

Lipid Pickering Nano-Emulsions Stabilized by Solid Lipid Nanoparticles as Potential Carrier for Biomedical Applications

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Abstract

In this paper, we investigated the behavior of Pickering nano-emulsions stabilized by solid lipid nanoparticles, when administrated by oral route. A first part of the study is dedicated to the characterization and stability studies of solid lipid nanoparticles (SLNs) and SLNs-stabilized Pickering nano-emulsions, against incubation in gastric and intestinal mediums. Characterizations were performed by dynamic light scattering and transmission electron microscopy. The SLNs measured around 40 nm, while the resulting nano-emulsions reached approximately 200 nm with low polydispersity. Transmission electron microscopy confirmed their spherical and homogeneous morphology. Differential scanning calorimetry revealed a thermal transition between 30°C and 40°C, indicating temperature sensitivity suitable for controlled drug release. The second part of the study was related to interactions, cellular uptake, and toxicology of the SLNs-stabilized Pickering nano-emulsions incubated with different cell lines: a healthy cell line MSC-2 and three cancer cell lines A549, U87 and MG-63. This work allows emphasizing the optimal conditions for cellular interactions without toxicity, showing the potential of these lipid Pickering nano-emulsions to be used for therapeutic and diagnostic purposes. *In vitro* digestion studies showed good stability of the emulsions, even in the presence of digestive enzymes. Cytotoxicity assays on healthy (MSC-2) and cancerous (A549, U87, MG-63) cell lines showed no toxicity at low concentrations. At 1/1000 and 1/10,000 dilutions, cell metabolism was slightly enhanced, suggesting active uptake. Fluorescence imaging confirmed efficient internalization of

intact nano-emulsions into cells. These findings highlight the biocompatibility and stability of the system. Overall, the developed Pickering nano-emulsions show strong potential as safe drug delivery carriers.

Keywords

Pickering Nano-Emulsion, Solid Lipid Nanoparticles, Biocompatibility, Cellular Uptake, Thermo-Sensitivity, *In Vitro* Digestion

1. Introduction

Current anticancer treatments are, still today, insufficient because of the heterogeneity of the tumor or the drug resistance, leading to frequent relapses [1]-[7]. To improve therapeutic efficacy and minimize side effects, current treatment options are encapsulation of anticancer drugs in therapeutic vectors that have been extensively studied [8]-[11]. An emerging solution lies in Pickering nano-emulsions, consisting in nano-emulsions stabilized by nanoparticles, used as a drug delivery system. Unlike conventional nano-emulsions stabilized by molecular surfactants, the Pickering nano-emulsions studied here are stabilized exclusively by solid lipid nanoparticles (SLNs), which are biocompatible and free of surfactants. These solid particles irreversibly adsorb at the oil/water interface, forming a robust physical barrier that prevents droplet coalescence. This mode of stabilization provides enhanced kinetic stability and greater resistance to biological environments, while avoiding the adverse effects commonly associated with traditional surfactants. Moreover, the solid and lipidic nature of the SLNs enables controlled thermal sensitivity through phase transition, offering a promising strategy for targeted drug release. These features clearly distinguish Pickering nano-emulsions from classical emulsions, both structurally and functionally.

Pickering nano-emulsions herein studied are composed of parenteral-grade lipid and biocompatible lipid nanoparticles, *i.e.* surfactant free, in order to maximize the physiological tolerance. The use of Pickering nano-emulsions has several advantages, including non-use of organic solvent, large scale production capacity, high bioavailability, protection against degradation of agents such as water and light, and better controlled release of the drug [12]-[16]. These nano-emulsions stabilized by solid lipid nanoparticles (SLNs) can be considered as a particular type of nano-emulsion that does not require the use of a large quantity of surfactants or inorganic nanoparticles (since the solid lipids used are also biocompatible). In general, many studies have shown the stabilization of nano-emulsions by colloidal particles giving Pickering nano-emulsions. Herein we propose a new generation of safe, non-toxic, biocompatible and surfactant-free nano-platform based on Pickering nano-emulsions [14]-[17], using several types of stabilizing particles [18]-[27].

The main objective of this work is to study Pickering nano-emulsions stabilized

by solid lipid nanoparticles (SLNs), with the additional advantage of a temperature sensitivity triggering the nano-emulsion destabilization and drug release, due to the SLNs phase transition [14]. SLNs are typically made up of physiological lipids that are biodegradable, non-toxic, and have a strong dispersibility in aqueous medium and offer simple and scalable preparation methods. In this study, nano-emulsions stabilized by SLNs were formulated, and an investigation was led regarding the *in vitro* digestion in the gastric phase and the intestinal phase. In parallel, complementary nano-emulsion characterizations of the phase behavior were carried out by differential scanning calorimetry. A last part of the investigation dealt with *in vitro* studies on a healthy and three different cancerous cell lines.

