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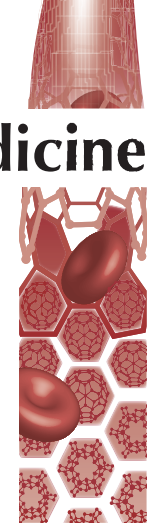

Enhanced intraperitoneal delivery of charged, aerosolized curcumin nanoparticles by electrostatic precipitation

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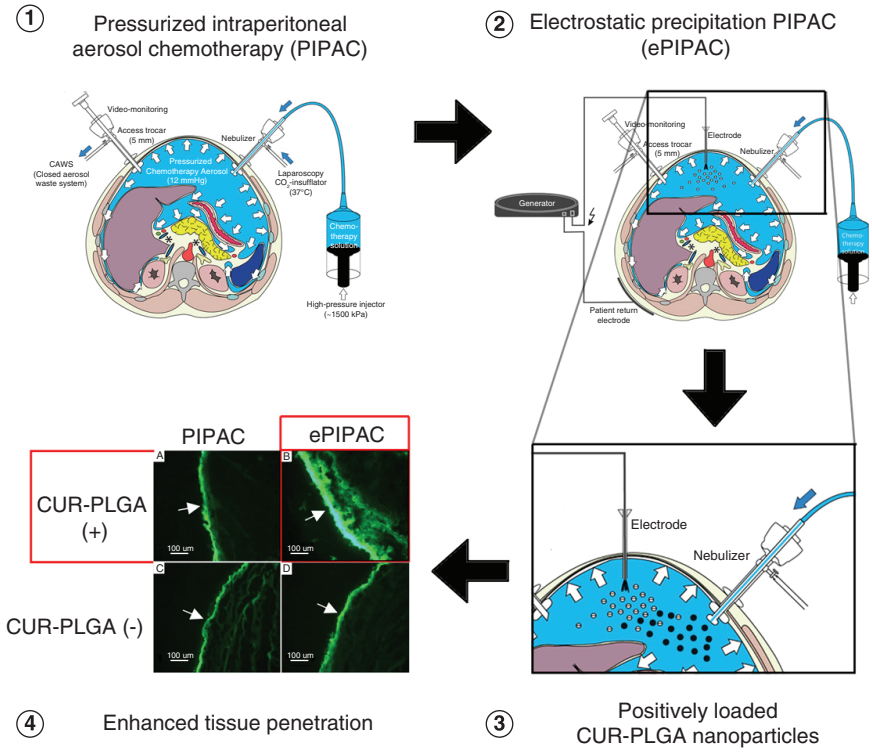
Aims: To investigate the potential of curcumin-loaded polylactic-co-glycolic acid nanoparticles (CUR-PLGA-NPs), alone and with electrostatic precipitation, for improving tissue uptake during pressurized intraperitoneal aerosol chemotherapy (PIPAC). **Methods:** Positively and negatively charged CUR-PLGA-NPs were delivered as PIPAC into inverted bovine urinary bladders *ex vivo*. The experiment was repeated with the additional use of electrostatic precipitation pressurized intraperitoneal aerosol chemotherapy (electrostatic PIPAC). **Results:** Positively charged CUR-PLGA-NPs increased depth of tissue penetration by 81.5% and tissue concentration by 80%. Electrostatic precipitation further improved the uptake of positively charged CUR-PLGA-NPs by 41.8%. **Conclusion:** The combination of positive charge and electrostatic precipitation have significant potential to improve tissue uptake of nanoparticles during intraperitoneal chemotherapy.

Graphical abstract:



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Peritoneal metastasis (PM) is associated with a poor prognosis and remains an unsolved challenge in modern oncology [1,2]. Relative to other metastatic locations, PM is resistant to systemic chemotherapy [3]. Novel therapeutic approaches are needed to achieve better tumor control. One such approach might be intraperitoneal chemotherapy (IPC) because it directly reaches the tumor foci within the abdominal cavity and increases the local drug availability, while maintaining a low systemic dose [4,5]. However, IPC has limitations, such as limited tissue penetration, poor homogeneity of distribution and dose-dependent local toxicity [6]. Thus, so far, IPC has not reached its full potential as a drug delivery technique.

Against this framework, pressurized intraperitoneal aerosol chemotherapy (PIPAC) is a promising approach because it takes advantage of physical laws to optimize drug delivery. By delivering drugs as aerosols under pressure [7,8], PIPAC provides the following benefits over liquid chemotherapy: a higher tissue drug concentration [9,10], a deeper tissue drug penetration [11] and fewer systemic adverse effects due to the lower dose applied. The superior pharmacological properties of PIPAC have been confirmed in a clinical setting: a study found that PIPAC is feasible in the vast majority of PM patients, is safe and well-tolerated and does not impair quality of life [12]. An objective histological response was reported in the majority of cases treated [12]. However, drug delivery throughout the peritoneal cavity with the current PIPAC technology is still not homogeneous because of inertial impaction and gravitational forces [13]. Thus, there is a need for further optimization of PIPAC.

One possible approach is the electrostatic loading of the therapeutic aerosol, followed by precipitation. This enhanced technique, electrostatic precipitation PIPAC (ePIPAC), has been shown to improve spatial aerosol homogeneity and enhance tissue penetration [13,14]. A second approach is to deliver chemotherapeutics in nanoparticles (NPs) – for example, with curcumin (CUR) [15–17]. Thus, the combination of electrostatics and formulation as NPs creates significant synergies.

