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Research paper

Liposomes augment biological benefits of curcumin for multitargeted skin therapy

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ABSTRACT

Curcumin, a multi-targeting pharmacologically active compound, is a promising molecule for the treatment of skin inflammation and infection in chronic wounds. However, its hydrophobic nature remains to be a challenge in development of its pharmaceutical products, including dermatopharmaceuticals. Here we propose deformable liposomes (DLs) as a mean to overcome the curcumin limitations in skin treatment. We explored the properties and biological effects of curcumin containing DLs (curcumin-DLs) with varying surface charge by preparing the neutral (NDLs), cationic (CDLs) and anionic (ADLs) nanocarriers. The vesicles of mean diameter 200–300 nm incorporated high curcumin load mirroring the type of employed surfactant. Curcumin-CDLs provided the most sustained *ex vivo* penetration of curcumin through the full thickness human skin. Although the curcumin-CDLs were the most potent regarding the *in vitro* anti-inflammatory activity, all curcumin-DLs were superior to curcumin in solution (control). No cytotoxicity in human skin fibroblasts was detected. All DLs significantly inhibited bacterial *Staphylococcus aureus* and *Streptococcus pyogenes* growth *in vitro*. The curcumin-CDLs were found superior to other DLs. The incorporation of curcumin in DLs enabled both its sustained skin penetration and enhancement of its biological properties. Cationic nanocarriers enhanced the activities of curcumin to the greatest extent.

1. Introduction

Curcumin is a polyphenol found in the rhizome of the turmeric plant *Curcuma longa L.* This natural compound is nowadays considered a "gold" molecule due to its anti-oxidant, anti-bacterial and anti-inflammatory properties, among other therapeutic activities [1]. Research over the past 30 years confirmed its therapeutic potential against several inflammatory-related diseases, such as the cancer, autoimmune, neurological, lung and liver diseases [2]. The anti-inflammatory properties of curcumin are the result of its interaction with several molecules involved in inflammation pathways. Curcumin is able to decrease the expression of various pro-inflammatory cytokines by down-regulating the nuclear transcription factor kappa B (NFκB) pathway [3]. Additionally, the suppression of cyclooxygenases, lipoxygenase and inducible nitric oxide synthase (iNOS) enzymes mediated by curcumin has shown to be beneficial in several inflammatory diseases [4–6]. In

Abbreviations: ADLs, anionic deformable liposomes; CCK-8, cell counting Kit-8; CDLs, cationic deformable liposomes; DLs, deformable liposomes; DOTAP, 1,2-dioleoyl-3-trimethylammonium propane; HFF, human foreskin fibroblasts; HLB, hydrophilic/lipophilic balance; IMDM, Iscove's modified Dulbecco's medium; LPS, lipopolysaccharide; NDLs, neutral deformable liposomes; NO, nitric oxide; NFκB, nuclear transcription factor kappa B; PBS, phosphate buffer saline; PCS, photon correlation spectroscopy; PI, polydispersity index; P20, polysorbate 20; PG, propylene glycol; SDC, sodium deoxycholate; SPC, soybean phosphatidylcholine; SA, stearylamine; SC, *stratum corneum*

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spite of the evidently high potential of this pleiotropic active compound in the treatment of inflammatory diseases, its limitations such as low solubility and poor bioavailability limit its oral administration. Therefore, topical administration of curcumin could be a promising approach as, for example, for localized treatment of chronic vaginal inflammation [4]. Topical administration onto skin appears equally encouraging and curcumin has shown to be a good candidate for the treatment of hyper-inflammatory wounds, such as chronic wounds and burns [7]. Moreover, curcumin is highly promising in controlling the scar formation and treatment of hypertrophic post burn scarring [8]. In addition to the well-established anti-inflammatory property of curcumin, its anti-bacterial activity became equally important considering the ongoing evolvement of bacterial resistance to antibiotics [9]. Curcumin as a topical anti-bacterial agent might be an attractive alternative to the overuse of antibiotics, both applied locally and systemically. Similar to its anti-inflammatory activity, the anti-bacterial potential of curcumin is related to its action at molecular level, including suppression of one of the essential proteins, FtsZ, which initiates bacterial cell division [10].

The wounded/impaired skin is highly prone to bacterial colonization due to the nutrient-rich environment provided by high levels of wound exudate [11]. Therefore, simultaneous anti-inflammatory and anti-bacterial actions of curcumin in the treatment of chronic wounds and burns would be highly beneficial.

However, the hydrophobic nature of curcumin limits its utilization for localized skin therapy, especially considering the hydrophilic wound dressings such as hydrogels. Moreover, curcumin is chemically unstable at the physiological pH if not protected by a carrier. Its direct applications locally onto the skin, in a vehicle without a carrier, might cause erythema, peeling and hot phenomenon, due to its photosensitivity [12]. Therefore, the focus is on finding novel approaches to enable curcumin solubilization and increase its stability to achieve optimal therapeutic outcome while minimizing the side effects.

Nanotechnology-based delivery systems are one of the novel strategies employed to overcome limited therapeutic potential of curcumin for topical administration. Up to now, several phospholipid-based nanocarriers have been employed to overcome its limitations in dermal delivery [1,7]. Among various nanocarriers of interest in dermal therapy, deformable liposomes (DLs) were proposed as a superior for dermal delivery of molecules, including poorly soluble molecules [13]. DLs can incorporate lipophilic molecules in their phospholipid bilayer thus improving their solubility and assuring protection from chemical instability [13–15]. Moreover, DLs can prolong drug retention time onto/in the skin and assure sustained/controlled drug release [16]. An additional advantage of DLs is the possibility of modifying their composition to tailor physicochemical properties, thus directly affecting their fate as dermal nanocarriers. The liposomal surface charge may enhance the interaction between liposomes and corneocytes in *stratum corneum* (SC), thus influencing the skin penetration of incorporated drug mediated by the liposomes [17]. Both the anti-inflammatory and anti-bacterial activities are exerted at cellular level and it is therefore crucial to assure sufficient cellular uptake of curcumin [18]. The cellular uptake of liposomally-associated molecules is influenced by the liposomal surface charge [19]. Considering the interaction between liposomes and corneocytes, the electrostatic interactions might occur between charged DLs and cell membranes. Positively charged DLs are expected to exhibit higher affinity for the negatively charged domains of the cell membranes thus enhancing a cellular uptake of curcumin [20].

The aim of the present study was to develop an efficient liposomal formulation for dermal delivery of curcumin focusing on its potential for treatment of inflamed and infected wounds. The neutral, cationic and anionic deformable liposomes (NDLs, CDLs and ADLs, respectively) containing curcumin were prepared and the role of the liposomal surface charge was evaluated in the *ex vivo* skin penetration studies using the full thickness human skin. The *in vitro* anti-inflammatory and anti-bacterial activities of curcumin-DLs were evaluated. In vitro cell viability study using human foreskin fibroblasts (HFF) was performed to assess the safety of the liposomal formulations in healthy human skin cells.

2. Materials and methods

2.1. Materials

Curcumin (≥94% curcuminoid content; ≥80% curcumin) was purchased from Sigma-Aldrich (St. Louis, USA). Lipoid S 100 (> 94% soybean phosphatidylcholine, SPC) was a generous gift from Lipoid GmbH (Ludwigshafen, Germany). Polysorbate 20 (P20), stearylamine (SA), sodium deoxycholate (SDC), propylene glycol (PG), methanol, disodium hydrogen phosphate dihydrate, monobasic potassium phosphate, sodium chloride, ammonium molybdate, Fiske-Subbarow reducer agent, phosphorus standard solution, RPMI 1640 medium, lipopolysaccharide (LPS) and Iscove's modified Dulbecco's medium (IMDM) were obtained from Sigma-Aldrich (St. Louis, USA). Hydrogen peroxide 30% was purchased from Merck KGaA (Darmstadt, Germany), sulphuric acid from May & Baker LTD (Dagenham, UK), sulfanilamide, naphthylethylenediamine dihydrochloride and phosphoric acid from Sigma Life Science (Sigma-Aldrich Norway AS, Oslo) and Albunorm® (human serum albumin, 200 mg/mL) from Octapharma AG (Lachen, Switzerland). HFF (CCD-1112Sk, ATCC® CRL-2429™) and murine macrophage RAW 264.7 cell lines were purchased from ATCC (Manassas, USA).