2. Materials and Methods

2.1. Materials

Suppocire® C (mixture of mono-, di- and triglyceride esters of fatty acids, C10 to C18) purchased from Cooper (Melun, France) and Kolliphor® HS15 (mixture of free polyethylene glycol 660 and 12-hydroxystearate of polyethylene glycol 660) purchased from Sigma-Aldrich (Saint Quentin Fallavier, France) were used. Labrafac® WL 1349 (MCT, Gattefossé S.A., Saint-Priest, France) was used as the oil phase for the formulation of Pickering emulsions. It is made up of medium chain triglycerides, *i.e.* a mixture of caprylic and capric acid triglycerides. Both for the formulation of the SLNs and for that of the nano-emulsion, MilliQ water obtained from a Millipore Super-Q unit C79625 was used. All ingredients used were of analytical grade and were used as received.

2.2. Methods

The formulation of the SLNs by spontaneous emulsification, and Pickering nano-emulsion by ultrasonication was carried out as described in previous reports [14]-[17] and is briefly described below.

2.2.1. Formulation of Solid Lipid Nanoparticles (SLNs)

Kolliphor HS15 and Suppocire C are mixed and melted at a temperature of 90°C (SLNs were made with SLR = 65% and the surfactant lipid to water ratio (SLWR = $100(w_{\text{surf.}} + w_{\text{lipid}})/(w_{\text{surf.}} + w_{\text{lipid}} + w_{\text{water}})$, where w_{water} is the weight of the aqueous phase) was 43.5%). The aqueous phase (MilliQ water) is also heated at the same temperature. The two phases are mixed and the nano-emulsions form spontaneously. The nano-emulsion is cooled to room temperature for one hour to allow the solidification of the droplets. The washing of the SLNs is carried out by dialysis in MilliQ water. We put the suspension of SLNs in 8 - 12 kDa porous dialysis tubes and immersed them in MilliQ water with constant stirring at 300 rpm for 48 hours, changing the water after 24 hours.

2.2.2. Formulation of Pickering Nano-Emulsions

After washing the suspension of SLNs, the dialysate obtained is used as aqueous phase and the particles of SLNs in suspension as sole stabilizer of the nano-emul-

sions. The volume fraction of dispersed phase is 20%. A first emulsion is made using an Ultraturax shaker (IKA T-25 Digital) operating at 22,400 rpm for 3 min, in ice bath. The resulting emulsion is homogenized by ultrasonication (power = 150 W, 20 kHz, cycles of 10 seconds “on”/20 seconds “off”, total time = 3 min, in ice bath). The nano-emulsions (O/W) obtained are then stored at 4°C until analysis. All the experiments were performed at 25°C, in triplicate.

2.3. SLN and Pickering Nano-Emulsion Characterization

2.3.1. Dynamic Light Scattering (DLS)

Thanks to a NanoZS Malvern apparatus (Malvern, Orsay, France) we determined the size distribution and the polydispersity index by dynamic light scattering (DLS). The helium/neon laser, 4 mW, was operated at 633 nm, with the scatter angle fixed at 173°. DLS data were analyzed using a cumulant-based method. The temperature was maintained at 25°C and the measurements were done in triplicate.

2.3.2. Transmission Electron Microscopy (TEM)

TEM observations are made at a voltage lower than that usually used in materials science to avoid any oil vaporization due to the electron beam in the TEM chamber. A Philips Morgagni 268D microscope operating at 70 kV was used for this purpose. The experiment is performed without any coloring agent. A drop of the suspension was placed on the carbon grid (carbon type-A, 300 mesh, copper, Inc. Redding, PA) and drying was carried out at room temperature. Under such conditions lipids gave significant contrast.

2.3.3. Differential Scanning Calorimetry (DSC)

The TA DSC Q200 allowed us to carry out the differential scanning calorimetry studies. The indium standard was used to calibrate the DSC temperature and enthalpy scale. We seal the powder samples in aluminum trays and heating is performed at a constant rate of 10°C/min, over a temperature range of 20°C - 170°C. Inert atmosphere was maintained by purging nitrogen at the flow rate of 15.8 mL/min, linear velocity 35 cm/sec and pressure 24.7 kPa.

2.4. *In Vitro* Digestion Test

Each nano-emulsion underwent a three-step *in vitro* digestion model simulating gastric and small intestine digestion. The particle size and charge of the samples were measured after incubation in each stage. Simulated gastric fluid (SGF) was prepared by dissolving NaCl (2 g) and HCl (7 mL) in water (total 1 L volume), then the pH was adjusted to 1.2 [28] [29]. The simulated intestinal fluid (SIF) contained 2.5 mL pancreatic lipase solution (60 mg, PBS), 3.5 mL bile extract solution (187.5 mg, PBS, pH 7.0, 37°C) and 1.5 mL salt solution (0.5 M CaCl₂ and 5.6 M NaCl in water).