Our hypothesis was that intraperitoneal delivery of NPs as pressurized aerosols could be further optimized by loading these NPs as either positively or negatively charged. The rationale is to synergize two drug delivery systems: the medical device technology (PIPAC or ePIPAC) and the formulation (positively vs negatively charged CUR-loaded polylactic-co-glycolic acid nanoparticles (CUR-PLGA-NPs). To test this hypothesis, we created cationic and anionic NPs. We studied their tissue uptake in an established *ex vivo* model of the human peritoneal space [18] after delivery as PIPAC versus ePIPAC. Our results provide the first evidence that a combination of ePIPAC with positively charged NPs has superior pharmacological properties for intraperitoneal drug delivery.

Materials & methods

Study design

This is a pharmacological study *ex vivo* evaluating the additional effect of electrostatic precipitation on tissue drug uptake and depth of tissue penetration of negatively and positively charged CUR-PLGA-NP. The nanoparticles were delivered as pressurized aerosols. Freshly prepared animal organs were used for testing [19]. The null hypotheses were that particle charge and electrostatic precipitation do not modify tissue uptake of CUR-PLGA-NPs.

Preparation of CUR-PLGA-NP

PLGA (Resomer RG503H) was purchased from Evonik Industries AG (Essen, Germany). CUR was obtained from Sigma Aldrich (Steinheim, Germany). Chitosan hydrochloride was supplied by Heppe Medical Chitosan GmbH (Halle/Saale, Germany). Polyvinyl alcohol (PVA, Mowiol 4–88) was provided by Kuraray (Hattersheim, Germany). Acetonitrile (ACN, HPLC gradient grade) was purchased from Fisher Scientific UK Ltd (Loughborough, UK). Water used was Millipore Q-Gard 2 by the water purification system of Merck Millipore (MA, USA).

Nanoparticles were prepared according to a previously reported modified solvent-displacement method [23]. Batch sizes of 500 mg PLGA were dissolved in acetonitrile under vigorous stirring at room temperature for 10 min. Then, 500 µl of a 1% (m/V) solution of CUR in acetonitrile was added. The total prepared amount of 30-ml polymer-drug solution was injected into 200 ml stirring aqueous phase containing 2% PVA using a syringe pump with a flow rate of 0.5 ml/min. The dispersion was stirred overnight for solvent evaporation and then purified three

times by centrifugation at 10,000 RCF for 20 min. For positively charged chitosan-coated nanoparticles, chitosan hydrochloride was dissolved in the aqueous phase in addition to PVA.

Characterization of CUR-PLGA-NPs

The fabricated particles were analyzed regarding size, size distribution and ζ-potential. Dynamic light scattering and electrophoretic light scattering were used for this purpose. Samples were investigated at a backscatter angle of 173° (Nano ZS, Malvern Panalytical GmbH, Malvern, UK, and Almelo, The Netherlands). After sputtering with a 10-nm gold layer under vacuum conditions (Q150R Rotary-Pumped Sputter Coater, Quorum Technologies Ltd, Lewes, UK), the surface morphology of dried nanoparticles was visualized by scanning electron microscope (Zeiss EVO 15, Carl Zeiss AG, Oberkochen, Germany) at a working distance of 16.5 mm, an acceleration voltage of 5 or 10 kV and a magnification of 20.000x, CUR-PLGA-NP have a spontaneous fluorescence which was imaged by a fluorescence microscope (AX10, Carl Zeiss AG) at an excitation wavelength of 470 nm. The amount of loaded CUR was quantified as follows: aliquots of samples were freeze-dried and subsequently dissolved in acetonitrile (1 mg/ml). After filtering (0.45 µm, RC membrane filter Titan 3, Thermo Fisher Scientific Inc., MA, USA), the samples were analyzed by fluorescence spectroscopy (plate reader Infinite M200, Tecan Trading AG, Männedorf, Switzerland). A wavelength of 415 nm was used for excitation, and the emission was detected at 515 nm. A calibration curve was taken to calculate the CUR concentrations. Drug loading (DL%) and encapsulation efficiency (EE%) were calculated using equations as follows:

$$\mathrm{DL}[\%] = \frac{\text{amount of drug}}{\text{1 mg formulation}} \times 100\%$$

$$\mathrm{EE}[\%] = \frac{\text{amount of drug recovered in formulation}}{\text{amount of initial drug}} \times 100\%$$

Sprayability of CUR-PLGA-NPs nanoparticles

CUR-PLGA-NP dispersions with concentrations of 0.5%, 0.75% and 1% (m/V) were sprayed onto a fluid surface (petri dish filled with water). Before and after spraying, CUR-PLGA nanoparticles were examined by dynamic light scattering using Zetasizer Nano ZS and visualized by SEM regarding their integrity. For the chitosan-coated CUR-PLGA-NPs, sprayability was investigated for different concentrations up to 2.4% (m/V). The solution containing 202.1 mg CUR-PLGA-NP, positive (NP+) or negatively (NP-) charged, in 150-ml solution was aerosolized at 20–24°C and 0.6 ml/s injection flow.

Ex vivo enhanced inverted bovine urinary bladder model

For this study, we used the previously established enhanced inverted bovine urinary bladder (eIBUB) model [19]. The bovine urinary bladder as an intraperitoneal organ is almost entirely covered by a visceral peritoneal layer. Once turned inside-out, the eIBUB creates a cavity lined by the peritoneum. After insufflation with CO_2, the eIBUB develops a volume similar to that of the human abdomen (3–5 l). Tissue concentration and depth of penetration of doxorubicin in the eIBUB have been shown to be similar to in a human patient [18]. Fresh bovine bladders were obtained from the slaughterhouse, stored on ice and delivered in the early morning to the laboratory. Experiments were performed within hours of removal. A 12-mm double-balloon trocar (Kii, Applied Medial, Düsseldorf, Germany) was introduced through the bladder neck and tightened by a Mersilene suture. A silicone tube was sewn onto the bladder bottom to collect liquid dripping down along the peritoneal surface.