2.2. Preparation of deformable liposomes

The DLs were prepared using the conventional film method reported earlier [21]. NDLs were made of SPC and P20 (total 200 mg) in a weight ratio of 85:15, respectively. CDLs were prepared by addition of SA in the same lipid mixture as used for NDLs. The ratio of SA to SPC was 1:9 (w/w). ADLs were made of SPC and SDC, maintaining the same weight ratio between lipid and surfactant (85:15, respectively) as for NDLs and CDLs. A complete overview of the liposomal composition is given in Table 1. Briefly, curcumin (20 mg) and lipids (200 mg: SPC and, when

Table 1
Composition of curcumin-DLs.

Liposomes[a]	Curcumin (mg)	SPC (mg)	P20 (mg)	SA (mg)	SDC (mg)	Extrusion through polycarbonate membrane[b]
NDLs	20	170	30	–	–	5 × 800 nm 4 × 400 nm
CDLs	20	153	30	17	–	5 × 800 nm 2 × 400 nm
ADLs	20	170	–	–	30	5 × 800 nm 7 × 400 nm

[a] Each liposomal formulation was prepared in triplicates. The final volume was 10 mL. For empty liposomes (without curcumin), the same lipid/surfactant composition was used.
[b] The extrusion is described as the number of extrusion cycles through the corresponding pore size membrane (nm).

applicable, P20, SA and SDC), were dissolved in methanol. A thin lipid film was obtained after evaporation of the solvent for 1 h in a rotary vacuum evaporator (Büchi Rotavapor R-124 with Büchi Vacuum Pump V-700, Büchi Labortechnik AG, Flawil, Switzerland). The lipid film was kept under vacuum (55 mbar) at 55 °C for additional 1 h and subsequently resuspended in 10 mL of phosphate buffer saline (PBS) (pH 7.4; 2.98 g/L Na_2HPO_4 $2H_2O$, 0.19 g/L KH_2PO_4, 8 g/L NaCl). The DLs were stored at 4 °C overnight. For assessment of liposomes' elasticity, antibacterial and anti-inflammatory activities of curcumin-DLs and *in vitro* cell viability study, the empty (curcumin-free) NDLs, CDLs and ADLs were prepared using the same liposomal composition as for curcumin-DLs (Table 1).

The size of all DLs was reduced by hand extrusion through the polycarbonate membrane (Nuclepore® Track-Etched Membranes, Whatman House, Maidstone, UK). The pore size of the membranes and the number of extrusion cycles were optimized for each formulation to obtain liposomes between 200 and 300 nm in size (Table 1). Five cycles of extrusion through 800 nm pore size membrane were performed for all DLs. Subsequently, NDLs were additionally extruded four times through 400 nm pore size membrane, while CDLs and ADLs were extruded through the same pore size membrane two and seven times, respectively. Prior to extrusion, CDLs (both empty and curcumin containing) were maintained at 55 °C for 10 min in a thermostat.

2.3. Characterization of liposomes

The vesicle size of all DLs was determined by photon correlation spectroscopy (PCS) using a NICOMP Submicron Particle Sizer Model 370 (NICOMP Particle Sizing system, Santa Barbara, California, USA) [22]. The liposomes were diluted with PBS to obtain a particle intensity of 250–350 kHz. All measurements were run in triplicates (run time of 10 min for each cycle) at room temperature (23–24 °C) and the results expressed as the intensity-weighted distributions.

Zeta potential determination was performed using Malvern Zetasizer Nano – ZS (Malvern, Oxford, UK) [21]. Samples were diluted 1:20 (v/v) with filtered water (0.2 μm syringe filter, Bulk Acrodisc® 25 mm Syringe Filter, Pall Life Sciences, East Hills, New York, USA) or filtered water containing SDC for ADLs. An attenuator of 6–7 was used for all measurement runs (equilibration time of 180 s, 25 °C). The measurements were performed in triplicates.

2.4. Entrapment efficiency of curcumin in DLs

DLs were centrifuged (Biofuge stratos centrifuge with a swinging bucket rotor 4 × 180 mL; Heraeus instruments GmbH, Hanau, Germany) at 3000*g* for 10 min (10 °C) to remove the free (unentrapped) curcumin. After dissolving the lipids in methanol, the incorporated curcumin in all DLs (supernatant) was quantified by UV–VIS spectrophotometry at 425 nm (SpectraMax 190 Microplate Reader, Molecular Devices, California, USA). Prior to the content determination, all samples were further diluted 1:1 (v/v) with PBS to avoid methanol evaporation. A standard curve of curcumin in methanol/PBS (1:1, v/v) was made in the concentration range of 1–20 μg/mL (R^2 = 0.9996).

2.5. Phospholipid content measurement

To express the entrapment efficiency as the curcumin/lipid ratio and follow a possible loss of lipids during the extrusion, the amount of SPC in all DLs was quantified by phosphorous assay [23]. Aliquots of liposomes (50 μL) were diluted to a final volume of 10 mL with distilled water and then incubated at 160 °C for 3 h after the addition of sulfuric acid (5 M). Hydrogen peroxide 30% was added and the samples incubated at 160 °C for additional 1.5 h. After the heating, ammonium molybdate (0.22%, w/v) and Fiske-Subbarow reducer were added and the solution incubated at 100 °C for 7 min. The samples were then analyzed spectrophotometrically on a SpectraMax 190 Microplate Reader (Molecular Devices, California, USA) at 830 nm using a standard curve of phosphorous standard solution.

2.6. Liposome elasticity measurements

The bilayer elasticity of all DLs was determined as reported earlier [24]. The liposomal dispersions were extruded through the polycarbonate membrane (pore size of 100 nm) at a constant external pressure of 2.5 bar. The amount of liposomal dispersion after 5 min of extrusion was determined (J). The vesicle mean diameter and polydispersity index were monitored by PCS measurements before and after the extrusion, respectively. The degree of membrane elasticity (E) of all curcumin-DLs was calculated using the following equation:

$$E = J \bullet (r_v/r_p)^2,$$

where J is the amount of liposomal dispersion (g) extruded in 5 min, r_v is the mean diameter (nm) of liposomes after the extrusion and r_p is the pore size membrane (nm).

Empty NDLs, CDLs and ADLs (without curcumin) were used as control, respectively.

Empty and curcumin-CDLs were kept for approx. 15 min at 55 °C prior to the measurements.

2.7. Ex vivo skin penetration studies

Curcumin penetration through the full thickness human skin was investigated in the Franz diffusion cells of 1.77 cm^2 diffusion area (PermeGear, Bethlehem, USA) [21]. Human skin, from the abdomen of female patients after plastic surgery, derived from the excess of skin panni. All patients were informed and gave consent to the use of the skin residue for this study. The excess of skin panni is normally discarded after surgery, therefore no ethical approval by the Norwegian Ethical Committee was required. The experiments were carried out in accordance with the Declaration of Helsinki Principles. The full thickness human skin, separated from the subcutaneous fat, was extensively rinsed with PBS (pH 7.4). The skin was stored at −20 °C and thawed at room temperature prior to the use. The human skin, with a thickness of 1.10–1.30 mm, was mounted with SC facing the donor chamber. The receptor chamber (volume of 12 mL) was filled with Albunorm® (5%, v/v) in PBS (pH 7.4) solution and the receptor medium was maintained at 32 °C during the experiment. All curcumin-DLs were tested. To explore the effect of the liposomal surface charge on the skin penetration of curcumin, deformable liposomes with neutral surface (NDLs) were used as a control. Each formulation (600 μL) was pipetted in the donor chamber; the experiments were performed for 24 h under occlusion and samples (500 μL) withdrawn from receptor medium at certain time intervals (1, 2, 3, 4, 5, 6, 7, 8 and 24 h, respectively). After each sampling, the receptor chamber was refilled with equal volume of fresh receptor medium to assure the sink conditions. At the end of the experiment, the penetrated curcumin in the receptor medium was quantified using a standard curve in Albunorm® (5%, v/v) in PBS (pH 7.4) as described in Section 2.4. The experiments were performed in triplicates.

