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In vitro cytotoxicity assays of solid lipid nanoparticles in epithelial and dermal cells

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Abstract. In recent years, the interest in nanostructured systems to drug delivery has increased because they offer several advantages over conventional dosage forms. Solid Lipid Nanoparticles (SLN) have been highlighted among these systems because they have advantages such as high physical stability, protection against drug degradation and ease of scale-up and manufacturing, without using organic solvent. The aim of this work was to evaluate the potential of SLN, by in vitro cytotoxicity assays, for dermal drug delivery. SLN of three different lipids were prepared by hot high pressure homogenization and the cytotoxicity was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test in mouse 3T3 fibroblasts and human HaCaT keratinocytes. SLN showed no cytotoxic potential suggesting a great potential for dermal application.

1. Introduction

In recent years, the interest in nanostructured systems to drug delivery has increased because they offer several advantages over conventional dosage forms such as increased therapeutic efficacy and reduction of collateral effects and toxicity [1]. Solid Lipid Nanoparticles (SLN) were developed at the beginning of the 1990s as an alternative carrier system to emulsions, liposomes and polymeric nanoparticles. They offer several advantages compared to other colloidal systems such as high physical stability, protection against drug degradation due to the solid state of lipid matrix and ease of scale-up and manufacturing, without using organic solvent. SLN are produced by replacing the liquid lipid (oil) of an o/w emulsion by a solid lipid (at both room and body temperature) or a blend of solid lipids [2].

Nanoparticles can cause cytotoxicity by adherence of the particle to the cell membrane, degradation and subsequent release of cytotoxic degradation products. Another mechanism is the internalization of nanoparticles by cells, intracellular degradation and subsequent toxic effects inside the cell [3]. The first step towards assessing the safety of a new formulation often involves *in vitro* cytotoxicity test methods. The majority of cytotoxicity assays used throughout published nanoparticles studies measure cell death via colorimetric methods that assess membrane integrity or mitochondrial activity.

Mitochondrial activity can be tested using tetrazolium salts as mitochondrial dehydrogenase enzymes cleave the tetrazolium ring. Only active mitochondria contain these enzymes; therefore, the reaction only occurs in living cells. The most widely used test is the MTT viability assay. MTT is pale yellow in solution but produces a dark-blue formazan product within live cells [4].

Schöler et al. found that the cytotoxicity of SLN assessed by MTT test on murine peritoneal macrophages was concentration dependent and influenced by the lipid matrix [5]. The cytotoxicity of SLN was also found to be dependent on the type and concentration of surfactant used [6-9]. Therefore, the choice of the lipid matrix and the surfactant is essential in order to formulate a safe preparation.

The aim of this work was to assess the cytotoxic potential of SLN, by *in vitro* cytotoxicity assays, using fibroblasts and keratinocytes that are the predominant resident cells in dermis and epidermis, respectively, in order to evaluate the potential of SLN for dermal drug delivery.

2. Methods

2.1. Solid Lipid Nanoparticles (SLN) preparation

The solid lipids used, cetyl palmitate (CP), myristyl myristate (MM) and cetyl esters (SS), were kindly donated by Croda (Brazil). SLN were produced by hot high pressure homogenization. The solid lipid heated to around 10°C above its melting point. Afterwards, the mixture was added to a hot aqueous solution of Pluronic F68 under high agitation in an Ultra-turrax® T18 to form a pre-emulsion. The pre-emulsion was homogenized using a Panda 2k (Niro Soavi, Italy), applying three homogenization cycles at 600 bar and cooled to form the SLNs.

2.2. Particle Size and Zeta Potential

The mean diameter (z-average diameter) and size distribution were measured by photon correlation spectroscopy (PCS) (Nano ZS Zetasizer, Malvern Instruments Corp, UK) at 25°C in polystyrene cuvettes with path length of 10 mm. The zeta potential was measured in capillary cells with path lengths of 10 mm, using the Nano ZS Zetasizer. Measurements were performed in deionized water obtained by a MilliQ system. All the measurements were performed in triplicate and data were expressed as the means \pm standard deviation (SD).

2.3. Physical stability of SLN

The physical stability of the SLN was evaluated by examining changes of mean particle size and zeta potential during storage at 4°C.