Drug delivery with PIPAC

Drugs were delivered into the eIBUB with nanoparticles as pressurized aerosols. The PIPAC technique has been described elsewhere [7,20]. Briefly, CO_2-pneumoperitoneum was established under standard laparoscopic pressure of 12 mm Hg. Therapeutic solutions were aerosolized at room temperature by a specific nebulizer (CapnoPen, Capnomed, Zimmern o.R., Germany) connected to a high-pressure angioinjector (Accutron Thera, Medtron, Saarbrücken, Germany). The closed system was maintained in steady-state for 30-min application time. At the end of the procedure, the toxic aerosol was discarded using a closed aerosol waste system (CAWS) with two microparticle filters [21]. All experiments were performed in a class 3 safety workbench certified for manipulating chemotherapeutic substances.

ePIPAC

The technique of ePIPAC has been described elsewhere [14,22]. Briefly, a brush electrode (Ionwand, Alesi Surgical, Cardiff, UK) was introduced through the bladder wall, and a return electrode was placed onto the external bladder surface. Both wires were connected to a generator (Ultravision, Alesi Surgical, Cardiff, UK) with a voltage 7500–9500 V. The maximum current was 9.8 uA, which is below the threshold of 10 uA specified in IEC 60601 for the allowable DC leakage current for electrosurgical products. The Ionwand emits a stream of electrons, resulting in the creation of negative gas ions. The gas ions collide with particulate matter, passing on the negative charge. The return electrode confers a weak positive charge on the subject, which results in the electrostatic attraction of the negatively charged aerosol particles to the tissue surfaces of the contained space – that is, the peritoneum. No ozone is generated in the CO_2 environment during ePIPAC. The electrostatic loading system was activated at the beginning of the aerosolization phase, and the electric current was maintained for 30 min.

Sampling and preanalytical processing

After the end of the experiment, the eIBUB was opened, and nine punch biopsies (three biopsies at three levels: top, middle and bottom) with a diameter of 8 mm were taken perpendicularly through the bladder wall for pharmaceutical assessment. Four additional samples in each location were taken for histological examination. All biopsies were placed on a colored inorganic substrate for proper orientation. The probes were immediately frozen at -80°C.

Measurement of depth of tissue penetration

Frozen biopsies were embedded in Tissue-Tek (Schwerte, Germany) for cryo-sectioning, and the probes cut in 10 µm thick sections (Leica cryocut CM3050S, Leica, Nussloch, Germany). The glass slides, each containing 6 to 12 sections of the same biopsy, were allowed to dry at room temperature. Then, biopsies were fixed (Cytoseal XYL, Thermo Scientific Richard-Allan Scientific, Thermo Fisher Scientific) and analyzed with fluorescence microscopy after sectioning or kept at 4°C. Three sections from each biopsy were examined. In the beginning, a microscopic overview photo at light microscopy was taken (magnitude 2.5x) to allow the whole section visualization. All sections were then measured under a fluorescence microscope (Leica Quantimed Q 600) using a fluorescence filter at a magnitude of 10x. The slides were measured by a previously trained researcher (A Castagna) and quality controlled by a pathologist. Each section was measured at three positions and each measurement repeated in triplicate. The analyzer was blinded to the origin of the sample. The depth of penetration was evaluated using the software Leica Qwin 2002.

Tissue homogenization

The biopsies were lyophilized in a Speedvac device (S-Concentrator, BA-VC-300H; H. Saur, Laborbedarf, Reutlingen, Germany) and centrifuged in vacuum conditions overnight (ca. 17 h, 1000 rpm, 100 mbar) at room temperature. The samples were weighed before and after the lyophilization procedure (Sartorius: R180D; Germany). The probes were then rehydrated in a solution composed of 200 µl of sterile distilled water (Ampuwa, Fresenius KABI, Bad Homburg, Germany) and 1300 µl acetonitrile overnight. The probes were transferred into 2 ml PowerBead Tubes (QiagenGmbH, Hilden, Germany, Metal 2.38 mm) and homogenized at room temperature in an automatic homogenizer (TissueLyser LT, Qiagen). The probes were then stored at –80°C until shipping.

Determination of CUR-PLGA tissue concentrations

The amount of CUR found in tissue biopsies was quantified, extracting CUR from the tissue using a solvent mixture of acetonitrile (1300 µl) and water (200 µl). After centrifugation of the tissue samples at 14,000 rpm for 30 min, 200 µl of supernatant was sampled and analyzed by fluorescence spectroscopy. A wavelength of 415 nm was used for excitation, and the emission was detected at 533 nm. A calibration curve of CUR dissolved in a solvent mixture of acetonitrile and water (ratio 13:2) was taken to calculate the CUR concentrations.

Statistical evaluation

This is an exploratory study, and no sample size was calculated beforehand. Blinding was applied whenever possible, in particular, for measurement of the depth of penetration. Descriptive statistics include mean and 95% CI. Comparative statistics were carried out using nonparametric tests. Statistical analysis was performed using SPSS software v. 25 (IBM, IL, USA).

