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Research paper

Surfactant-free redispersible nanoparticles in fast-dissolving composite microcarriers for dry-powder inhalation

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ABSTRACT

Spray-drying was investigated for the stabilization of surfactant-free nanoparticles as carriers for dry-powder inhalers. The microparticles rapidly dissolve after inhalation yielding dispersed nanoparticles.

Nanoparticles were prepared by a solvent displacement technique avoiding any surfactants. Microcarriers were prepared by spray-drying nanoparticle suspensions with lactose, mannitol or α-cyclodextrin as stabilizers. Nanoparticle size and ζ-potential before and after spray-drying were analyzed with photon correlation spectroscopy and laser Doppler anemometry, respectively. Cell uptake into macrophages was studied using U 937 cells by confocal microscopy.

Stabilization of nanoparticle suspensions by spray-drying with α-cyclodextrin yielded redispersible particles smaller than 200 nm. α-Cyclodextrin was a more efficient stabilizer than commonly used excipients. Microparticles with a mass median aerodynamic diameter of 4.3 μm showed properties suitable for dry-powder inhalation. The cell culture experiments with redispersed nanoparticles seem to suggest less interaction and uptake with macrophages compared to polymeric microparticles.

In conclusion, nanoparticles can easily be transferred to dry-powders suitable for inhalation by spray-drying. This allows the pulmonary application of nanoparticles in high concentrations.

1. Introduction

Controlled and targeted drug delivery to the lung using nano-sized carrier systems has become an attractive area of research. Apart from facilitating uptake into lung tissue, constant drug levels over time would be desirable to increase patient compliance by reduction of the inhalation frequency [1,2].

Until now, no sustained release pulmonary drug delivery system is commercially available. High clearance of therapeutics from the lungs occurs either through phagocytosis by macrophages, via rapid uptake in the alveolar region [3] or through elimination by mucociliary clearance in the bronchial region [4].

Nanoparticles (NP) were widely investigated for controlled drug delivery to the lungs [1,2,5]. They are considered to be superior to microparticles (MP) for several reasons. Makino et al. have shown that there is no active uptake of NP with a size below 200 nm by macrophages [6]. For sustained release, NP smaller than 200 nm should be administered to the lung to avoid clearance by macrophages. Another aspect is the interaction of particles with mucus. The penetration through the mucus layer is dependent on size and surface characteristics, making NP superior to MP with respect to penetration [7].

Biodegradable polymeric NP delivery to the lungs suffers from two main drawbacks: Firstly, depending on their size, they show high surface energy leading to aggregation if stored in dry state. Secondly, they are too small to deposit in the lung and are easily exhaled [1]. To overcome these problems, NP are often applied to the lung in suspension. Hereby, distribution of generated droplet size varies with nebulizer technique, and applied stress during nebulization can lead to stability problems of the NP suspensions, such as aggregation [8].

When NP are embedded into microparticles, the carrier size determines the deposition in the lungs. The size distribution of microcarriers (MC) is independent on the application device and can be produced within the desired size range for inhalation by spray-drying. Particle sizes can be adjusted by concentration, viscosity and atomization pressure [9]. Preparation of MC for NP delivery was first described by Freitas et al. who spray-dried solid lipid NP [10]. Problems of those delivery systems were identified as follows: (1) MP do not disintegrate after spray-drying [11–14] and can therefore be taken up by macrophages. (2) The use of high concentrations of stabilizers (stabilizer/NP ratio 100:1) [15] is only feasible for highly potent drugs (3). Different surfactants were used to prevent NP aggregation. Yet, non-ionic surface active stabilizers can disturb mucus or alveolar surfactant layers leading to irritation of the sensitive pulmonary epithelium [16,17]. Therefore, the

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objective of this investigation was to prepare surfactant-free composite MC containing easily redispersable NP suitable for dry-powder inhalation.

Particle size of NP after administration of MC was thought to be an important parameter to ensure uptake into broncho-alveolar tissue for targeted delivery of particles below 200 nm. Different carbohydrates were studied as stabilizers to prevent NP aggregation: lactose, well known in pulmonary delivery, mannitol, which is less hygroscopic, and α-cyclodextrin (α-CD), a cyclic oligosaccharide. These excipients were expected to stabilize the NP by steric effects and stabilization of hydrogen bonds in analogy to lyophilization [18].