2.8. Inhibition of LPS-induced NO production measurements

The *in vitro* anti-inflammatory activity of curcumin-DLs was assessed in terms of their effect on the inhibition of NO production in LPS-induced murine macrophage RAW 264.7 cells [4]. The macrophages (1×10^5 cells/mL) were cultured in a 24-wells plate with RPMI 1640 medium supplemented containing 10% fetal bovine serum and 2.5 mM glutamine for 24 h at 37 °C/5% CO_2. RPMI medium was replaced with LPS (1 μg/mL) containing RPMI (I mL) to induce NO production. Additionally, the test formulations (curcumin-DLs) were added (10 μL) at various lipid concentrations, namely 1, 10 and 50 μg/mL, corresponding to curcumin concentrations of 0.05, 0.5 and 2.5 μg/mL. The

Table 2
Characteristics of curcumin-DLs.

Liposomes	Diameter (nm)[a]	PI[b]	Zeta potential (mV)	Curcumin per lipid (mg per mg)	SPC recovery (%)
NDLs	286.1 ± 60.8 (87%) 130.8 ± 14.5 (13%)	0.17 ± 0.02	−2.3 ± 0.2	0.07 ± 0.00	80.2 ± 0.6
CDLs	231.5 ± 68.1 (90%) 392.3 ± 61.8 (10%)	0.22 ± 0.04	33.7 ± 1.1	0.04 ± 0.01	97.1 ± 3.3
ADLs	299.3 ± 22.8 (100%)	0.20 ± 0.03	−34.9 ± 0.5	0.11 ± 0.01	89.1 ± 0.9

Results are expressed as mean ± SD (n = 3).
[a] The diameter is indicated as peaks in size distributions (nm). The weight intensity of each peak (%) is indicated in parentheses.
[b] Polydispersity index.

controls were treated under the same conditions. As a negative control, the cells treated with LPS were used. After 24 h incubation, the NO production was measured in terms of nitrite formation in the media Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride, 2.5% phosphoric acid) (1:1 as volume ratio with RPMI). The absorbance was read at 540 nm using an Epoch Microplate Spectrophotometer (BioTek Instruments, Vermont, USA). The effect of the curcumin-DLs on the inhibition of NO production was expressed as percentage of produced NO, in comparison to 100% NO detected in the control (cells treated with 1 μg/mL LPS).

2.9. In vitro cell viability testing

The cell viability of HFF cells exposed to curcumin-DLs was tested to evaluate possible cytotoxic effect of curcumin-DLs on healthy skin cells. The HFF cells (50,000 cells/mL) were cultured in a 96-wells plate with IMDM supplemented with 10% (v/v) fetal bovine serum at 37 °C in 5% CO_2. After 24 h, 10 μL of test formulations were added and the cells incubated further for 12 or 24 h, respectively. All tested formulations were identical to those used in the NO production testing (Section 2.9). Living cells were quantified using the Cell Counting Kit-8 (CCK-8) (Sigma-Aldrich Chemie, Steinheim [21]. CCK-8 (10 μL) was added to the cells and the absorbance was read at 450 nm using an Epoch Microplate Spectrophotometer (BioTek Instruments, Vermont, USA) after 4 h incubation according to the protocol recommended by the manufacturer.

2.10. Anti-bacterial susceptibility testing

The *in vitro* anti-bacterial activities of all curcumin-DLs were assessed against two clinical strains of Gram-positive bacteria, namely *Staphylococcus aureus subsp. aureus* Rosenbach MSSA476 (ATCC- BAA-1721, LGC standard AB, Sweden) and *Streptococcus pyogenes* (ATCC 19615), using the modified broth dilution method [25]. For start, a bacterial suspension with a turbidity of 0.5 McFarland was prepared in sterile saline solution (0.85%, w/v), corresponding to 1-2·10^8 colony forming unit (CFU)/mL. The bacterial suspension containing *S. aureus* and *S. pyogenes* was further diluted in Mueller Hinton broth or Mueller Hinton broth with 5% horse blood, respectively, to obtain the concentration of approx. 10^6 CFU/mL. Subsequently, two-fold serial dilutions of the test formulations were prepared in growth medium using a 96-well plate. All tested DLs were of 100 nm mean diameter (additional extrusion cycles applied) to avoid possible liposomes precipitation during the experiment. Curcumin and lipid contents in the additionally extruded liposomal formulations were determined as described in Sections 2.4 and 2.5, respectively. Two lipid concentrations of curcumin-DLs were tested, namely 2 and 4 mg/mL, corresponding to the curcumin concentrations of 100 and 200 μg/mL, respectively. Moreover, as the controls, the following samples (in growth medium) were tested: (i) PG (20%, w/v) solution in PBS, (ii) empty NDLs, CDLs, ADLs, at similar lipid concentration as in curcumin-DLs, (iii) curcumin in PG solution (20%, w/v) in similar concentrations as curcumin in DLs. As a control, one row of the 96-well plate was filled only with the growth medium. The bacterial suspension, previously prepared as described above, was then added to each well to a final concentration of 5x10^5 CFU/mL and incubated for 4 h at 37 °C. The bacterial survival after applying the tested formulations was evaluated by serial dilution of the bacterial suspension obtained from each well and subsequent plating on blood agar plates following overnight incubation at 37 °C. The percentage of bacterial survival was determined by comparing the surviving bacteria to the control were no antibacterial agent was added (100%).

2.11. Statistical analyses

Statistical analyses were performed using one-way ANOVA test followed by Bonferroni's multiple comparisons test performed on GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla CA, USA). Results were expressed as mean ± SD. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Liposomal characteristics

Considering that our aim was to assure local effects of curcumin, we chose to develop curcumin-DLs within a size range of 200–300 nm, which are expected to provide high drug reservoir in the deeper skin layers [26,27]. Table 2 presents the vesicle size of all curcumin-DLs. A bimodal size distribution (Nicomp distribution) was observed for both NDLs and CDLs, with a minor smaller vesicle population peak outside the targeted size range. However, the main peaks in size distribution were in the range of 200–300 nm, as targeted. The polydispersity index (PI) below 0.25 confirmed a satisfactory homogeneity for all curcumin-DLs regardless of the liposomal surface charge.

The surface charge of all DLs mirrored the type of surfactants/lipids used for their preparation (Table 2). CDLs bear a strong positive surface charge, whereas ADLs a strong negative charge; both the results of the inclusion of a positively charged lipid (SA) and negatively charged ionic surfactant (SDC), respectively. The slightly negative zeta potential observed for NDLs was still considered to be neutral, due to the presence of a neutral lipid (SPC) and a non-ionic surfactant (P20).

Curcumin is expected to accommodate itself in the phospholipid bilayers of liposomes, similar to other polyphenolic compounds, such as resveratrol [28]. As reported in Table 2, the amount of incorporated curcumin varied among the DLs. ADLs exhibited the highest entrapment of curcumin, whereas the lowest was found for CDLs. The presence of SA in CDLs has previously been shown to cause low entrapment

efficiency for celecoxib, drug with similar lipophilicity as curcumin [29]. SA might be responsible for causing the repulsions within the lipid bilayers, thus triggering alterations in bilayers packing [30]. Therefore, curcumin might encounter impediments when accommodating itself in the lipid bilayer of CDLs.

The SPC recovery after the extrusion was found to be more than 80% for all liposomal formulations (Table 2). This confirmed that the incorporated curcumin was closely associated with the phospholipid bilayer, in accordance with previously published data on conventional liposomes [4].