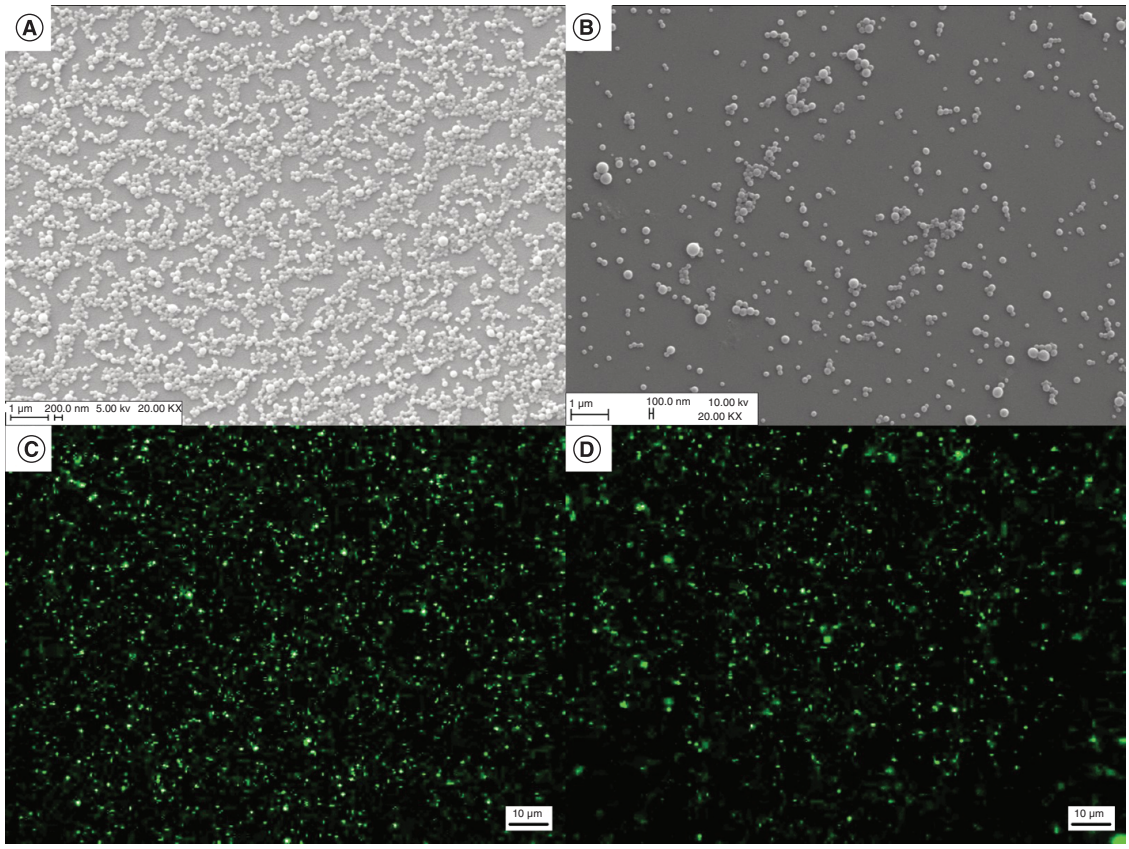

Figure 1. Visualization of curcumin-loaded polylactic-co-glycolic acid nanoparticles. (A) Scanning electron microscope image of negatively charged, uncoated curcumin-loaded polylactic-co-glycolic acid nanoparticles (CUR-PLGA-NPs). **(B)** Scanning electron microscope image of positively charged, chitosan-coated CUR-PLGA-NPs. **(C)** Fluorescence of CUR-PLGA-NPs imaged by fluorescence microscopy. **(D)** Fluorescence of chitosan-coated CUR-PLGA-NPs imaged by fluorescence microscopy. Magnification 20,000x.

Table 1. Properties of negatively and positively charged curcumin-loaded nanoparticles.

Particle properties	Negatively charged particles	Positively charged particles
Hydrodynamic diameter (nm)	189 ± 5	210 ± 5
PDI	0.040 ± 0.018	0.059 ± 0.013
Zeta potential (mV)	-27 ± 2	+18 ± 3
Particle yield [%] (m/m)	36.9	59.67
DL [%] (m/m)	0.45	0.57
EE [%] (m/m)	16.8	33.1

Hydrodynamic diameter, PDI and ζ-potential were averaged from all batches; the yield, drug loading, and encapsulation efficiency were based on the pooled samples (average of 18 batches).
DL: Drug loading; EE: Encapsulation efficiency; PDI: Polydispersity index.

Results

Characterization of nanoparticles

SEM visualization of the CUR-PLGA-NPs showed that both negatively (Figure 1A) and positively (Figure 1B) charged particles are spherical with homogeneous surfaces. The size distribution of chitosan-coated positively charged CUR-PLGA-NPs was qualitatively more heterogeneous than negatively charged particles. Both negatively and positively charged CUR-PLGA-NPs could be visualized by fluorescence microscopy and were thus suitable for subsequent measurement of the depth of tissue penetration.

Average particle properties are shown in Table 1. Particle sizes were determined by light scattering to be between 189 and 210 nm, whereby positively charged particles were approximately 20 nm larger than negatively charged

