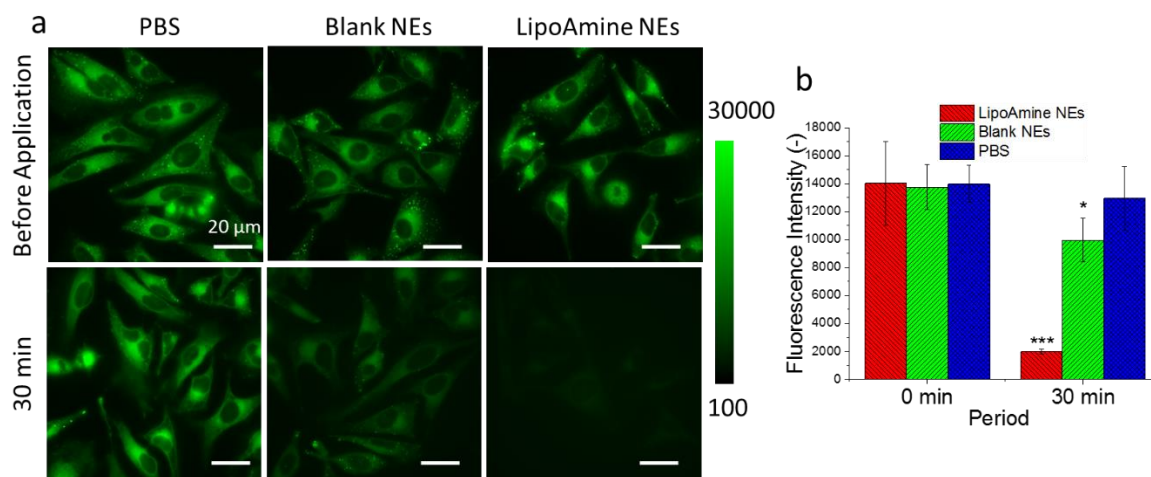


Fig. 6. Extraction of PA from HeLa cells with LipoAmine NEs. (a) Fluorescence images of cells stained with PA, before and after incubation with LipoAmine NEs, blank NEs or PBS for 30 min. Excitation was

at 470 nm and the emission filter was 531/40 nm. NEs were 200-fold diluted from the original formulation. (b) Mean fluorescence intensity of 10 cells. Statistical analysis (vs. data before NEs application): $p < 0.1$ (*), $p < 0.001$ (***)).

PA *in situ* extraction by LipoAmine NEs from tissues. Further investigation of the capability of LipoAmine NEs to extract model aldehyde dye, was conducted on model epithelial tissue from avian origin (chicken skin). Transmission microscopy image of the skin showed characteristic morphological features of epidermis tissue, of stratum corneum with specific textures (Fig. 7a). Only faint (negligible) auto-fluorescence was observed in these imaging conditions (Fig. S4). After incubation with PA solution, all tissues gave good fluorescence signal under UV lamp (excitation wavelength of 365 nm) (Fig. S5c) and especially from the texture parts of the tissue (Fig. 7), suggesting PA accumulation. After 6 h incubation with LipoAmine NEs using special 3D printed chambers (Fig. 7b), the PA fluorescence intensity in the skin tissue dropped significantly in the area contacted with the NEs (Fig. 7c), which was supported by quantitative image analysis of the extracted area ($p < 0.001$, Fig. 7d). In contrast, blank NEs produced less significant decrease in the fluorescence intensity of PA in the tissues ($p < 0.01$), whereas almost negligible changes were observed for PBS (Fig. 7). In addition, strong blue shifted fluorescence was observed in the extraction medium from LipoAmine NEs in comparison to that of NEs under UV lamp excitation (Fig. S5e), indicating *in situ* formation of PA-LipoAmine conjugate. Thus, LipoAmine NEs were able to extract aldehyde compound from the skin surface, highlighting the potential of our drug-sponge NEs as a skin detoxification tool.