Different pH conditions were maintained during the *in vitro* digestion test to simulate the physiological environment of the human gastrointestinal (GI) tract. The gastric phase was performed at pH 1.2, which mimics the acidic environment

of the stomach, where enzymes such as pepsin are active and able to initiate the breakdown of lipids and proteins. In contrast, the intestinal phase was adjusted to pH 7.0, reflecting the neutral to slightly basic pH of the small intestine, where pancreatic lipase and bile salts function optimally to further digest lipids and facilitate absorption. This pH variation is crucial for assessing the structural stability and integrity of the nano-emulsions under realistic digestive conditions and for predicting their behavior and drug release profile during oral administration.

2.4.1. Gastric Phase

Simulated gastric fluid (SGF, 20 mL) was added to the sample (20 mL). The pH was adjusted to 1.2, and the mixture was then incubated for 2 h (37°C, 100 rpm) under agitation.

2.4.2. Small Intestinal Phase

The sample (30 mL) obtained from the gastric phase was incubated in a water bath to maintain it at 37°C. The pH was then adjusted to 7.0. Bile extract and salt solutions were added into the sample. The resulting mixture was re-adjusted to pH 7.0, and then pancreatic lipase solution was added.

2.5. Cell Culture

U-87 (ATCC HTB-14), MG-63 (ATCC CRL1427), A-549 (ATCC CCL-185) and MSC-2 (respectively glioblastoma, osteosarcoma and lung tumour cell lines, and mesenchymal stem cells) were purchased from ATCC and used for the *in vitro* biological evaluation. Mesenchymal Stem Cell Growth Medium MSC-2 was purchased from PromoCell (Heidelberg, Germany). All the cell handling procedures were performed in sterile conditions under a laminar flow hood, and the cells were grown in a controlled atmosphere at 37°C, 5% CO₂, and humidity conditions. Cells were detached from culture flasks by trypsinization, centrifuged, and resuspended in an appropriate volume of cell culture media to perform a Trypan Blue Dye Exclusion test to assess cell viability and to count the cells.

One millilitre vial containing 1×10^6 cells is defrosted. About 500,000 cells of each line were seeded in a T75 flask. The cells were incubated at 37°C in a water saturated atmosphere at 5% CO₂. They are cultivated 3 - 4 days until they reach 80% confluence while changing the environment regularly. When the cells have reached confluence, they are harvested with trypsin. The flasks are removed from the incubator and 6 mL of culture medium are added to inactivate trypsin. The content is transferred to a 50 mL Falcon tube. Finally, the tube is centrifuged and the cell pellet is recovered.

2.6. Cell Viability

Cell viability tests are performed using a healthy (MSC-2) and cancerous (A549, U87, MG-63) cell lines. The cells are treated with the suspension dilutions of NE-SLNs (1:100, 1:1000, 1:10,000), 24 - 72 h before performing the Alamar Blue cytotoxicity assay. One tenth of the volume of reagent is directly added to the cells of

the culture medium. The cells are then incubated for one to four hours at 37°C in a cell culture incubator protected from direct light. The results are recorded using fluorescence. The fluorescence excitation wavelength is 540 - 570 nm and the emission wavelength is 580 - 610 nm. All the experiments were triplicated.

2.7. *In Vitro* Imaging

The trypsin is inactivated by adding the culture medium and everything is transferred to a 50 mL Falcon tube. The tube is centrifuged. Then, the cell pellet is recovered and resuspended in 3 mL of culture medium. Sterilized slides are placed in the wells of 24-well plates and are intended to receive the cultured cells. Around 3×10^4 cells are put in each well. Then, 1 mL of culture medium containing the suspension of Pickering nano-emulsions (at a dilution of 1/2000) is added to each well. After 1 h, 24 h and 48 h the lamellae are removed and then 4% PFA is added for 20 minutes to fix the cells. After washing with PBS, DAPI is added to mark the nuclei of the cells in blue. Finally, the cells are washed with PBS and the slides are dried before visualization under a fluorescence microscope.

3. Results and Discussion

3.1. Characterizations of SLNs and Pickering Nano-Emulsions

3.1.1. SLNs Size and Polydispersity Index

SLNs size relies on the formulation parameters, notably values of SLRs that were varied by fixing the proportion of solid lipids (Suppocire® C) and modifying those of nonionic surfactant (Kolliphor® HS15). The representative value of SLR, of 65%, was selected (from previous report [14]) to produce SLNs sizing to 40 nm for which the size distribution is reported below.

3.1.2. Pickering Nano-Emulsion Size and Polydispersity Index

The sizes of SLNs (40 nm) are used for the preparation of Pickering nano-emulsions. A volume fraction of dispersed phase between the aqueous suspension (containing SLNs) and Labrafac® WL 1349 was fixed ($\phi_d = 20\%$). As a result of nano-emulsification by ultrasonication, nano-emulsions obtained were exhibiting a milky and translucent aspect. Results are reported in **Figure 1**, showing narrow size distributions of SLNs suspension and Pickering nano-emulsions, with mean sizes of 40 nm and 200 nm, respectively (and values of PDI equal to 0.098 and 0.116 respectively). These results show the feasibility of the formulation of Pickering nano-emulsions, providing monodisperse dispersion of oil nano-droplets, stabilized by SLNs and surfactant-free.