2.4. Cytotoxicity assays

2.4.1. *Cell lines and culture.* BALB/c 3T3 cell line was purchased from National Institute of Health-Baltimore, USA (NIH) and, HaCaT cell line was kindly gifted from Dr. Liudmila L. Kodach (Academic Medical Center, Amsterdam University). Both cell lines were routinely grown in DMEM supplemented with fetal bovine serum, 15% for BALB/c 3T3 and 10% for HaCaT and, antibiotics (100 U/mL penicillin, 10 μ g/mL streptomycin) in a humidified incubator with 5% carbon dioxide, at 37°C.

2.4.2. *Treatment of cells with CP, MM and SS.* Cells, BALB/c 3T3 10^4 cells/well and HaCaT 7×10^3 cells/well, were incubated in 96-well plates until reach the semiconfluence and then treated with different concentrations of SLN (10–500 μ g/mL final concentrations) for 24 h. Cell viability was assessed by the MTT reduction assay.

2.4.3. *MTT reduction assay.* The medium containing riboflavin was removed and 100 μ L of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (0.5 mg/mL of culture medium) was added to each well. After incubation for 4 h at 37°C, the medium was removed and the

formazan crystals solubilized in 100 μ L of ethanol. The plate was shaken for 5 min on a plate shaker and the absorbance was measured at 570 nm in a microplate reader (ELx800, BioTek Instruments, Inc., Winooski, VT) [10]

2.4.4. *Statistical analysis.* Cell viability data were expressed as the means \pm standard deviation (SD) of experiments. A percentage of viability compared with control wells (the means optical density of untreated cells was set to 100% viability) was calculated from the concentration-response curves by linear regression analysis. Data from each assay were analyzed statistically by ANOVA. Multiple comparisons among group mean differences were checked with the Tukey test. Differences were considered significant when the $p < 0.05$.

3. Results and discussion

3.1. SLN characterization

SLN were prepared with three different lipids, cetyl palmitate (CP), myristyl myristate (MM) and cetyl ester (SS). The mean diameter, polydispersity index (PI) and zeta potential of SLN are listed in Table 1. The SLN dispersions were obtained with high homogeneity (low PI) and both mean diameter and zeta potential of the SLN dispersion were close. As shown in Figure 1, SLN dispersions did not show any significant changes of particle size and zeta potential when maintained at 4°C in test period (six months), indicating a high physical stability. This can be explained by high zeta potential of the SLN dispersions and by the steric stabilization provided by surfactant used (Pluronic F68).

Table 1. z-average diameter, polydispersity index (PI) and zeta potential and of SLN

SLN	Diameter (nm)	PI	Zeta Potential (mV)
CP	189.0 \pm 1.8	0.149 \pm 0.037	-34.7 \pm 4.1
MM	185.4 \pm 6.3	0.188 \pm 0.012	-31.9 \pm 2.1
SS	197.5 \pm 3.5	0.234 \pm 0.023	-30.5 \pm 1.5

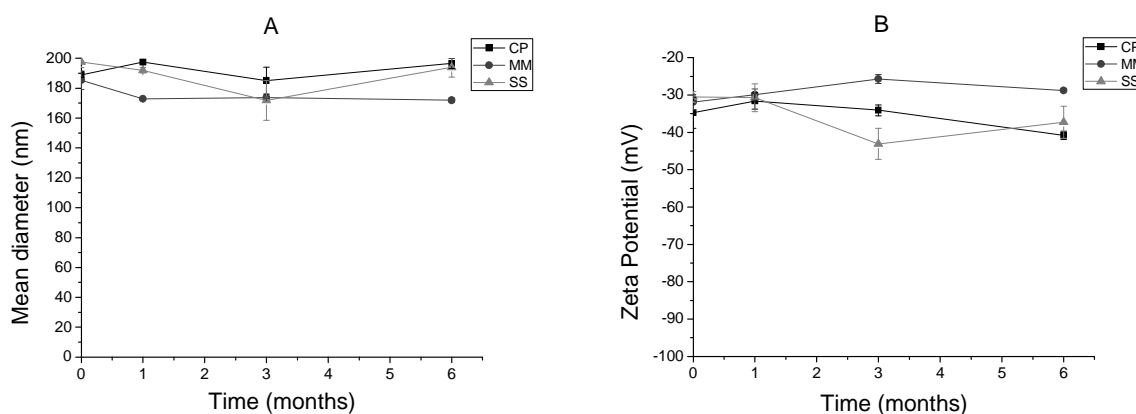


Figure 1. Physical stability of SLN during storage at 4 °C. (A) Mean diameter; (B) Zeta potential.