The membrane elasticity is the distinctive characteristic of DLs enabling them to squeeze through the skin pores much smaller than their size and consequently enhance the transport of incorporated drug into the deeper skin layers [17]. The DLs elasticity is determined by the presence of surfactant in the lipid bilayer. Surfactants possess high radius curvature thus destabilizing the lipid bilayer and improving liposome membrane elasticity by influencing the interfacial tension of DLs. The type of employed surfactant [30] can therefore affect the membrane elasticity. In this study, we employed different surfactants to prepare DLs bearing different surface charges. We incorporated P20 in both NDLs and CDLs, whereas SDC was employed to prepare ADLs. CDLs additionally contained SA to confer the positive surface charge to the vesicles. To determine the DLs elasticity and liposomal deformability, the liposomal dispersions were passed through the polycarbonate membrane (100 nm as pore size) using a constant external pressure of 2.5 bar. The DLs elasticity was determined in respect to both the vesicle size (r_v) after the extrusion as well as the amount of liposomal dispersions (J) extruded in 5 min. Table 3 presents the degree of membrane elasticity (E) of all DLs. The determined parameters after the extrusion, used to calculate the E, are included. Considering empty DLs (without curcumin), E was mostly dependent on the J. The highest E was obtained with empty ADLs, whereas empty CDLs exhibited the lowest E value. CDLs were composed of both P20 and SA. Although P20 increased the elasticity of NDLs, the same effect was not observed when the same surfactant was incorporated in CDLs. The presence of SA in CDLs might cause repulsions when accommodating itself in the lipid bilayers, consequently causing alterations in the lipid packing. This might reduce the CDLs elasticity. The presence of curcumin in all DLs influenced their degree of membrane elasticity, as expected (Table 3). The incorporation of curcumin in both NDLs and ADLs reduced their E values by more than five- and three-fold, respectively, as compared to empty vesicles. Interestingly, the entrapment of curcumin in CDLs enabled an increase in membrane elasticity by more than 30-fold in comparison to empty CDLs. A possible explanation might be that the presence of curcumin in CDLs can change the surfactant arrangement in the lipid bilayer and limit the lipid packing alterations caused by SA. The degree of membrane elasticity of all curcumin-DLs was in agreement with E values typical for elastic vesicles reported in literature [24]. The PI of all vesicles (below 0.25; Table 2) may suggest that DLs are oligolamellar in nature and that the large number of bilayers might affect the membrane elasticity. However, the E values of all DLs suggest that oligolamellarity did not limit the elasticity of DLs. Moreover, all curcumin-DLs exhibited similar E values. In this study, our focus was on the role of liposomal surface charge in the dermal delivery of curcumin. Therefore, the similar membrane elasticity observed for all curcumin-DLs allowed us to compare directly the different DLs excluding any possible effects related to their membrane elasticity.

Table 3
The membrane elasticity of curcumin-DLs.

Liposomes	Surface charge	r_v/r_p	J (g)	E
Empty DLs	Neutral	1.76 ± 0.04	4.73 ± 0.11	14.59 ± 0.30
	Cationic	1.75 ± 0.02	0.05 ± 0.00	0.16 ± 0.01
	Anionic[a]	1.44	12.70	26.41
Curcumin-DLs	Neutral[a]	1.71	1.61	4.72
	Cationic	1.64 ± 0.07	2.36 ± 0.14	6.38 ± 0.90
	Anionic[a]	1.39	2.83	5.46

The degree of membrane elasticity (E) was calculated considering both the r_v/r_p and J. r_v is the vesicle diameter (nm) after extrusion, r_p is the pore size membrane (nm) and J is the amount (g) of liposomal dispersion extruded in 5 min (constant pressure of 2.5 bar). Empty DLs bearing different surface charge were used as control.
Results are expressed as mean ± SD (n = 2). [a]n = 1.

3.2. Ex vivo human skin penetration of curcumin mediated by DLs

The use of human skin in the Franz diffusion cells is one of the most appropriate skin models to obtain diffusion kinetics closer to the *in vivo* conditions [31]. We therefore, applied this *in vitro* model in our studies. Curcumin, a lipophilic compound, is not expected to partition from the skin into the aqueous receptor fluid. We therefore incorporated 5% (v/v) of Albunorm® in receptor medium, corresponding to 10 mg/L of albumin, to overcome the scarce solubility of curcumin in an aqueous media and avoid negligible diffusion kinetics. Moreover, addition of solubilizing agents in the receptor medium could overcome putative poor *in vitro/in vivo* correlation [32]. Commonly used solubilizing agents are alcohol derivatives [32,33], however their presence does not mimic the physiological composition of receptor fluid. We therefore used Albunorm® as a source of albumin, which is physiologically present in the receptor fluid; thus mimicking the *in vivo* conditions to a higher extent while avoiding impairment of the membrane barrier function [34]. The cumulative amount of penetrated curcumin from the different DLs through the full thickness human skin is presented in Fig. 1. The amount of penetrated curcumin from a control solution of curcumin in propylene glycol was 6.80 ± 0.77 μg/cm^2 (data not shown in Fig. 1).

Although cumulative, the amount of penetrated curcumin at 24 h was interestingly lower than after 8 h possibly due to degradation of free curcumin at the physiological pH in the receptor medium. Curcumin was found to be stable up to 8 h and the amount of penetrated curcumin through the full thickness human skin was low for all DLs. These results are in accordance with the previous reports on human skin penetration profiles of curcumin when incorporated in lipid-core nanocapsules [35]. Although the penetration of curcumin through the full thickness human skin mediated by our DLs was limited, the findings confirmed the ability of DLs to deliver the incorporated active compound in the deeper skin layers reaching the dermis layer [36]. We have proven that our curcumin-DLs possess elastic membranes contributing to the curcumin delivery into the deeper skin layers, as previously shown when DLs were tested on mimicked *stratum corneum* model [24]. However, the low amount of curcumin that reached the receptor medium also indicates a retention of curcumin within the skin thus showing good suitability of these nanocarriers for dermal therapy of curcumin. The comparison of the different DLs indicates different behavior of nanocarriers as dermal delivery systems. CDLs enabled the most sustained skin penetration of curcumin, whereas ADLs exhibited the highest skin penetration enhancement of curcumin (Fig. 1). The similar degree of membrane elasticity observed for all curcumin-DLs (Table 3) indicates that the differences in the skin penetration profiles of curcumin might be mainly attributed to the liposomal surface charge. We have earlier confirmed the capability of ADLs to deliver lipophilic compound rhodamine into the deeper skin layers through *ex vivo* human skin in Franz diffusion cells [22].

3.3. Effect of curcumin-DLs on NO production in LPS-induced macrophages

Considering the treatment of chronic wounds, a local therapy based on a formulation that inhibits NO production is expected to reduce the persistent inflammation distinctive in chronic wounds and burns. Fig. 2 shows the effect of curcumin-DLs on NO production in LPS-induced macrophages. Regardless of the liposomal surface charge, a

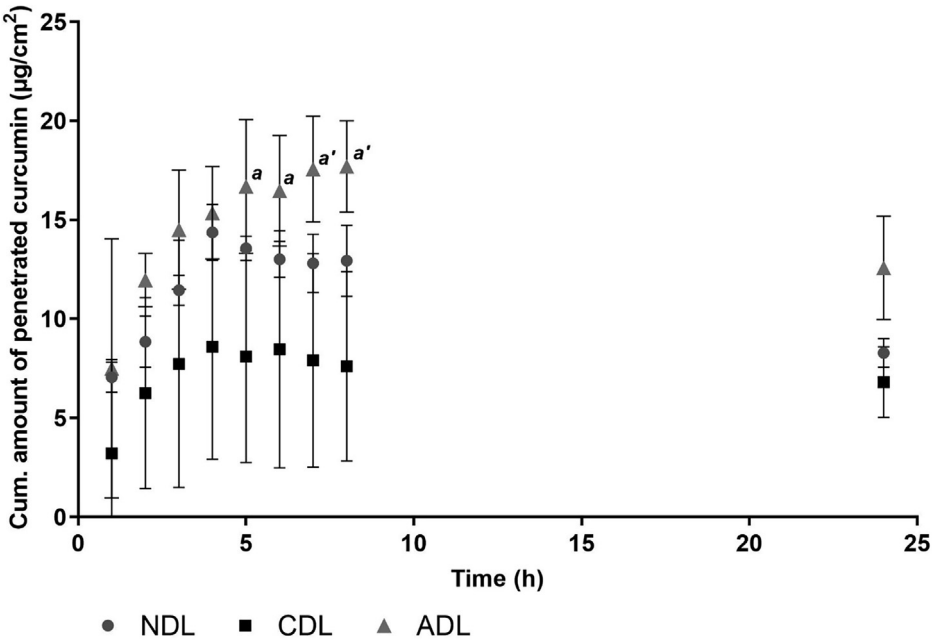

Fig. 1. Curcumin penetration from different deformable liposomes through the full thickness human skin over 24 hrs. Results are expressed as mean ± SD (n = 3). [a:] $p < 0.05$, [a':] $p < 0.005$ *vs.* CDL.