3.1.3. Transmission Electron Microscopy of SLNs and Pickering Nano-Emulsions (TEM)

The morphological characterizations of the nano-emulsions were carried out by transmission electron microscopy (TEM). The nano-emulsions appeared as round spheres with a size consistent with the dynamic light scattering (DLS) results. TEM images of Pickering nano-emulsions have been reported in **Figure 1**, showing spher-

ical, contrasting and distinct features with a size of approximately 160 nm and appearing homogeneously dispersed. These results also seem in agreement with the DLS measurements, proving by direct observation the feasibility of the formulation of the Pickering nano-emulsion by SLNs, and confirming our previous hypotheses. Moreover, they are also in line with the microscopic observations reported in the literature for Pickering emulsions stabilized by reference solid particles. As conventional nano-emulsions (stabilized by surfactants) are particularly stable for steric reasons, this could also be the case here for similar reasons due to the aggregation of SLNs at the interface.

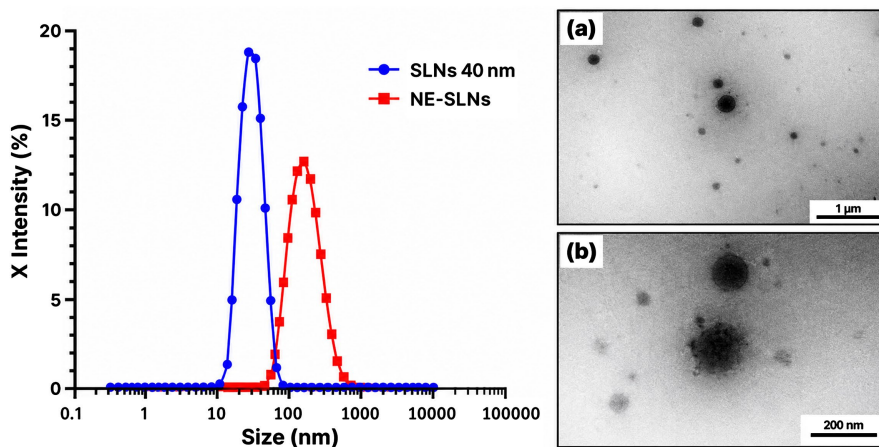


Figure 1. Size distributions measured by dynamic light scattering for the SLN suspension (SLNs 40 nm) and the Pickering nano-emulsion (NE-SLNs), together with a transmission electron microscopy (TEM) image of a Pickering nano-emulsion stabilized by solid lipid nanoparticles (SLNs). The same micrograph is shown at two different magnifications: (a) general view at 1 μm scale illustrating droplet distribution, and (b) zoomed-in view at 200 nm scale highlighting a nanodroplet surrounded by SLNs adsorbed at the interface. These observations confirm the typical structure of Pickering nano-emulsions.

3.2. DSC

The DSC thermograms of the nano-emulsion suspension are shown in **Figure 2**, where the heating rate of 5 °C/min shows no thermal events in the temperature range 0 °C to 70 °C, and significant melting from 80 °C onwards. The results were compared with Suppocire C and bulk Kolliphor HS15 (previous report [14]), for which endothermic peaks are observed between 21 °C and 33 °C.

For nano-emulsions, thermal events were due to Suppocire® C and Kolliphor® HS15, as these were the only components that underwent a solid-liquid transition in this temperature range. The melting and crystallization differences between Suppocire® and Kolliphor® HS15 in bulk compared to SLNs and nano-emulsions were due to compartmentalization in the nanometric size domain. These results mean that the SLNs may still be in a glassy state below 30 °C and, between 30 °C and 40 °C, partially melt. This informs us of the fact that, in the temperature range where our experiments were conducted (*i.e.* $T < 40^\circ\text{C}$), we can consider that the particles are either glassy or at least partially melted.

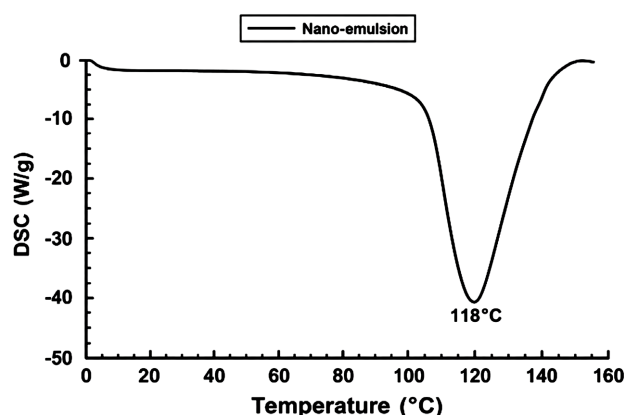


Figure 2. Differential scanning calorimetry thermograms of nano-emulsion.