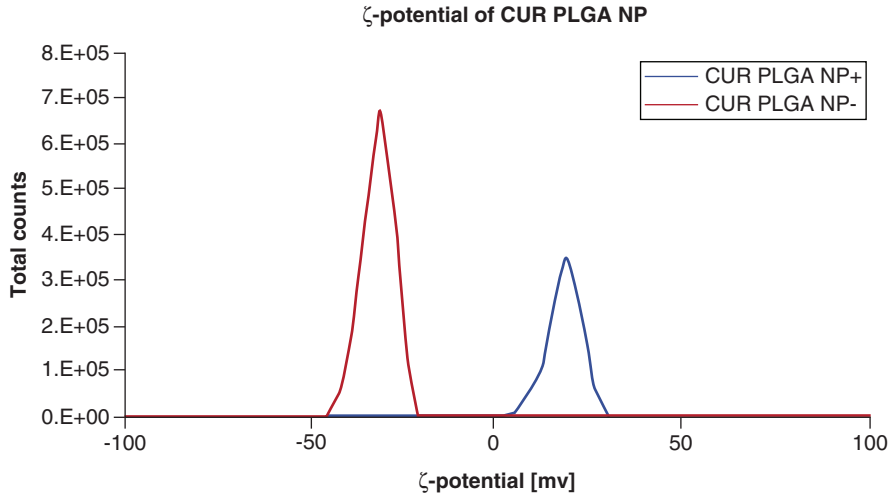

Figure 2. ζ-potential distributions of negatively and positively charged curcumin-loaded polylactic-co-glycolic acid nanoparticles. The blue curve represents the ζ-potential distribution of positively charged chitosan-coated CUR-PLGA-NPs, the brown curve the negatively charged CUR-PLGA-NPs.
CS: Chitosan; CUR: Curcumin; NP: Nanoparticle; PLGA: Poly(lactic-co-glycolic acid).

particles, probably due to the chitosan coating. The polydispersity indices of less than 0.1 implied very narrow size distributions for both types of particles. The surface charges, estimated with a zeta (ζ) potential of -27 ± 2 mV and +18 ± 3 mV, indicated stable nanoparticle dispersions. ζ-potential distributions are presented in Figure 2.

Drug loading

Drug loading was determined to 0.57% (m/m) and 0.45% (m/m) and the encapsulation efficiency to 16.8% (m/m) and 33.1% (m/m) for positively and negatively charged particles, respectively. The proportion of 57% and 45% encapsulation is fair for the expected theoretical value [24]. However, the encapsulation efficiency is relatively low, which can be attributed to the centrifugation conditions and the smaller particles' loss.

Sprayability of PLGA nanoparticles

The narrow size distributions (polydispersity index <0.1) of the NPs was not influenced by spraying. The size distribution was also not modified by the high-pressure conditions during the passage through the aerosolizer (Figure 3A). The dispersions also demonstrated unchanged ζ-potentials of -25 to -35 mV, reflecting stable colloidal behavior after spraying.

Visualization by scanning electron microscopy (Figure 3B) did not reveal any apparent changes in the particles' shape or any destruction or agglomeration. Thus, CUR-PLGA-NPs were aerosolized with the currently available PIPAC technology without any noticeable shift in particle quality. The chitosan-coated CUR-PLGA-NPs could be aerosolized up to the highest tested concentration of 2.4% (m/V). Upstream pressure maxima needed for aerosolization ranged from 7 to 17 bar. Thus, the preconditions for following penetration tests using biological models were granted – specifically, suitable particle properties, successful sprayability, compatibility with a medical device (no clogging) and persistent particle stability.

Feasibility of CUR-PLGA-NPs electrostatic precipitation as a pressurized aerosol in the *ex vivo* model

Both PIPAC and ePIPAC experiments were feasible with no difficulty in the IBUB model. Reproducible aerosolization of charged CUR-PLGA-NPs was also possible in this model. Macroscopically, we observed yellow staining of the peritoneal lining, which was more pronounced after ePIPAC, suggesting a superior efficacy of ePIPAC vs. PIPAC delivery. Subjectively, maximal staining was observed for the combination of ePIPAC with NP+ (Supplementary Figure 1).