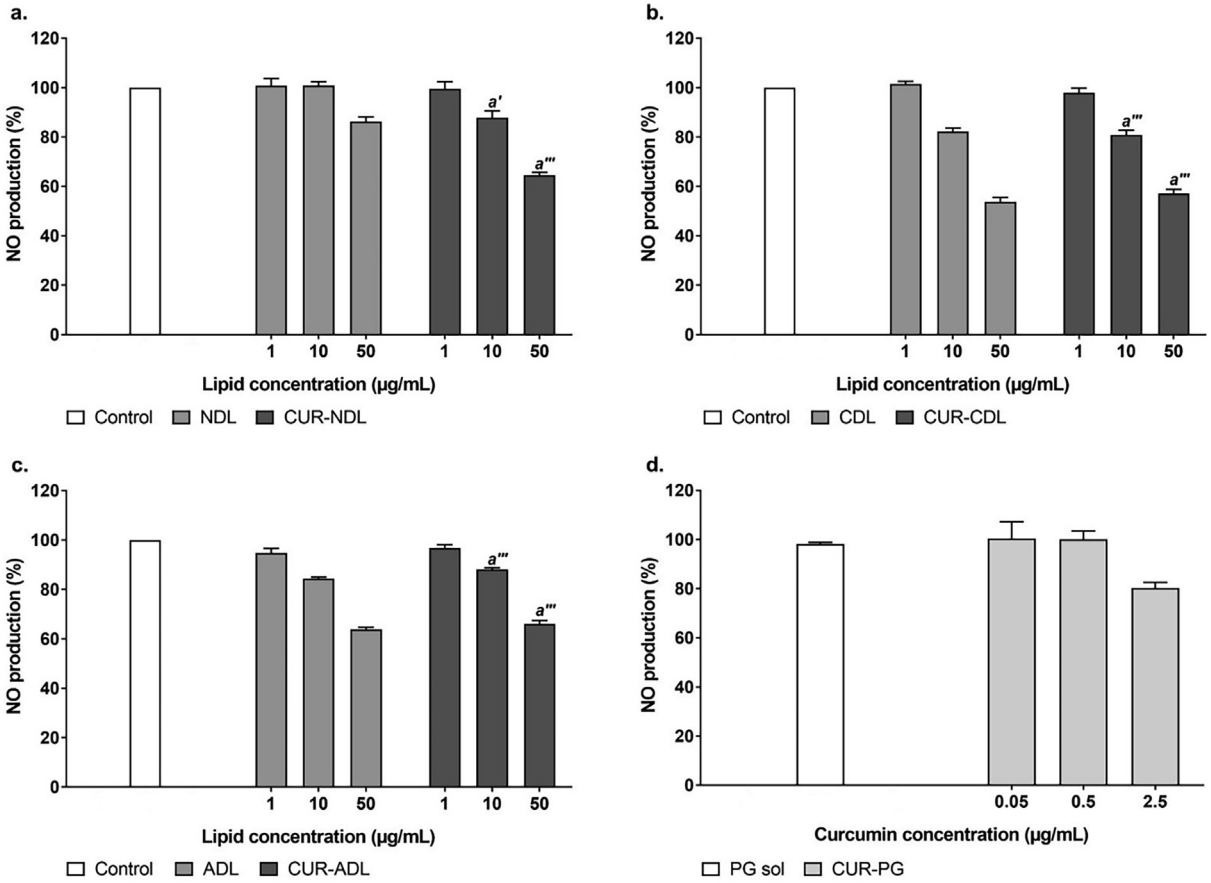

Fig. 2. Effect of different curcumin-DLs on NO production in LPS-induced macrophages. LPS-induced macrophages were treated with: (a) curcumin neutral deformable liposomes (CUR-NDLs), (b) curcumin cationic deformable liposomes (CUR-CDLs) and (c) curcumin anionic deformable liposomes (CUR-ADLs), incubated for 24 h. Untreated LPS-induced macrophages were used as a control. NDLs, CDLs and ADLs are empty deformable liposomes, tested at the same lipid concentrations as curcumin-DLs. (d) curcumin in PG solution (CUR-PG) at the same curcumin concentration as in deformable liposomes. PG sol is PG (20% w/v) solution in PBS. Results are expressed as mean ± SD (n = 3). [a:] $p < 0.03$, [a':] $p < 0.002$, [a'':] $p < 0.0002$, [a''':] $p < 0.0001$.

concentration-dependent inhibition of NO production was observed for all curcumin-DLs (Fig. 2a–c). At the lowest lipid concentration (1 μg/mL), no inhibitory effect on NO production was observed for all DLs. However, they exhibited an inhibitory effect at 10 μg/mL, which was stronger than the effect of free curcumin (curcumin in PG solution) (Fig. 2d). This effect was more evident at the highest tested lipid concentration (50 μg/mL). Free curcumin (curcumin in solution) has been confirmed to possess anti-inflammatory activity, exhibiting even stronger effect compared to other well-known anti-inflammatory compounds, L-nitro-arginine methyl ester [4]. In this study, we observed that DLs enhanced curcumin's anti-inflammatory activity, as as we have previously shown for conventional neutral liposomes destined for vaginal administration [4].

Comparing liposomes of different surface charge, the strongest inhibitory effect was obtained with CDLs (Fig. 2b). At the highest lipid concentration, CDLs inhibited NO production by almost 10% more than both NDLs and ADLs (Fig. 2a, c). The stronger effect of CDLs might be explained by the higher affinity of positively charged DLs towards the negative cell membrane, facilitating their interactions. Moreover, the more sustained skin penetration of curcumin observed CDLs (Fig. 1) might prolong the retention time of curcumin at the targeted site of action. This would facilitate and prolong CDLs interaction with macrophages, thus allowing continuous cellular uptake of curcumin and enhancing its effect at cellular level, as also observed for the anti-bacterial effect (Fig. 4a, c). Studies regarding possible curcumin uptake by macrophages would be necessary to confirm our hypothesis, however this correlation between sustained release and enhanced anti-inflammatory activity has been previously observed with other nanocarriers, such as curcumin-loaded propylene glycol liposomes both *in vitro* and *in vivo* in rat paw edema model [12].