3.3. *In Vitro* Digestion

Pickering nano-emulsions stabilized by SLNs are complex nanoparticulate lipid systems. Oral lipids administered in different forms, such as bulk oils, lipid emulsions and nano-emulsions (and their subsequent digestion and adsorption of active encapsulated substances) play an essential role in the design of these drug delivery systems. We investigated the size variations occurring in the simulated gastric medium with and without pepsin (as major hydrolysis agent in digestion).

It appears from these results that the droplets have retained their integrity until the end of the treatment. In both cases, a slight evolution of the size distribution is observed, increase in the size and distribution of the population, emphasized in presence of enzyme. This modification can be due to the droplet destabilization process enhancing Ostwald ripening, originated from temperature increase or interactions with components of the incubation medium. In that case of Pickering nano-emulsions stabilized with thermo-sensitive SLNs, we have shown [14] that temperature plays a significant role in the destabilization of such nano-emulsions; however, the impacts of the presence of enzymes on the destabilization (**Figure 3(b)**) also testify that the composition of the incubation medium has a non-negligible impact. To study the behavior of NE_{SLNs} during digestion, they were subjected to the simulated gastric medium (SGF) and then to simulated intestinal medium (SIF). The results shown in **Figure 3(a)** and **Figure 3(b)** show a gradual size increase upon the simulated digestion process, suggesting that nano-emulsions have retained their integrities and that enzymes induce their slight destabilization.

The incubation times selected for the gastric and intestinal phases of the *in vitro* digestion test were based on the typical residence times of the respective parts of the human GI tract: 2 hours for the stomach and 2 - 4 hours for the small intestine. This standard simulation model enables a more realistic evaluation of the stability and integrity of lipid-based delivery systems, such as Pickering nano-emulsions. Enzymatic digestion was mimicked using pepsin in the gastric phase and pancreatic lipase in the intestinal phase, alongside bile salts and a calcium/sodium salt mixture. These conditions enabled us to observe the effects of enzymatic action

and pH variation on droplet size distribution, emulsion destabilization and the release behavior of the encapsulated compounds.

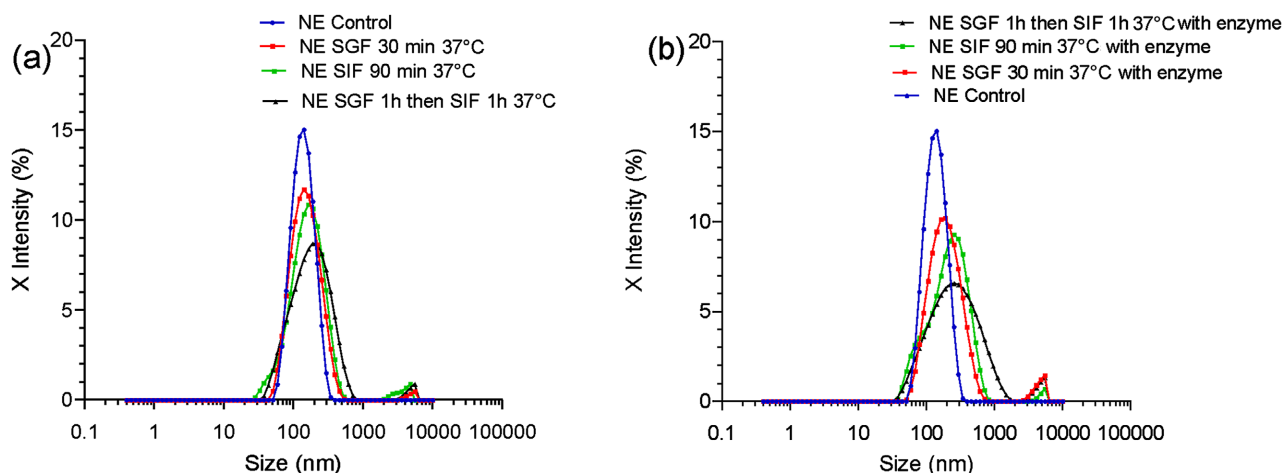


Figure 3. *In vitro* digestion test of Pickering nano-emulsion: (a) Without enzyme and (b) With enzyme.

3.4. Cell Viability

The biocompatibility of nano-emulsions has been tested on four cell lines. The healthy mesenchymal cell line MSC-2 and three cancer cell lines MG-63, A549 and U87 were used. As a non-radioactive colorimetric test, the Alamar Blue test was selected to assess cell viability and proliferation, as it indicates the metabolic capacity of the cell population used for the cytotoxicity test. Resazurin is directly incubated in the cell culture after the nano-emulsion exposure period. The living cells convert, after 1 - 4 hours, this molecule into a fluorescent product (resorufin) whose quantity is proportional to the number of living cells. The concentration of resorufin was quantified by optical absorbance at 570 nm and 595 nm. The mean value and the standard deviation are the results of three measurements. The test was carried out in three independent experiments and in each experiment the samples were tested in triplicate. The data were expressed as a percentage. As shown in **Figure 4**, the NE_{SLNs} at a dilution of 1/1000 and 1/10,000 showed no cytotoxicity at the different measurement times (24 h, 48 h and 72 h), whereas for the 1/100 dilution and the positive control a decrease in cell viability was observed. On the other hand, it should be noted that the concentrations 1/1000 and 1/10,000 of the NE_{SLNs} increase cell metabolism rates probably due to an active absorption process.