3.2. Cytotoxicity of SLN

The results obtained in cytotoxicity assays are presented in Figure 2. The cytotoxic effect was influenced by the lipid matrix, as verified by statistical analysis. The lipid that showed smaller reduction in cell viability in the tested concentration range was the SS. Although in some concentrations of SLN cell viability was reduced, until the highest concentration tested (500 μ g/mL) the IC₅₀ (half maximal inhibitory concentration) was not reached for the three lipids in both cell showing no cytotoxic potential of the tested SLN dispersions. When compared to results obtained by

Marcato et al. [11] in MTT test with polymeric nanoparticles, using the same cell lines, the SLN were less cytotoxic.

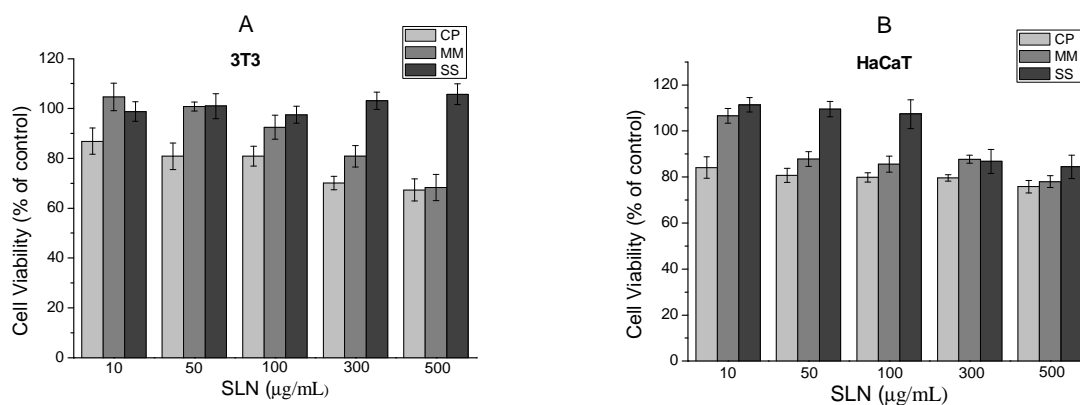


Figure 2. Cytotoxicity of SLN in (A) 3T3 fibroblasts and (B) HaCaT keratinocytes.

4. Conclusions

The results obtained in this work suggest that SLN have a great potential for dermal drug delivery because they showed no cytotoxic potential in fibroblasts and keratinocytes cells.

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References

- [1] Mishra B, Patel B B and Tiwari S 2010 *Nanomed.-Nanotechnol. Biol. Med.* 6 9
- [2] Mehnert W and Mäder K 2001 *Adv. Drug Deliv. Rev.* 47 165
- [3] Pardeike J, Hommoss A and Müller R H 2009 *Int. J. Pharm.* 366 170
- [4] Lewinski N, Colvin V and Drezek R 2008 *Small* 4 26
- [5] Schöler N, Hahn H, Müller R H and Liesenfeld O 2002 *Int. J. Pharm.* 231 167
- [6] Müller R H, Maassen S, Schwarz C and Mehnert W 1997 *J. Control. Release* 47 261
- [7] Shöler N, Hahn H, Müller R H and Liesenfeld O 2002 *Int. J. Pharm.* 231 167
- [8] Weyenberg W, Filev P, Plas D V, Vandervoort J, Smet K D, Sollie P and Ludwig A 2007 *Int. J. Pharm.* 337 291
- [9] Kristl J, Teskac K, Milek M and Rascan I M 2008 *Toxicol. Appl. Pharmacol.* 232 218
- [10] Mosmann T. 1983 *J. Immunol. Methods* 65 55
- [11] Marcato P D, Caverzan J, Rossi-Bergmann B, Pinto E F, Machado D, Silva R A, Justos G Z, Ferreira C V and Durán N 2011. *J. Nanosci. Nanotechnol.*, in press.