To verify suitability of MC for inhalation, cascade impaction with a next generation impactor under standard conditions for the HandiHaler® was performed. Particle uptake into macrophages was studied in a cell culture model of differentiated U 937 cells against PLGA reference MP at time points between 30 min and 8 h.

The hypothesis of our investigation was to stabilize NP suspension by spray-drying with suitable excipients to generate fast-dissolving MC suitable for dry-powder inhalers. Such formulations could be of interest for pulmonary application of nanocarriers.

2. Materials and methods

2.1. Materials

RG 502H® a poly(lactide-*co*-glycolide) (PLGA) with a weight average molecular weight (M_w) of 12 kDa was purchased from Boehringer Ingelheim (Ingelheim am Rhein, Germany). Acetone, acetonitrile, ethyl acetate, N-methyl pyrrolidone and dichloromethane of analytical or HPLC grade were obtained from Acros Organics (Belgium). Mannitol (Merck, Darmstadt, Germany) and lactose (Sigma, Schnelldorf, Germany) were used as received. α-Cyclodextrin (α-CD) was a gift from Schwarz Pharma (Monheim, Germany). Nile red for particle staining (Acros Organics, Belgium) and tetra methyl rhodamine-5-carbonyl azide (TMR) (Invitrogen, Karlsruhe, Germany) for polymer labeling were used as received.

For cell culture experiments, RPMI-1640 medium from PAA (Pasching, Austria) was supplemented with 10% fetal calf serum (FCS) (Biochrom, Berlin, Germany) and 10 mM glutamine (Merck, Darmstadt, Germany). For cell activation/differentiation, phorbol-12-myristate-13-acetate (PMA) from Sigma (Schnelldorf, Germany) was used. Human serum gained from a healthy volunteer by centrifugation ($2000 \times g$) of a whole blood sample was used for opsonization of these particles. Informed consent was obtained according to the Declaration of Helsinki as amended in Seoul 2008 for humans.

2.2. Polymer labeling

For qualitative cell uptake studies using confocal laser scanning microscopy (CSLM), PLGA was labeled with a red fluorescent dye as described earlier [19]. In brief, 5 mg tetra methyl rhodamine-5-carbonyl azide was added to a dry solution of 495 mg polymer in N-methyl pyrrolidone. The reaction mixture was stirred at 80 °C for 4 h. After cooling to room temperature, the solution was poured into ultrapurified water (Millipore, conductivity 0.055 μS/cm) and the precipitate was collected by filtration. The product (TMR-PLGA) was washed several times and freeze-dried in Edwards Freeze-Dryer Modylo (Condensator temperature −55 °C, 2×10^{-6} bar) for 10 days.

2.3. Surfactant-free NP preparation

Nile red–labeled NP (NR-NP) were prepared by a modified solvent displacement technique [20]. Briefly, 2 ml of organic phase (20 mg/ml PLGA, 0.02 mg/ml nile red, 20% ethyl acetate and 80% acetone) was injected (10 ml/min) into 5 ml of ultrapurified water.

Tetra methyl rhodamine-labeled NP (TMR-NP) were prepared by injection of 36 mg PLGA and 4 mg TMR-PLGA dissolved in 2 ml acetone into 5 ml ultrapurified water.

The aqueous phase was stirred at 500 rpm, and the needle (Fine-Ject® 0.6 × 30 mm) was moved in the aqueous phase during the injection. The samples were left under stirring for at least 3 h in a fume hood to evaporate the organic solvents. After this the samples were centrifuged at 4 °C for 5 min with 27*g* to remove aggregates. The supernatant was used for further experiments.

2.4. Spraying and spray-drying

Spray-drying was performed using a Büchi Mini Spray Dryer B-290® (Büchi, Flawil, Switzerland). Mannitol, lactose or α-CD were dissolved in ultrapurified water and filtered through a 0.2 μm filter to remove insoluble material. Then the solution was mixed with the nanosuspension in the desired 20:80 (NP/stabilizer) weight ratio and diluted to 2.5% w/V of the solids.

As shear forces during atomization can lead to aggregation, suspensions were sprayed with a pump rate of 1.7 ml/min through a two-fluid nozzle by an air stream of 536 normliters compressed air per minute. The liquid was collected in a beaker and analyzed for NP size distributions and ζ-potentials.