3.4. Effect of curcumin-DLs on healthy human derived skin cells

The incorporation of cationic lipid and surfactants, especially ionic, in DLs destined for dermal therapy might rise toxicity concerns [20]. Moreover, curcumin as polyphenolic compound might exhibit side effects on skin cells when administered at high concentrations [6]. To explore possible cytotoxic effect of curcumin-DLs to skin fibroblasts, different surface charged DLs were incubated with HFF cells for 12 or 24 h, respectively. As shown in Fig. 3, all DLs were found to be nontoxic for HFF cells at the applied concentrations, in agreement with previous findings on other type of nanocarriers containing curcumin [35]. Moreover, a proliferative effect of curcumin-DLs was observed (Fig. 3a, b, c). Gopinath and co-workers [37] reported similar findings on fibroblast proliferation mediated by curcumin formulated in collagen films. More recently, Manca and co-workers [38] studied the effect of curcumin-in-hyalurosomes on human keratinocytes and found an increase in cell viability, as observed for our liposomal formulations. Curcumin-NDLs (Fig. 3a) and curcumin-ADLs (Fig. 3c) showed a concentration-dependent cell proliferation enhancement. After 12 h exposure, both curcumin-NDLs and curcumin-ADLs exhibited greater proliferative effect than the control (untreated HFF cells) at lipid concentrations of 10 and 50 μg/mL. NDLs, at 10 and 50 μg/mL, enhanced the number of living cells by 24 and 32% compared to control, respectively. ADLs exhibited weaker cell proliferative ability compared to NDLs, however they still enabled 12 and 14% stronger proliferation than control at 10 and 50 μg/mL, respectively. The same trend was observed when comparing curcumin-DLs with free curcumin (curcumin in PG solution) (Fig. 3d). At the highest lipid concentration (50 μg/mL), NDLs enhanced cell proliferation by 32%, whereas ADLs enhanced by 14%. This cell proliferation activity was more evident after 24 h exposure. For both NDLs and ADLs, an enhancement in cell proliferation by 50% compared to free curcumin was observed (Fig. 3a, c, d). Our results are in agreement with published data on other types of nanocarriers, showing that curcumin incorporation in phospholipid-based nanosystems is beneficial for cell proliferation [39]. Regarding CDLs (Fig. 3b), the tested lipid concentrations of 1 and 10 μg/mL exhibited similar proliferative effect as observed for NDLs and ADLs. Interestingly, the CDLs at the highest lipid concentration (50 μg/mL) showed lower proliferation effect than at concentration of 10 μg/mL. By increasing the surfactant concentration, the interaction between the amphiphilic ionic surfactant and the cellular lipid bilayer is more likely to occur, resulting in a disruption of the plasma membrane [40]. This lower proliferative effect of CDLs at higher concentrations (50 μg/mL) was not observed for NDLs and ADLs at the same concentration, indicating that the liposomal surface charge might influence the extent of the interaction between nanocarrier and cell membrane. Due to the negative charge of the cellular membrane, electrostatic interactions are more likely to occur with positively charged liposomes thus facilitating the possible cytotoxic effect exerted by CDLs. However, CDLs at the highest lipid concentration assured equal or higher cell viability than control after both 12 or 24 h exposure. This excludes any concerns related to potential cytotoxic effect of CDLs. To the best of our knowledge, the cytotoxicity of stearylamine liposomes (CDLs), has only been tested in human lung epithelial cells by Tahara and collaborators, who found no negative effect of stearylamine liposomes on the cell viability after 4 h exposure [41].

The findings from the cell viability study showed a clear lack of cytotoxicity for CDLs (Fig. 3b) and even more, demonstrated a cell proliferative effects.

3.5. Anti-bacterial activity of curcumin-DLs

Curcumin as an antimicrobial agent is clinically not yet fully utilized and the potential of nanocarriers in modulating its antimicrobial effects are currently, although very promising, not fully explored [10]. Therefore, broader use of curcumin in wound therapy can contribute to reduced use of antibiotics while maintaining antimicrobial efficacy of wound dressing. All liposomal formulations with curcumin were tested against two clinical strains, *S. aureus* and *S. pyogenes*. We aimed firstly to evaluate the anti-bacterial effect of curcumin when incorporated in DLs and secondly, to explore the effect of the liposomal surface charge on curcumin-DLs anti-bacterial activity.

All curcumin-DLs inhibited both *S. aureus* and *S. pyogenes* growth compared to control (untreated bacteria) (Fig. 4a, c). No differences in anti-bacterial activity were observed between the two tested concentrations of curcumin (100 and 200 μg/mL, respectively), indicating that lower curcumin concentration was already sufficient to exert the effect. This might also be beneficial considering the possibility of side effects related to high curcumin concentration at the site of administration [12]. Curcumin-NDLs exhibited the weakest inhibition of *S. aureus* growth compared to both CDLs and ADLs (Fig. 2a). The same trend was observed with *S. pyogenes* (Fig. 4c). These findings might indicate that the liposomal surface charge influences the liposomal interaction with the bacterial cell membrane. The neutral liposomal membrane of NDLs might limit this interaction thus reducing curcumin availability at the targeted bacterial membrane resulting in a weak anti-bacterial activity. The strongest activity was observed for curcumin-CDLs against both *S. aureus* and *S. pyogenes*. Moreover, CDLs inhibited bacterial growth to a higher extent than free curcumin (curcumin in PG solution) (Fig. 4b, d). Therefore, the positive surface charge of CDLs facilitated their interactions with the negatively charged bacterial cell membrane thus increasing the anti-bacterial activity of the incorporated curcumin. The effect could have been even more pronounced if the incubation time was prolonged. However, we were very encouraged by the promising findings achieved after only 4 h incubation time allowing detection of differences between the DLs bearing different surface charges.

Additionally, we tested all liposomal formulations against a clinical strain of Gram-negative bacteria, namely *Pseudomonas aeruginosa* (ATCC 27853), using the agar diffusion method to determine eventual zones of inhibition, as described earlier [42]. However, none of the

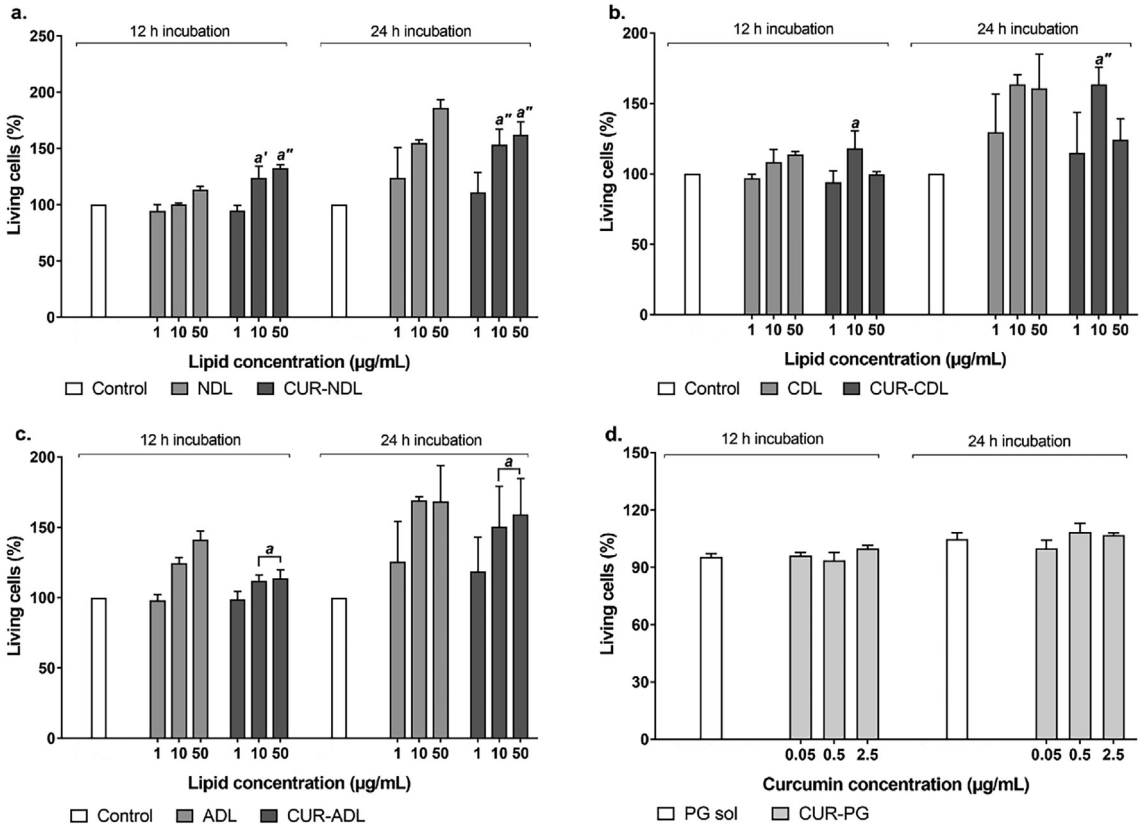

Fig. 3. Effect of curcumin-DLs on viability of human foreskin fibroblast (HFF) cells after 12 and 24 h exposure. HFF cells were exposed to: (a) curcumin neutral deformable liposomes (CUR-NDLs), (b) curcumin cationic deformable liposomes (CUR-CDLs) and (c) curcumin anionic deformable liposomes (CUR-ADLs), respectively. Untreated HFF cells were used as control. NDLs, CDLs and ADLs represent empty deformable liposomes, tested at the same lipid concentrations as curcumin-DLs. (d) represents the percentage of living cells after exposure to PG (20% w/v) solution (PG sol) in PBS and curcumin in PG solution (CUR-PG) at the same curcumin concentration as in deformable liposomes. Results are expressed as mean ± SD (n = 3). $^{a:}$ $p < 0.03$, $^{a':}$ $p < 0.002$, $^{a'':}$ $p < 0.0002$.