3.5. *In Vitro* Imaging

The following sections on *in vitro* cell experiments and imaging focused on the study of the impact of this system (Pickering nano-emulsion) on living systems. Previous studies [30] [31] have shown that the interactions between droplets and cells in culture are limited and that the cellular uptake relied on the nature of the oily body and surface composition. In the present study, Pickering nano-emul-

sions were incubated with the cells for 2 h at 37°C. To allow the visualization of the droplets under the microscope, a lipophilic dye was introduced into the oily core at 0.5% by weight in the oil. This fluorophore is a modified dye (NR668) which, once encapsulated in the nano-emulsions, has been shown to not leak into surrounding tissues or lipophilic receptors [31]–[33]. Thus, this tool ensures an effective control of the location of the nano-emulsion droplets in fluorescence imaging.

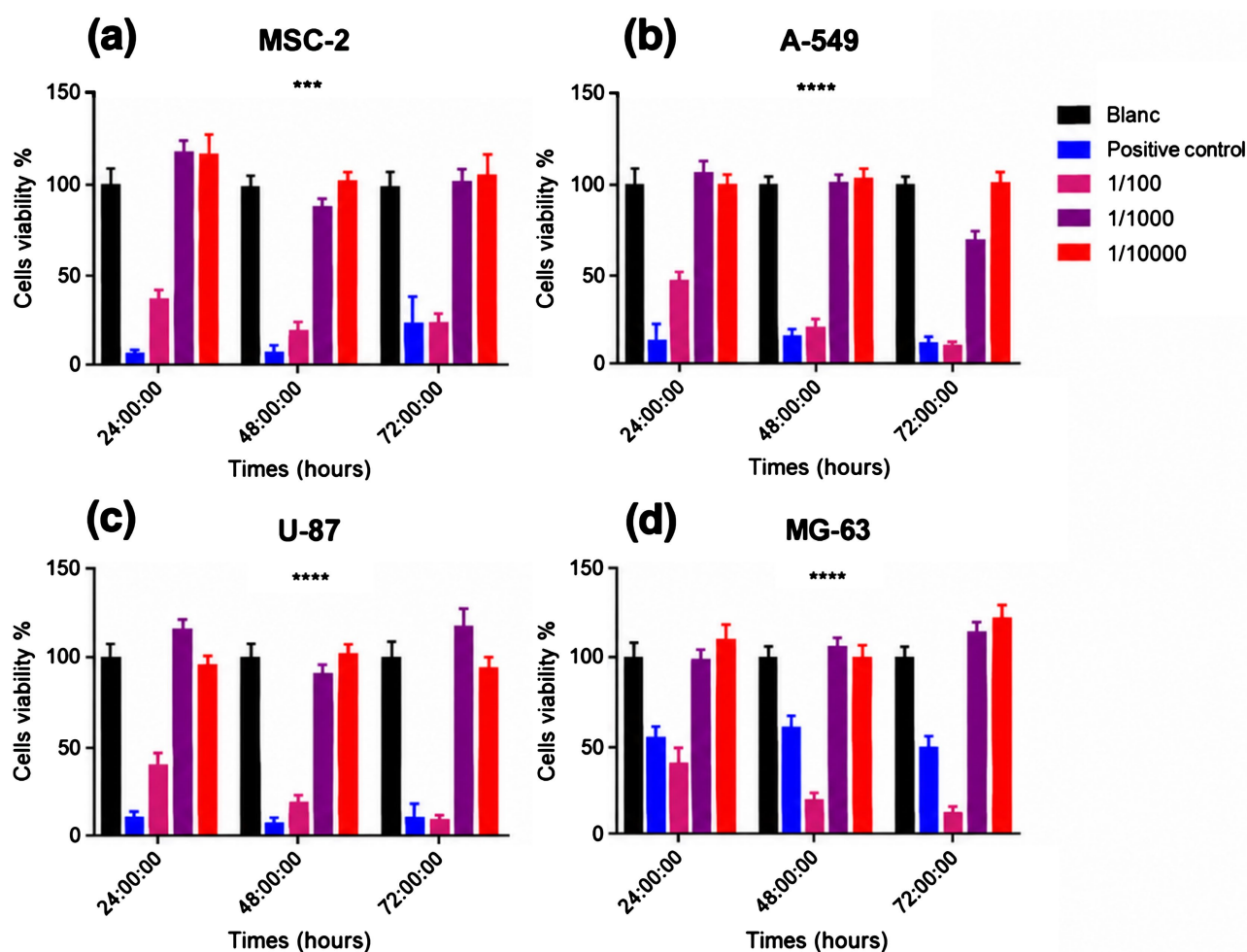


Figure 4. Cell viability of MSC-2, A-549, U-87, and MG-63 cells assessed by the Alamar Blue assay after 24, 48, and 72 hours of exposure to the Pickering nano-emulsion at increasing concentrations. Cell culture medium was used as the control condition. Results are expressed as mean $\pm$ standard deviation (SD), $n = 3$. *A statistically significant difference compared to the control ($p < 0.005$).