Secondly, the suspensions were spray-dried under protection from light. α-Cyclodextrin was additionally tested in ratios of 40:60 and 10:90 (NP/carbohydrate). The conditions of spray-drying were set as follows: inlet temperature 70 °C, aspirator 100%, pump 1.7 ml/min, nozzle cleaner 1. The samples were collected with a high-performance cyclone. The outlet temperature reached approximately 41 °C.

Polymeric reference particles were spray-dried from a 2.5% PLGA (1/100 TMR-PLGA) solution in DCM with a pump rate of 10%. The inlet temperature was set to 60 °C resulting in an outlet temperature of approximately 41 °C. The aspirator was set to 100%, and the spraying nozzle was cooled with water. To atomize the polymer solution, 536 normliters compressed air per minute was used.

To ensure removal of residual solvents, the microparticles were freeze-dried in Edwards Freeze Dryer Modylo® (−55 °C, 2×10^{-6} bar) over night. The dry particles were sieved through 90-μm sieve prior to further use.

2.5. Particle characterization

NP size distributions and ζ-potentials were measured using a Zetasizer NanoZS® (Malvern Instruments, Herrenberg, Germany) with photon correlation spectroscopy (PCS) and laser Doppler anemometry, respectively. NP suspensions were diluted in a 1:10 ratio with ultrapurified water. The sugar matrix of the spray-dried product was dissolved by gentle shaking in ultrapurified water, and the NP were diluted to desired concentrations. Results are shown as intensity weighted distributions of particle diameters (*Z*-Ave).

The size distributions of spray-dried microparticles (RI: 1.600) were measured by laser diffraction in isopropanol (RI: 1.390) or hexane (RI: 1.380) using a Mastersizer X® (Malvern Instruments, Herrenberg, Germany). The particle concentrations were adjusted to obtain optimal obscuration levels. The suspensions were stirred during measurement to prevent particle sedimentation. The analysis based on volume weighted size distributions were reported as $D(v,X)$ values, where X represents the fraction of particles smaller than the given size.

Aerodynamic properties play a crucial role in the pulmonary deposition of inhaled particles. Therefore, powders were characterized using a cascade impaction apparatus E (Next Generation

Impactor, Copley Scientific, Therwil, Switzerland), described in the European Pharmacopoeia. Approximately 20 mg samples were accurately weighed into polyethylene capsules (size 3) and delivered with a Handihaler® (Boehringer Ingelheim, Ingelheim am Rhein, Germany) at an air flow of 39 l/min for 6.15 s, to reach a total flow of 4 l and the recommended pressure drop of 4 kPa. Acetonitrile was used to rinse the collection pans several times, collected in volumetric flasks and filled up with acetonitrile to compensate evaporation. Quantitative analysis of particle entrapped nile red dissolved in acetonitrile was performed with Safire II fluorescence plate reader, Tecan Deutschland (Crailsheim, Germany) after dilution of the samples to the linear range between 0.5 and 125 ng/ml.

MMAD and GSD calculation was performed by Copley Inhaler Testing Data Analysis Software (CITDAS®) Version 2.0 (Copley Scientific).

To gain information on particle morphology, the particles were investigated in dry state after sputter-coating with platinum using a JSM-7500F Scanning Electron Microscope® (Jeol, Japan) equipped with an ALTO-2500® liquid N_2-cryo-transfer system (Gatan, USA) with acceleration voltage between 2 and 4 kV in cryo mode.

2.6. Particle clearance by macrophages

The promonocytic cell line, U 937 (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany), was seeded in 8-well camber slides in a density of 5×10^4 cells/well and fed with 400 µl RPMI-medium supplemented with fetal calve serum (FCS) and glutamine. For each time point and particle type, two wells were seeded. For differentiation into macrophages, the cells were cultured for 4 days with 10 ng phorbol-12-myristate-13-acetate (PMA) per milliliter [21].