curcumin-DLs exhibited stronger inhibition of *P. aeruginosa* growth than the free curcumin (curcumin in PG solution) (data not shown). Krausz and collaborators [43] also observed that Gram-negative bacteria, particularly *P. aeruginosa*, were less susceptible than Gram-positive bacteria to curcumin-containing nanoparticles.

4. Discussion

Curcumin is one of the "hot" molecules with potential in treatment of various diseases, especially chronic, ranging from cancer to skin diseases [44]. However, its poor solubility, low chemical stability and short half-life following systemic absorption contribute to curcumin being considered a pharmaceutical challenge [1,5]. Numerous delivery systems have been proposed as means to tailor its biological properties [39,44,45]. We were particularly interested in a potential of curcumin as active ingredient in wound dressings. Various topical formulations have been evaluated for their potential to enhance curcumin's therapeutic activities relevant for wound healing [6]. The conventional liposomes can incorporate lipophilic compounds, such as curcumin, in their phospholipid bilayer thus enhancing their solubilization and providing their stability and protection [5]. Liposomal bilayers resemble the membrane structure of corneocytes thus enabling their accumulation into the SC providing a drug depot on the skin surface [15]. However, our target were the bacterial infections common in wound bed, which are often not reachable and require drug/carrier penetration to achieve total eradication [39]. Deformable liposomes have been shown to be superior to conventional liposomes in improving dermal drug delivery, assuring prolonged retention time of the drug within the skin and sustained/controlled drug release [16]. Incorporation of curcumin in DLs should provide solubilization of curcumin, increase its stability and assure sustained release over the desired time. This can subsequently provide a high curcumin concentration within the skin, including wound bed, minimizing systemic absorption and undesired side effects. Moreover, the liposomal surface charge may contribute to their behavior and potential as dermal nanocarriers [17]. Considering that curcumin acts at cellular level, the liposomal surface charge might facilitate the interaction between curcumin carrier and targeted cell membrane [19]. Consequently, the targeted delivery of curcumin at the cellular level mediated by DLs is expected to improve its therapeutic effects.

The characterization of liposomes in terms of their physicochemical properties is fundamental to develop effective liposomal formulations destined for dermal therapy. The vesicle size has been shown to play an important role in the dermal delivery mediated by nanocarriers. In addition, effective nanocarriers for dermal drug delivery need to carry high drug load to assure the therapeutic level of drug/active substance at the targeted skin layer(s). It is known that for DLs, the incorporation of surfactants in the phospholipid bilayer can affect the entrapment of various substances [17]. This mainly affects incorporation of lipophilic/amphiphilic drugs/substances within the phospholipid bilayers of liposomes. Consequently, a competition might occur between the lipophilic drug/substance and surfactant thus affecting the incorporation of lipophilic substance in the DLs. This especially applies to curcumin, a highly lipophilic compound with high log P (3.29) [46].

The skin penetration potential of liposomally-associated substance/drug is fundamental to assure targeted drug delivery and, consequently, effectiveness of nanoformulation. Although diseased skin often exhibits compromised SC barrier and the drug/substance penetration might be therefore enhanced, the assessment of the skin penetration potential of nanocarriers using the full thickness human skin can serve as a pilot

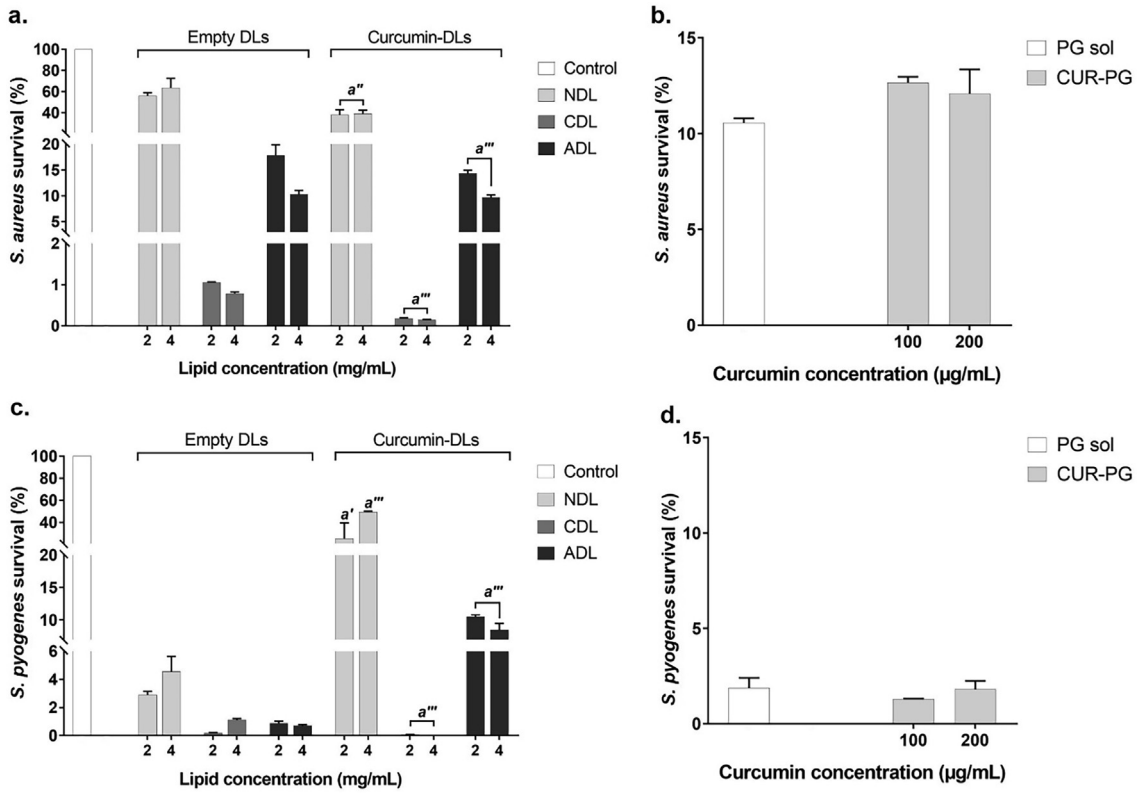

Fig. 4. Effect of curcumin-DLs on *S. aureus* (upper part) and *S. pyogenes* (lower part) growth after 4 h incubation. (a) and (c) show the effect of curcumin-DLs on *S. aureus* and *S. pyogenes*, respectively. Control refers to untreated bacteria. Empty DLs are curcumin-free NDLs, CDLs and ADLs, respectively, with similar lipid concentrations as curcumin-DLs. (b) and (d) represent the effect of free curcumin (CUR-PG), meaning curcumin in PG solution (20% w/v) with same concentrations as in deformable liposomes. PG sol is PG (20% w/v) solution in PBS. Bar graphs showing mean ± SD (n = 2). [a:] $p < 0.03$, [a':] $p < 0.002$, [a'':] $p < 0.0002$, [a''':] $p < 0.0001$.

study assisting in the selection of the appropriate nanocarrier. This is mainly applicable during the screening of nanocarriers with different properties, as, in our case, with different composition and surface charge.