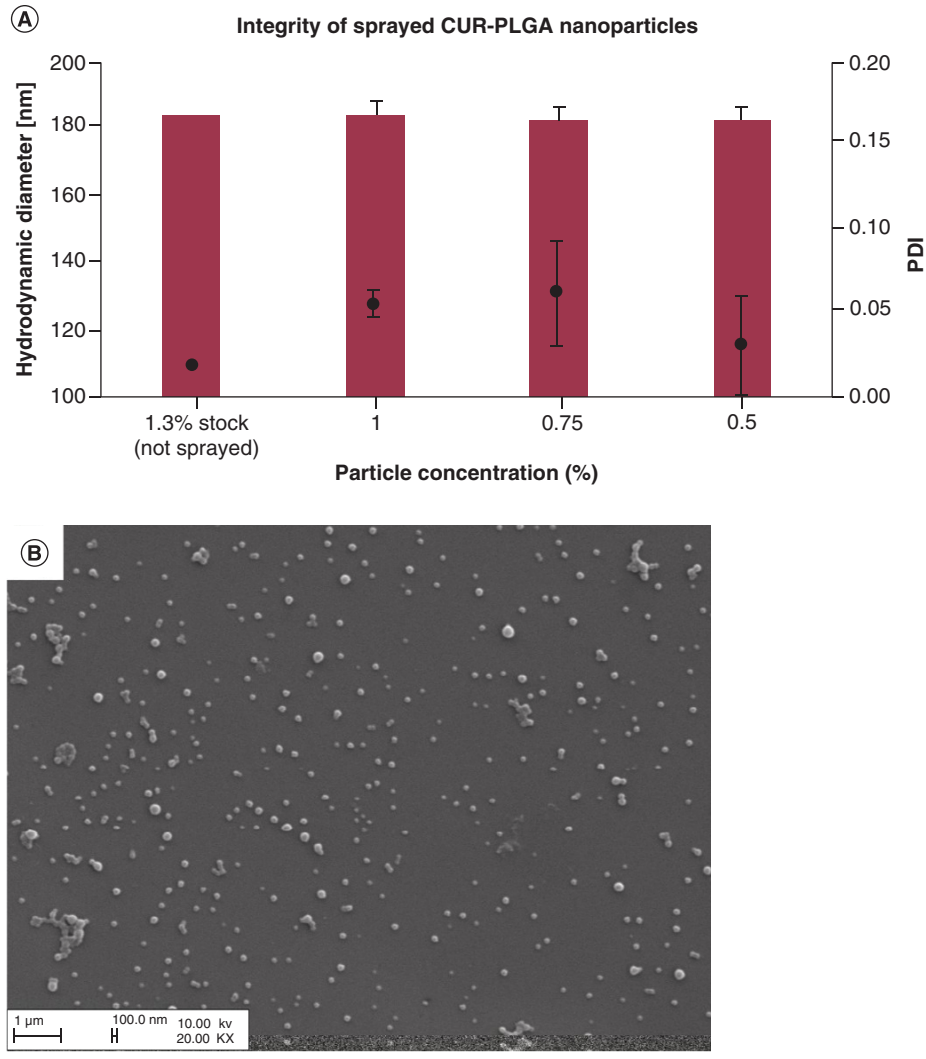

Figure 3. Integrity of sprayed curcumin-loaded polylactic-co-glycolic acid nanoparticles. (A) Hydrodynamic diameters and polydispersity indexes of CUR-PLGA naoparticles before and after aerosolization at different concentrations. **(B)** Visualization of nebulized nanoparticles of 1% (m/V) dispersion by scanning electron microscopy. CUR: Curcumin; PLGA: Poly(lactic-co-glycolic acid).

Depth of tissue penetration

For the analysis of the tissue penetration, pooled samples of 18 batches per particle type were used. Qualitatively, samples sprayed using the ePIPAC technique showed a higher fluorescence intensity and a deeper penetration into the peritoneal tissue in both the NP- and the NP+ groups (Figure 4).

Quantitatively, as shown in Figure 5, the depth of penetration values was significantly higher in the ePIPAC group (mean 156 µm, 95% CI: 149–163 µm) vs the PIPAC group (mean 119 µm, 95% CI: 113–123 µm); $p < 0.001$. The maximal depth of tissue penetration was reached by the combination of ePIPAC with chitosan-coated, positively charged NP (mean 208 µm, 95% CI: 199–217 µm).

Quantitative tissue concentration analysis

Positive versus negative charged CUR-PLGA-NPs did not significantly modify tissue concentration, with values remaining at 12.6 (95% CI: 7.2–18.0) ng/ml and 7.0 (95% CI: 6.1–7.9) ng/ml, respectively ($p = 0.36$). Electrostatic precipitation of the CUR-PLGA-NPs did not improve tissue concentration (ePIPAC: 9.1; 95% CI: 5.3–13.0) ng/ml versus PIPAC 10.5 (95% CI: 6.1–14.9) ng/ml, $p = 0.69$). Data are shown in Figure 6.

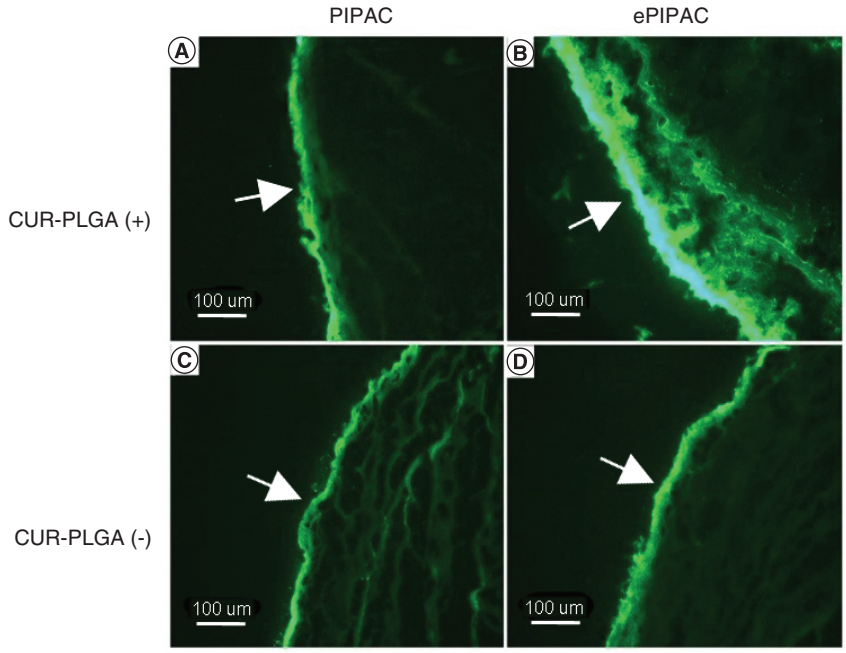

Figure 4. Depth of tissue penetration after aerosolization of positively (upper panels) or negatively (lower panels) charged curcumin-loaded polylactic-co-glycolic acid nanoparticles with pressurized intraperitoneal aerosol chemotherapy (A & C) versus electrostatic precipitation pressurized intraperitoneal aerosol chemotherapy (B & D). CUR has a spontaneous fluorescence. Qualitatively, the most intense staining is observed after ePIPAC delivery of positively charged CUR-PLGA nanoparticles (NPs) **(B)**. The fluorescence obtained after PIPAC with both positively and negatively charged CUR-PLGA-NPs **(A & C)**, and with ePIPAC combined with negatively charged CUR-PLGA-NPs **(D)** was less intense. These findings confirm the macroscopic assessment (see Supplementary Material 1).
Arrows: spray direction. CUR: curcumin; ePIPAC: Electrostatic precipitation PIPAC; NP: Nanoparticle; PIPAC: Pressurized intraperitoneal aerosol chemotherapy; PLGA: Poly(lactic-co-glycolic acid.