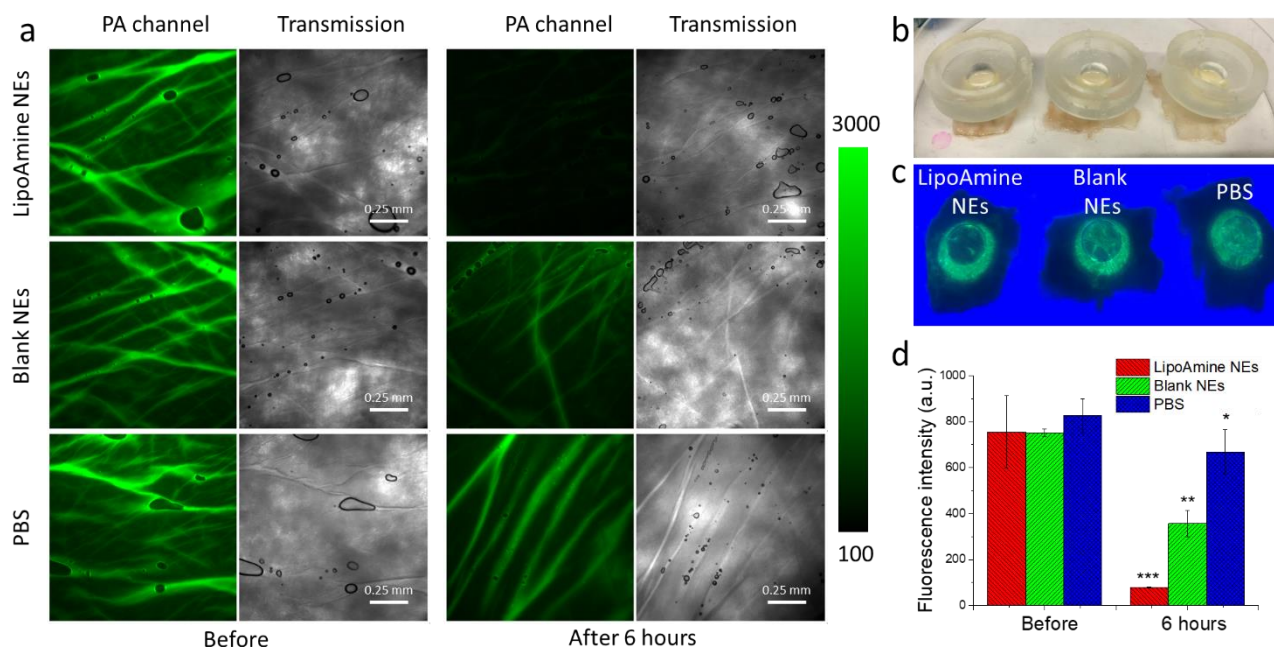


Fig. 7. *In situ* extraction of PA by LipoAmine NEs from epithelial tissue (chicken skin). (a) Transmission (grey) and fluorescence (green) images of *in situ* extraction of model aldehyde compound PA: the skin tissue was pre-incubated with 3 μ M of PA for 30 min and treated for 6 h with LipoAmine NEs, blank NEs or PBS. NEs were 100-fold diluted from the original formulation. Excitation wavelength was 470

nm and the emission filter was 531/40 nm. Scale bar: 250 μ m. (b) Experimental setup for in situ extraction of PA from chicken skin using 3D printed incubation chambers. (c) Chicken skin fluorescence after PA extraction under UV lamp (excitation wavelength: 254 nm). (d) Mean fluorescence intensity measured in the center area (3 images analyzed per condition). Statistical analysis (vs. data before application): $p < 0.1$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

Detoxification of cells from 4-hydroxynonenal by LipoAmine NEs. In order to test the potential of LipoAmine loaded NEs to capture toxic biological aldehydes, 4-hydroxynonenal (4HN) was studied as a drug model. This compound is of significant biological importance, because it is associated with numerous diseases.⁴⁸ Reports showed that this compound could induce apoptosis, causing around 60% of cell death at 20 μ M concentration in human osteosarcoma cell line MG63.⁷⁵

Firstly, *in situ* reaction was conducted between 4HN and LipoAmine inside NEs. The conjugate of 4HN with LipoAmine was successfully identified by mass spectrometry from the DCM extraction phase of the reaction mixture (Fig. S6), suggesting the potential of using LipoAmine NEs as extraction agent to detoxify cells from 4HN.

Treatment of Hela cells with growing concentrations of 4HN revealed that the latter was cytotoxic, with 45% cell viability for 20 μ M of 4HN (Fig. 8a), consistent with the earlier data on a different cell line (MG63).⁷⁵ This concentration was then applied to investigate the detoxification potential of LipoAmine NEs at different NEs concentrations. In the absence of 4HN, the viability of the cells incubated with NEs containing or not LipoAmine (Fig. 8b) was similar, showing that LipoAmine did not have significant impact on the cytotoxicity of NEs. However, both blank NEs and LipoAmine NEs displayed some cytotoxicity at high concentrations (10% from the original formulation), in line with the earlier studies.⁷⁶ This could be due to the surfactant of NEs, which might cause at high concentrations harmful effect on the cell membranes.

In the presence 4HN, significantly higher cell viability (around 62%) was observed for LipoAmine NEs compared to the blank NEs (around 42 %) at optimal NEs concentration (4% dilution from the formulation). One should note that the detoxification effect of LipoAmine NEs was observed only at concentrations ≥ 4 % of the NEs dilution. We can speculate that at lower concentrations the extraction of 4HN was too weak. At the highest used concentration of the NEs, cells with and without 4HN showed similar level of cell viability, being much higher compared to 4HN alone. The statistical analysis between the individual groups confirmed the detoxification effect of LipoAmine NEs against 4HN (Fig. S7).

Several conditions were selected to demonstrate the detoxification effect by naked eye in a 6-well plate (Fig. S8) The well 4 of the plate without 4HN showed much darker yellow color compared to the well number 1 with 4HN in PBS, indicating strong cytotoxicity effect of the latter. For blank NEs, the toxicity effect of 4HN was also clearly observed (wells 3 and 6). However, in the presence of 10 % LipoAmine NEs (i.e. 10-fold diluted NEs), the wells showed similar strong yellow color with or without 4HN (wells 2 and 5, respectively). These results are consistent with the quantitative analysis shown above.