The vesicle surface charge have been shown to influence skin penetration of drugs mediated by liposomes, as well as improve the stability of liposomes [47]. Considering the negative charge of the corneocytes membrane, electrostatic interactions between liposome and SC cells might be favorable when the liposomal surface charge is positive [17]. This stronger interaction might cause a long-term retention of curcumin on/within the skin thus sustaining its penetration through/into the skin layers. Recently, Jose and co-workers [48] studied cationic deformable liposomes containing curcumin. The authors prepared cationic deformable liposomes made of 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) and sodium cholate with a vesicle size of 100 nm, whereas our curcumin-CDLs differed in lipid/surfactant composition and were bigger in size (approx. 231 nm). In spite of dissimilarities in liposomal composition and size, the sustained skin penetration of curcumin observed for our CDLs in human skin was in accordance with their findings using pig skin. The authors proposed liposomal co-delivery of curcumin and siRNA as a superior approach in treatment of skin cancer [48]. The skin penetration enhancement effect of negatively charged DLs for incorporated lipophilic compounds has been previously observed *in vitro* in pig skin [49,50]. Ascenso and co-workers developed ultradeformable vesicles for tretinoin, which were able to target SC and viable epidermis layers; vesicles were found non-toxic in mice, reducing the drug induced irritation [49]. Paolino and collaborators [36] reported an enhancement in *ex vivo* human skin penetration of lipophilic compound for DLs comprising SDC, in accordance with our current and earlier findings [22]. Moreover, the authors confirmed the findings in *in vivo* experiments using rat model. Choudhary and colleagues reported recently that the curcumin-loaded liposomes were able to provide localized permeation and deep penetration into the skin, thus verifying skin localization. The same liposomes were found superior in ability to enhance wound closure in animal experiments [51].

Inflamed skin, especially in chronic wounds, often exhibits delayed healing due to persistent inflammation mediators. NO is known to play a central role in the inflammation pathways thus worsening healing of chronic wounds [6]. During the inflammation process, LPS-induced macrophages release toxic amount of NO. NO production can help as cytotoxic mediator thus preventing microorganism invasion. However, an excessive NO production can interact with oxygen radicals (superoxide) forming an extremely reactive radical (peroxinitrite), which induces inflammatory cellular cytokines thus causing cell death and impaired healing [52]. Moreover, in chronic wounds, an excessive production of NO also causes an impairment of collagen synthesis [53], further delaying the wound healing process.

Curcumin-DLs significantly increased the inhibitory effect of curcumin on NO production. We postulate that the effect might be contributed to (i) increased curcumin stability due to the liposomes' ability to protect it from UV- and pH-induced degradation, (ii) enhanced curcumin uptake in macrophages, and (iii) improvement of curcumin aqueous solubility thus maximizing its bioavailability and tissue distribution, in agreement with Mehanny and colleagues [1]. Recent studies on liposomes containing glucocorticoid by Gauthier and collaborators showed the ability of phosphatidylserine-modified liposomes to enable faster uptake in macrophages, by inducing pro-resolution phenotype in human primary macrophages, thus promoting targeted delivery of the incorporated drug and enhancing its anti-inflammatory effect Especially interesting was the finding that liposomes exhibited a very limited effect keratinocytes [54].

All curcumin-DLs were found non-toxic and safe for dermal administration (Fig. 3). Moreover, delivery systems exhibited cell proliferative activities. The enhanced proliferative effect observed for curcumin-DLs is highly beneficial in treatment of chronic wounds and burns, but is equally relevant in other inflammatory skin disorders such as atopic dermatitis, *ichthyosis vulgaris*; namely all conditions characterized by a conspicuous human skin cells death. In these conditions, a cell proliferation helps and accelerates the normal reconstitution of the skin layers.

Skin, especially skin with the compromised barrier as in chronic wounds, is prone to colonization by bacteria. Bacterial infections contribute to delayed healing thereof increasing the incidence of morbidity and mortality in patients [52]. Gram-positive bacteria, specifically *S. aureus* and *S. pyogenes* are responsible for skin infections in general [11,55,56]. Therefore, curcumin-containing liposomal formulations that prevent/treat microbial contamination can contribute to improved skin therapy and prevention of formation of bacterial biofilms in chronic wounds. Krausz and colleagues reported that curcumin nanoparticles inhibited *in vitro* growth of methicillin-resistant *S. aureus* and *P. aeruginosa* in a dose-dependent fashion, and even more, inhibited bacterial growth and enhanced wound healing in murine wound model [43]. Additionally, dermal (localized) therapy for chronic wound treatment might limit the high incidence of bacterial resistance related to the overuse of antibiotics orally and systemically. Liposomal curcumin can therefore be a promising alternative to common antibiotics with evolved resistance against broad spectrum of bacteria, including *S. aureus*. Functional biomaterials that offer opportunities to combat drug-resistant bacterial infections are the future of intelligent wound dressings [9].

We could not prove the antimicrobial potential of curcumin against *P. aeruginosa,* however, the properties of Gram-negative bacterial cell membrane, both in terms of the surface charge and composition, might limit interaction with nanocarrier [43].

Although we challenged the curcumin-DLS against bacteria during a rather short time (4 h) all curcumin-DLs inhibited bacteria growth by more than 50%. We assume that the longer incubation time may result in even stronger effect; however, the differences among the DLs and the role of surface charge might become less evident. On the other hand, free curcumin would require more time to initially interact with the pathogen surface membrane and exert the therapeutic effect [56] even if stability issues would be neglected.

Very recently, Madan and co-workers suggested that liposomal curcumin has potential in treatment of acne, caused by *Propionibacterium acnes* (*P. acnes*). The authors confirmed improved anti-bacterial and anti-inflammatory activities of curcumin in liposomal gels in the rat ear model of acne vulgaris [57]. The liposomes employed in their study were also positively charged. Niranjan and colleagues challenged nano-curcumin in polyvinyl and chitosan patch as a superior wound dressing in Albino wistar rats wound model. The novel dressing exhibited superior antimicrobial, anti-inflammatory and wound healing properties [58]. The importance of nanosystem as a carrier for curcumin has been recently proven in rat model of diabetes mellitus Type 1, where curcumin-in-nanoparticles in hydrogel has effectively improved the healing process in diabetic skin wound with substantial differences in the wound healing kinetics compared to wounds that were treated by curcumin-in-hydrogel alone [59].

We have proven that curcumin incorporated in cationic liposomes offers an interesting novel multitargeting alternative in wound therapy.Considering that DLs suspensions are liquid in nature, their topical administration onto the skin surface can be improved by their incorporation into a secondary vehicle, such as hydrogel, to develop final skin formulations. The selection of the optimal final formulation and a pilot scale clinical evaluation, utilizing the effect of the liposomal surface charge, would be a consequent step in further studies on the effectiveness of curcumin-DLs in hydrogel systems destined for localized skin therapy.

5. Conclusions

All prepared deformable liposomes enabled relatively high curcumin entrapment, thus assuring optimal curcumin concentration at the skin site while limiting the systemic absorption, as conformed in the *ex vivo* skin penetration studies. Moreover, we were able to discriminate between the skin penetration potentials of different nanocarriers. Cationic deformable liposomes exhibited the most sustained curcumin penetration through the full thickness human skin, assuring a high retention of curcumin within the skin. All liposomal formulations exerted concentration-dependent anti-inflammatory and anti-bacterial activities, superior to activities of non-liposomal curcumin. Additionally, all liposomal formulations were non-toxic for the human skin fibroblast cells, and even more, exhibited a cell proliferation effect. We confirmed that incorporation of curcumin in cationic deformable liposomes enhances its multi-targeting properties. Based on the preliminary findings, the curcumin-containing deformable liposomes can be useful in the treatment of various skin diseases which pathologies involve microbial infection and inflammation.

Declaration of Competing Interest

None.

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