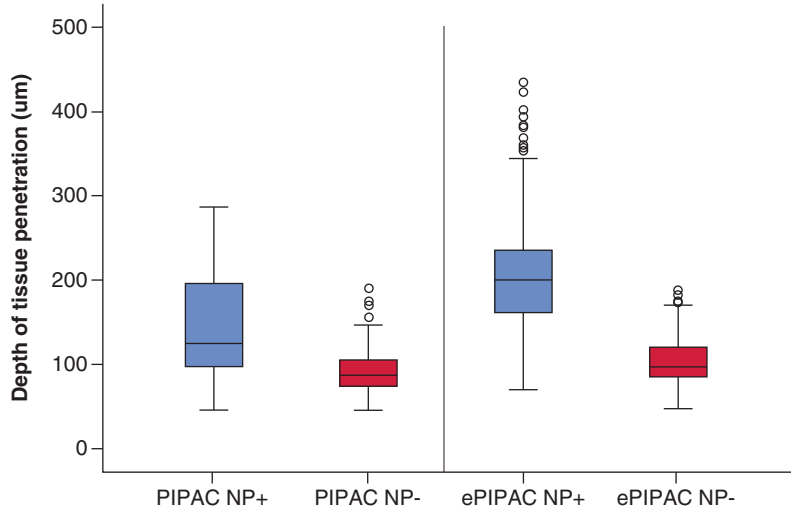

Figure 5. Depth of tissue penetration of curcumin-loaded polylactic-co-glycolic acid nanoparticles depending on the mode of delivery. The maximal tissue penetration is achieved by the delivery of positively charged curcumin-loaded polylactic-co-glycolic acid NPs as ePIPAC. The depth of tissue penetration is doubled to 208 μm by delivering positively charged NPs with ePIPAC versus negatively charged NPs with PIPAC.
CUR: Curcumin; ePIPAC: Electrostatic precipitation PIPAC; NP: Nanoparticle; PIPAC: Pressurized intraperitoneal aerosol chemotherapy; PLGA: Poly(lactic-co-glycolic acid).

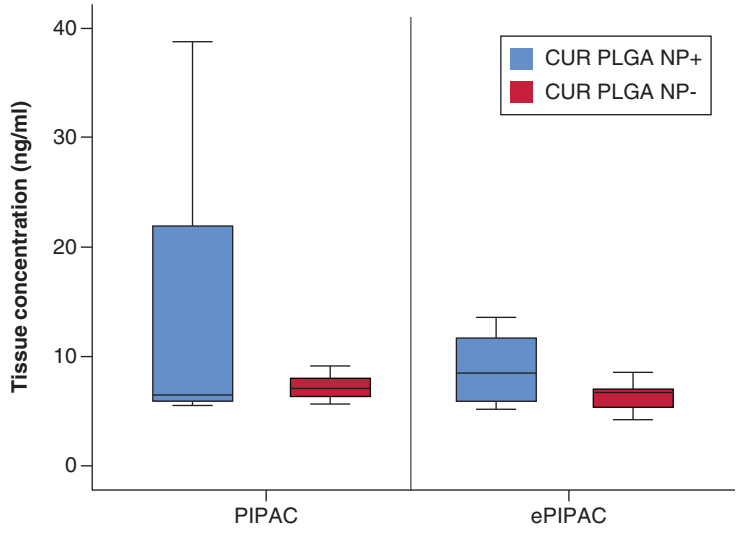

Figure 6. Tissue drug concentration of curcumin depending on the charge (positively vs negatively charged nanoparticles) and the mode of delivery (pressurized intraperitoneal aerosol chemotherapy vs electrostatic pressurized intraperitoneal aerosol chemotherapy). There is no significant difference in tissue concentration of CUR-PLGA-NPs, depending on the charge or the mode of administration. CUR: Curcumin; ePIPAC: Electrostatic precipitation PIPAC; NP: Nanoparticle; PIPAC: Pressurized intraperitoneal aerosol chemotherapy; PLGA: Poly(lactic-co-glycolic acid).

Discussion

The efficacy of intraperitoneal chemotherapy is limited by a low tissue concentration, mainly explained by limited drug penetration into the target tissue [6]. Efficacy can be improved by PIPAC [7], a new drug delivery technique with superior pharmacological properties compared with liquid intraperitoneal chemotherapy [8,25]. A further opportunity to optimize intraperitoneal drug delivery in patients with peritoneal metastasis is nanoparticles [26]. A combination of PIPAC with nanoparticles might further improve the target effect of the drug in the peritoneal tissue. The efficacy of this combined approach has recently been proven in an animal model of disseminated peritoneal ovarian tumors, where PIPAC enhanced intratumoral deposition of fluorescent pARG-HA nanoparticles [27]. It has also been suggested that adding electrostatic loading and following precipitation of the therapeutic aerosol might optimize tissue effects of PIPAC [22]. This hypothesis was confirmed in an animal model [14,28], and ePIPAC has successfully entered clinical practice [29,30].

The proof-of-principle of combining PIPAC, electrostatic precipitation and nanoparticle formulation for enhancing tissue delivery was recently delivered. Simulations *in silico* showed that ePIPAC improved the spatial distribution of nanoparticles [13]. In a rodent model, ePIPAC significantly increased tissue penetration and provided a more homogeneous delivery of Nab-paclitaxel into the peritoneal tissue. Aerosolization of cisplatin-loaded polyarginine-hyaluronic acid nanoscale particles (Cis-pARG-HA NPs) using PIPAC efficiently eradicated peritoneal metastasis in a rat model of human ovarian cancer. At the same time, this effect was not observed for the administration of free cisplatin [27].