The results are reported in Figure 5, in which the nuclei of the cells and the nano-emulsion droplets appear in blue and red, respectively. After incubation with the NE_{SLNs} suspension for 2 h, 24 h and 48 h in the culture medium at 37°C, the A549, MG-63 and U87 cancer cells were fixed and the cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI). The images of the cells were acquired by fluorescence microscopy with a wavelength of 650 nm for the dye (Nile Red lipophilic derivative NR668 we used in previous reports [30] [31]), and a

wavelength of 560 nm for the DAPI. Two formulations were used: (i) one with NR668 incorporated into the oil core of NE_{SLNs} and (ii) one with NR668 incorporated into SLNs.

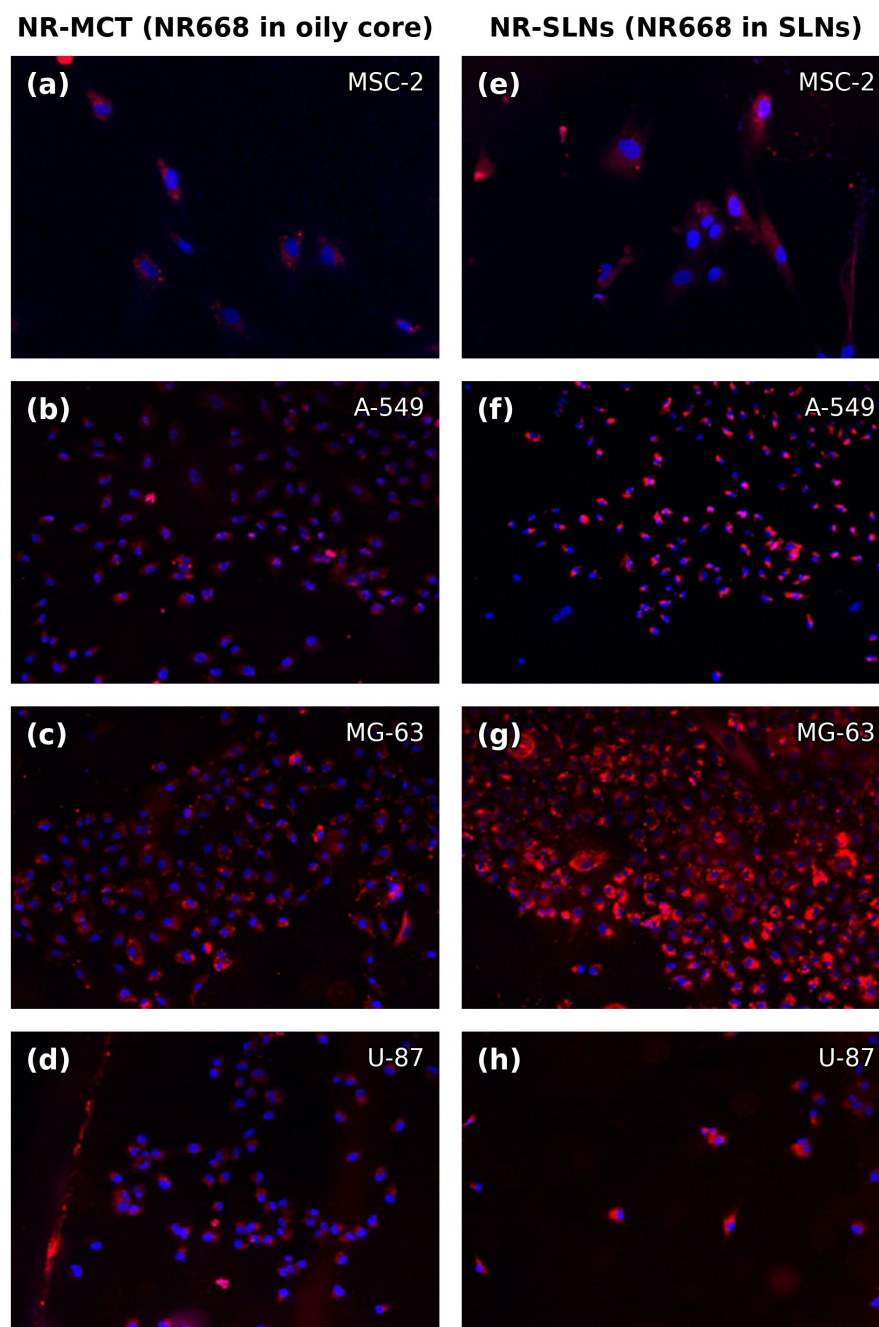


Figure 5. Fluorescence microscopy images of MSC-2, A-549, MG-63, and U-87 cells incubated with NR668-labeled Pickering nano-emulsions. Two labeling strategies were used: (a)-(d) NR-MCT, where NR668 was incorporated into the oily core (MCT) of the nano-emulsion; and (e)-(h) NR-SLNs, where NR668 was incorporated into the solid lipid nanoparticles. Blue: nuclei stained with DAPI; red: NR668 fluorescence. Images were taken after 2 h of incubation. The fluorescent signal confirms cellular internalization of the nano-emulsions and the integrity of the nanodroplets during uptake.

