Curcumin nanoparticles attenuates inflammation caused by macrophages: effects of nanoparticles’ surface charges

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ABSTRACT SUMMARY
Curcumin nanoparticles (Cur-NPs), with a mean diameter of 30 nm, containing different surface charges were prepared by employing different polymer coatings. Positively charged Cur-NPs demonstrated a higher efficacy to attenuate both inflammation and oxidation caused by lipopolysaccharide (LPS)-induced macrophage cell line (NR 8383) compared to negatively charged and neutral particles.

INTRODUCTION
Respiratory diseases, such as asthma or chronic obstructive pulmonary disease (COPD), are gaining more attention as the general population is constantly exposed to highly polluted air which contains toxic inhalable compounds such as cigarette smoke, diesel exhausts or heavy metals [1]. These pollutants induce inflammations in human lung through the excessive secretions of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), IL-6 and IL-8. Additionally, chronic inflammations could also contribute to the development of lung carcinogenesis [2].

Curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a natural plant compound derived from plant Curcuma longa. It is commonly used as spice, food additive and fabric coloring [3]. Curcumin also has been widely used for modern medicine treatment including cancer, asthma, cystic fibrosis and COPD [4]. It possesses strong anti-oxidant and anti-inflammatory properties, which potentially protects cells from DNA damage and lipid peroxidation via scavenging oxygen-derived free radicals. Clinical data has demonstrated that curcumin is highly safe for human consumption even at high doses (8 g/day) [5].

In a previous study, inhalable Cur-NPs were produced by polymeric encapsulation to improve the stability of curcumin and achieve delivery to lung in vitro [6]. In this new study, the effects of surface charges of Cur-NPs on the reduction of inflammation caused by macrophages are presented.

EXPERIMENTAL METHODS
Cur-NPs were formulated using solvent and anti-solvent precipitation method as described previously [6]. For the preparation of particles with variable surface charges different polymers were used as coating: 0.3% w/v of polyvinylpyrrolidone, (PVP, positive charge), polyvinyl alcohol (PVA, negative charge) or dextran (neutral charge).

The polydispersity index (PI), size distributions and surface charges measurement of Cur-NPs were determined by Dynamic Light Scattering (DLS) at 25 °C [6]. The encapsulation efficiency of curcumin nanoparticles was measured high performance liquid chromatography (HPLC). A defined mass of Cur-NPs (5 mg) was mixed with methanol (5 mL) and filtered using a 0.45 μm filter. The HPLC conditions were as follow: 75:25% methanol: acetonitrile as mobile phase, flow rate of 1 mL/min, using an isocratic pump at 25 °C and a C18 column (Nova-Pak, 150 x 4.6 mm). The content of curcumin was quantified by UV at 420 nm from the peak area correlated with predetermined standard curve.

The free radical scavenging activity of Cur-NPs with different surface charges was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. For this, stock solutions of 60 μM DDPH and 20 μM Cur-NPs were prepared separately. Then, 0.5 mL of DDPH solution was mixed with 0.5 mL Cur-NPs, shaken thoroughly and incubated for 30 min in the dark. As a control, well-known antioxidants agents such as vitamin C, lipoic acid and L-nitro-arginine methyl ester (NAME) were used for comparison. The antioxidant activity of curcumin nanoparticles and these antioxidants agents were measured at a wavelength of 520 nm.

The reduction of cytokine TNF-α production in LPS-induced NR8383 macrophages cells by Cur-NPs was also evaluated. Briefly, 5 x 10⁵ cells were seeded in 6 well plates and induced with 5 μg/mL LPS. Cells were then treated with 10 μM Cur-NPs. At pre-determined time point, 500 μL of cells-free media were collected for cytokine assay. The amount of TNF-α was measured using an enzyme immunoassay kit according manufacturer protocol. LPS-induced macrophage cells without Cur-NP treatments were expressed as 100% TNF-α production.

RESULTS AND DISCUSSION
The physiochemical properties of Cur-NPs are summarized in Table 1. All Cur-NPs with different surface charges had similar mean hydrodynamic diameter of approximately 30 nm. The particles also showed high monodispersity, as reflected in low PI, ranging from 0.061–0.090. The encapsulation efficiency for all samples prepared was high (> 90%). Cur-NPs coated with PVP were positively charged (+5.5±1.2 mV), while particles coated with PVA showed negative charges (−20.1±1.0 mV).

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Table 1. Characterizations of Cur-NPs.

<table>
<thead>
<tr>
<th>Cur-NPs with different surface charges</th>
<th>Mean particle size (nm)*</th>
<th>PI</th>
<th>Surface charge (mV)</th>
<th>EE (%)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>27.2±2.1</td>
<td>0.061±0.001</td>
<td>+5.5±1.2</td>
<td>96.5±3.5</td>
</tr>
<tr>
<td>Negative</td>
<td>28.0±0.7</td>
<td>0.063±0.001</td>
<td>-20.1±1.0</td>
<td>93.3±3.2</td>
</tr>
<tr>
<td>Neutral</td>
<td>28.9±1.3</td>
<td>0.09±0.007</td>
<td>0.3±0.1</td>
<td>90.3±1.9</td>
</tr>
</tbody>
</table>

* Determined using DLS, ** EE, encapsulation efficiency

Antioxidant activities are expressed as the amount of DPPH scavenged by antioxidant. The radical scavenging activities of Cur-NPs were investigated for their effect in reducing the production of DPPH and compared with well-known antioxidants, such as vitamin C and lipoic acid. Vitamin C demonstrated a potent radical scavenging effect compared to lipoic acid and NAME, a nitric oxide synthase (NOS) inhibitor. Approximately 20% DPPH radical was present after treatment with vitamin C. For Cur-NPs, positively charged particles were found to be the most effective (27%), followed by the negatively charged (43%) and neutral particles (60%), respectively (Figure 1).

The effects of Cur-NPs on the production of TNF-α in LPS-induced macrophage were further investigated. The treatment of macrophages with Cur-NPs demonstrated a time-dependent decrease in TNF-α production. Cur-NPs with positive surface charges were the most effective, followed by the negatively charged and neutral Cur-NPs, respectively, regardless of treatment time compared to non-treated control (Figure 2).

CONCLUSION
Different surface charges of Cur-NPs with similar mean diameter (30 nm) were successfully produced. The inflammation and oxidation induced by in LPS in rat macrophages were attenuated using Cur-NPs treatment in the following decreasing order of surface charges: positive > negative > neutral, respectively. These charged Cur-NPs could serve as a platform for further development as inhalable formulation to attenuate the excessive inflammatory cytokines commonly found in respiratory diseases such as COPD and cystic fibrosis.

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REFERENCES