Inter-nanocarrier and nanocarrier-to-cell transfer assays demonstrate the risk of an immediate unloading of dye from labeled lipid nanocapsules

Titre	Inter-nanocarrier and nanocarrier-to-cell transfer assays demonstrate the risk of an immediate unloading of dye from labeled lipid nanocapsules
Type de publication	Article de revue
Auteur	Simonsson, Carl [1], Bastiat, Guillaume [2], Pitorre, Marion [3], Klymchenko, Andrey S [4], Bejaud, Jérôme [5], Mély, Yves [6], Benoît, Jean-Pierre [7]
Pays	Pays-Bas
Editeur	Elsevier
Ville	Amsterdam
Type	Article scientifique dans une revue à comité de lecture
Année	2016
Langue	Anglais
Date	Jan-01-2016
Pagination	47-56
Volume	98
Titre de la revue	European Journal of Pharmaceutics and Biopharmaceutics
ISSN	0939-6411
Mots-clés	Cell culture [8], Fluorescent dye [9], FRET [10], Labeling of nanocarriers [11], Lipid nanocapsule [12], Size-exclusion chromatography [13]

Résumé en anglais	Release studies constitute a fundamental part of the nanovector characterization. However, it can be difficult to correctly assess the release of lipophilic compounds from lipid nanocarriers using conventional assays. Previously, we proposed a method including an extraction with oil to measure the loading stability of lipophilic dyes in lipid nanocapsules (LNCs). The method indicated a rapid release of Nile Red from LNCs, while the loading of lipophilic carbocyanine dyes remained stable. This method, although interesting for a rapid screening of the fluorescence labeling stability of nanocarriers, is far from what happens in vivo, where lipid acceptor phases are nanostructured. Here, lipophilic dye loading stability has been assessed, by monitoring dye transfer from LNCs toward stable colloidal lipid nanocompartments, i.e. non-loaded LNCs, using new methodology based on size exclusion chromatography (SEC) and Förster Resonance Energy Transfer (FRET). Dye transfer between LNCs and THP-1 cells (as model for circulating cells) has also been studied by FACS. The assays reveal an almost instantaneous transfer of Nile Red between LNCs, from LNCs to THP-1 cells, between THP-1 cells, and a reversal transfer from THP-1 cells to LNCs. On the contrary, there was no detectable transfer of the lipophilic carbocyanine dyes. Dye release was also analyzed using dialyses, which only revealed a very slow release of Nile Red from LNCs, demonstrating the weakness of membrane based assays for investigations of the lipophilic compound loading stability in lipid nanocarriers. These results highlight the importance of using relevant release assays, and the potential risk of an immediate unloading of lipophilic fluorescent dyes from lipid nanocarriers, in the presence of a lipid acceptor nanocompartment. Some misinterpretations of cellular trafficking and in vivo biodistribution of fluorescent nanoparticles should be avoided.
URL de la notice	http://okina.univ-angers.fr/publications/ua14252 [14]
DOI	10.1016/j.ejpb.2015.10.011 [15]
Autre titre	Eur. j. pharm. biopharm.

Liens

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[15] http://dx.doi.org/10.1016/j.ejpb.2015.10.011