Figure 5 shows that intense red fluorescence has been observed in the cell cytoplasm. These results demonstrate that NE_{SLNs} are effective for contrast cell imaging using Nile Red. A homogeneous distribution of NE_{SLNs} loaded with NR668 was observed. However, this alone does not allow us to conclude that the NE_{SLNs} are intracytosolic. To prove that the NE_{SLNs} are intracytosolic, we encapsulated NR668 in the core of the nanodroplets or in the stabilizing particles. The intense red fluorescence that has been obtained confirms both intracytosolic localization and the integrity of NE_{SLNs}, whatever the type of cell tested. NE_{SLNs} are efficiently absorbed into cells. These results demonstrate that Pickering nano-emulsions can be used to effectively encapsulate and deliver active drug ingredients in cells.

In general, when the core of nanodroplets is composed of triglycerides and without modification of the formulation, nano-emulsions exhibit a stealth behavior towards cells, mainly due to the PEGylated surface. In the case of Pickering nano-emulsions, a specific interaction is observed. In addition, we observe co-localization of the signal when the fluorescent probe is in the nano-emulsion core or when it is in the SLNs, showing that, when the droplets integrate the cells, they are not destroyed. Therefore, these nano-emulsions open new doors for effective drug delivery applications. For example, when a drug needs a carrier to cross the cell membrane, these nano-emulsions combine a retention and permeation-enhancing effect that passively targets certain tumours. They may therefore be an alternative to active targeting technologies for crossing the tumour cell membrane once they reach the tumour microenvironment.

4. Conclusions

This study explores the potential of SLN-stabilized Pickering nano-emulsions for use in biomedical applications. This development aims to reduce the potential toxicity of surfactant- and polymer-free lipid nanocarriers, while also enabling the stabilization of these nanocarriers to be controlled by exploiting the partial melting of SLNs at different temperatures. DSC data show that SLNs significantly alter the melting point of Pickering nano-emulsions. Since the emulsion interfaces are dynamic, the limitation of cell internalization is circumvented by using stabilizing particles, resulting in a modified nano-emulsion surface. Upon contact with cultured cells, these nano-emulsions interact strongly and are internalized by the cells in their native form. In fact, in this work, we demonstrated that SLN-stabilized Pickering nano-emulsions are biocompatible and are internalized by cells in intact form.

Pickering nano-emulsions show strong potential for the targeted delivery of anti-tumor agents. Their temperature sensitivity enables optimized drug release in pathological areas. They can also carry antioxidant or anti-inflammatory compounds to modulate oxidative stress or inflammation. The absence of chemical surfactants enhances their biocompatibility and reduces side effects. A tailored formulation could allow for selective release at the disease site. These features position them as promising carriers for smart and personalized therapies.

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Data Availability Statement

All generated and required physicochemical data are reported in this manuscript.

Author Contributions

Conceptualization, S.M.D., N.B.-J., M.D. and T.V.; methodology, S.M.D., Y.I.-G. and T.V.; software, S.M.D.; validation, S.M.D., N.B.-J., M.D. and T.V.; formal analysis, S.M.D., N.B.-J. and T.V.; investigation, S.M.D.; resources, M.D., N.B.-J. and T.V.; writing—original draft preparation, S.M.D., N.B.-J., M.D. and T.V.; writing—review and editing, S.M.D., N.B.-J., P.M.S., M.J.A.C., M.D. and T.V.; visualization, S.M.D., N.B.-J., M.D. and T.V.; supervision, S.M.D., N.B.-J., M.D. and T.V.; project administration, S.M.D., N.B.-J., M.D. and T.V.; funding acquisition, S.M.D., N.B.-J., M.D. and T.V. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

SLNs: Solid Lipid Nanoparticles;

NE: Nano-Emulsion;

NE_{SLNs}: Nano-Emulsion Stabilized by Solid Lipid Nanoparticles;

MCT: Medium Chain Triglycerides;

SGF: Simulated Gastric Fluid;

SIF: Simulated Intestinal Fluid;

NR668: Nile Red 668 (lipophilic fluorescent probe);

MSC-2: Mesenchymal Stem Cells (line 2);

A549: Human Lung Carcinoma Cell Line;

U87: Human Glioblastoma Cell Line;

MG-63: Human Osteosarcoma Cell Line.