In the present study, we investigated the possibility of changing the electric charge (positive vs negative) of nanoparticles to increase tissue uptake. For this purpose, CUR-PLGA-NPs were coated with chitosan to create a positive versus negative ζ-potential. We then used an established *ex vivo* model of the peritoneal cavity, the eIBUB model [18,19], to compare the efficacy of ePIPAC versus PIPAC for delivering these CUR-PLGA-NPs. The main finding of our study is that both a positive charge and the electrostatic precipitation increase the depth of tissue penetration of CUR-PLGA-NPs. Our results are in line with prior data [13,27].

After delivery as ePIPAC, the depth of tissue penetration of cationic CUR-PLGA-NPs doubled to more than 200 µm compared with the penetration of negatively charged NPs. This penetration is likely to be deep enough to effectively target the invasion front of peritoneal metastasis, a hypervascularized zone at 100–200 µm from the boundary to surrounding tissue [31]. The depth of tissue penetration of CUR-PLGA-NPs+ administered as ePIPAC (>200 µm) can be contrasted, for example, with doxorubicin, a molecule with a size of approximately 3 nm [31], which impregnates only four to six cell layers (20–30 µm depth) after intraperitoneal liquid delivery [5]. This improvement might have far-reaching consequences in future therapy of peritoneal metastasis.

Conclusion

The enhanced depth of tissue penetration of positively charged NPs after ePIPAC delivery may be clinically relevant. The tumor invasion front is located approximately 150 µm below the tumor rim in patients developing peritoneal

metastasis, whereas this front is deeper in patients without peritoneal recurrence (>300 μm) [32]. The evaluation of the clinical significance of these results will require further investigation, but it is evident that intraperitoneal drug delivery to the target tissue can be optimized by a combination of medical devices, formulation of the drug and control of the peritoneal environment. Specifically, a combination of PIPAC, positively charged NPs and the electrostatic precipitation considerably enhanced the depth of tissue penetration of CUR-PLGA-NPs.

Further formulation work is needed to increase the encapsulation efficiency of CUR-PLGA-NPs. Prior work reported similar encapsulation efficiency of 23% (m/m) with an initial drug loading of 1% (m/m), using harsher centrifugation conditions [33]. The effect of centrifugation on particle recovery, depending on particle volumes, has been described in the literature [34]. The difference between the two systems might be due to the more substantial amount of chitosan-coated particles, which tend to trap more drug within the particle. Larger particles are also expected to achieve higher yields due to purification by centrifugation and thus higher encapsulation efficiencies.

Future perspective

Pressurized intraperitoneal aerosol chemotherapy (PIPAC) is rapidly diffusing into clinical practice worldwide. The innovative combination of PIPAC, electrostatic precipitation and positively charged nanoparticles creates many opportunities for developing more effective, less invasive and less toxic therapies for peritoneal surface malignancies. After a thorough preclinical toxicity assessment, we expect the first combinations to be tested in controlled clinical studies within the next 5 years. There is considerable work ahead for appropriate selection of the most suitable agents, formulations and optimal conditions of delivery as PIPAC or electrostatic PIPAC, for each disease condition and therapeutic indication.

Summary points

- Peritoneal metastasis is an unmet medical need due to the limited efficacy of systemic chemotherapy.
- Pressurized intraperitoneal aerosol chemotherapy (PIPAC) is a promising locoregional drug delivery technique that takes advantage of physical laws to optimize drug delivery.
- PIPAC increases tissue drug concentration, improves tissue drug penetration and reduces systemic adverse effects due to the lower dose applied.
- Current PIPAC technology is hampered by inhomogeneous spatial distribution because of inertial impaction and gravity.
- The addition of an electrical force to aerosol particles, exerted by an electrostatic field (ePIPAC), improves homogeneity of aerosol distribution within the abdomen and further enhances tissue penetration.
- Nanoparticles (NPs) have the potential to improve further the target tissue effect of drugs distributed intraperitoneally.
- We have created cationic and anionic curcumin (CUR) polylactic-co-glycolic acid (PLGA) nanoparticles.
- It was possible to aerosolize these CUR-PLGA-NPs using current PIPAC and ePIPAC technology without damaging them.
- Target tissue effect (CUR concentration and depth of penetration) was maximal when CUR-PLGA-NPs+ were administered as ePIPAC.
- The combination of these two drug delivery systems (ePIPAC and cationic NPs) creates significant opportunities for better therapies of peritoneal metastasis.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/nnm-2020-0373

Author contributions

A Castagna prepared the samples, performed the depth of tissue penetration measurements and contributed to the writing of the manuscript. AJ Zander engineered the curcumin nanoparticles, performed concentration measurements and contributed to the writing of the manuscript. I Sautkin performed the aerosolization experiments. M Schneider designed the methods, coordinated the pharmaceutical project and contributed to the writing of the manuscript. R Shegokar participated to data analysis, prepared study protocol and contributed to the writing of the manuscript. A Königsrainer critically reviewed the manuscript. MA Reymond contributed to the study design, statistical analysis and redaction of the manuscript.

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Financial & competing interests disclosure

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Ethical conduct of research

This study did not involve data relating to human or live animal experimental investigations. The bovine organs used for the experiments were obtained from a slaughterhouse and came from animals used in the food chain. According to German law, such a study does not require the authorization of the Institutional Review Board or the Animal Protection Committee.

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