Our results provide evidence that LipoAmine NEs are able to remove 4HN toxin from cells, which is linked to their capacity to form imine bonds with 4HN inside the oil NEs core.

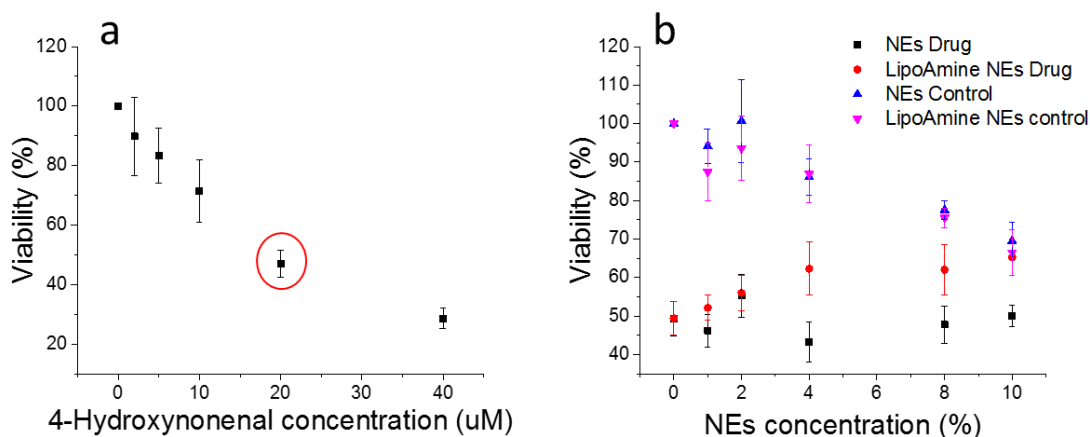


Fig. 8. (a) Viability of HeLa cells incubated for 4 h with different concentrations of 4-hydroxynonenal (4HN). (b) Detoxification study with LipoAmine NEs based on four different conditions: cells with 4HN (20 μM) treated with blank NEs (NEs 4HN); cells with 4HN (20 μM) treated with LipoAmine NEs (LipoAmine NEs 4HN); cells without 4HN treated with NEs (NEs Control); cells without 4HN treated with LipoAmine NEs (LipoAmine NEs Control).

Conclusion

In the present study, we developed a “drug sponge” platform for reversible uptake and delivery of aldehyde-bearing molecules, exploiting dynamic imine bond formation inside oil core of NEs. In this system a highly lipophilic amine (LipoAmine) was designed and its capacity to form reversible imine bonds inside NEs was investigated using a model fluorescent aldehyde, push-pull pyrene PA. We found that PA aldehyde undergoes emission color change from green to blue on incubation with LipoAmine NEs, suggesting *in situ* formation of the imine bond. The obtained PA-LipoAmine conjugate in form of reduced amine derivative was successfully identified by the mass spectrometry. It is important to note that imines cannot really form in aqueous solution because the reversible reaction between the amine and an aldehyde does not favor formation of imine poorly stable in water. In the oil core of NEs the situation is very different, because highly lipophilic LipoAmine after forming imine with PA traps it in the highly apolar oil phase and thus shifts the reaction equilibrium towards imine formation. PA-LipoAmine conjugate inside NEs was found stable at neutral pH of 7.4, while at acidic pH (pH 6 and 4), a characteristic red shifted emission indicated the cleavage of the imine bond and the release of free PA. Cell imaging experiments showed that weakly acidic conditions (pH 6) trigger the release of PA into the cells, while at neutral pH, the release of PA was minimal. Thus, drug-sponge NEs based on dynamic imine chemistry could become a platform for loading and further pH-controlled release of active molecules bearing aldehyde function. Furthermore, LipoAmine NEs showed great potential for the uptake of aldehyde compounds from cells. Using fluorescence microscopy, we showed that LipoAmine NEs can extract PA aldehyde from cells on the time scale of 30 min. Remarkably, PA capture protected

cells from phototoxic effects, suggesting that LipoAmine NEs can detoxify cells from phototoxic molecules. LipoAmine NEs also effectively removed PA from epithelial tissue (chicken skin), providing the first demonstration of detoxification of skin from model aldehyde using dynamic imine chemistry inside NEs. Moreover, successful extraction was also achieved for a highly toxic aliphatic aldehyde 4HN, which allowed to obtain a proof of concept for detoxification of the cells from toxic aldehydes. Taken together, these results show that the dynamic covalent chemistry of imine bonds inside NEs could be exploited to develop detoxification platforms, for example by simple application of NEs to the skin surface. This concept of drug-sponge NEs could be extended to other dynamic covalent bonds and green chemistry reactions, which would allow specific capture and release of other biologically active molecules.

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Conflict of Interest

The authors declare no conflict of interest.

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