Reference MP and MC, disintegrating to NP, were suspended in RPMI-medium (1 mg/ml) by sonication for 2 min in an ultrasound bath to ensure homogenous distribution within the sample. A volume of 200 µl of particle suspension and 200 µl RPMI-medium supplemented with 20% human serum (for opsonisation) were added to each well and incubated at 37 °C in a 5% CO_2-atmosphere. At defined time points, the cells were washed two times with PBS pH 5 and two times with PBS pH 7.4 to remove particles, which were not taken up. The cells were fixed by incubation with 4% paraformaldehyde for 20 min and washed two times with PBS pH 7.4 prior to nucleus staining with DAPI (20 min). The cells were washed with PBS pH 7.4 and embedded in FluorSave® (Calbiochem, San Diego, CA, USA) and stored in the dark until examination by microscopy. All washing and incubation volumes were 400 µl.

Confocal laser scanning microscopy (CLSM) was performed with a Zeiss Axiovert® 100M microscope equipped with a Zeiss LSM 510 scan module (Zeiss, Oberkochen, Germany). The blue channel was used to locate the cell nuclei (ex: 364 nm, BP 385–470 nm) and the red channel (ex: 543 nm, LP 560 nm) was used to locate the labeled nano- and microparticles. *Z*-stacks (one slice/µm) were performed to ensure that particles and nuclei were on the same level, and the particles were therefore taken up by the macrophages. To obtain representative data, 10–20 images per well were monitored. At least three pictures were taken per time point. The same settings were used for all time points and images.

2.7. Statistics

Experiments were performed at least in triplicate unless indicated otherwise. Statistical significance was investigated by Mann–Whitney test with GraphPad Prism 5 (Vers.5.02, GraphPad Software Inc., San Diego, USA) at confident intervals $p < 0.05$.

3. Results and discussion

3.1. Surfactant-free NP preparation

As non-ionic surfactants may be irritant or harmful to the sensitive airway epithelia [16,17], alternatives in the composition of vectors for drugs to the lungs have to be investigated. A valuable technique to prepare particles in nanoscale under avoidance of surfactant is the solvent displacement technique, first described by Fessi et al. [22]. In contrast to other methods, neither shear stress nor chlorinated solvents are necessary to generate NP with small particle sizes and narrow size distributions.

The aim of this study was to produce fluorescently labeled NP and to investigate their stabilization by spray-drying. The rationale behind this approach is the rapid dissolution of MC in lung fluids generating high concentrations of NP which would ensure efficient drug delivery to alveolar and bronchial tissues. The preparation process is a fast and simple method yielding narrow and highly reproducible size distributions for the NR-NP with a *Z*-Ave of 165 ± 9 nm and a polydispersity index below 0.1. The nanosuspensions were stabilized by electrostatic repulsion with a ζ-potential of −39.0 ± 3.8 mV (Table 1). TMR-NP were of the same size and ζ-potential range, although the distribution with a PDI of 0.140 was wider. The particles sizes were in the desired sub 200 nm range and could be expected to show less uptake by macrophages [6].

3.2. Spraying and spray-drying

To avoid macrophage uptake and clearance, the MC should disintegrate rapidly and release the NP at the site of action. Disaggregation is dependent on the solubility of the matrix material. The used carbohydrates are easily soluble in water. Mannitol (238 ± 1 mg/g solution) and lactose (290 ± 20 mg/g solution) [23] are well-known excipients in preparations for inhalation [5]. α-Cyclodextrin in comparison with β-cyclodextrin has also a high solubility in water (145 mg/ml vs. 18.5 mg/ml) and shows reduced toxicity [24].

As polymeric NP are sensitive, several factors can disturb NP integrity during spray drying, for example atomization may lead to aggregation or disruption of particles. This was tested by spraying the particle suspensions without heating in a 20:80 (NP/stabilizer) ratio and collecting the mist with a beaker for PCS measurements. For all stabilizers, the particle sizes slightly increased during the spraying process by less than 10% and therefore were still in the desired size range (Fig. 1). The increased PDI suggested slight aggregation during the spraying process, but the values below 0.2 indicated that the size distribution was still monomodal.

High temperature of the product may lead to aggregation and fusion of NP, especially when the glass transition temperature (T_g) of the polymer is exceeded. By using a concurrent spray-dryer, the product is cooled by the evaporation of the dispersant until product and air are in equilibrium. The product temperature can be estimated by the outlet temperature, which is 10–15 °C higher than the product temperature [25]. The outlet temperature was chosen to be 40–42 °C and therefore to be below the T_g of RG 502H (45 °C).

Table 1
Nanoparticle size distribution and ζ-potential.

	Size, *d* (nm)	PdI	ζ-potential (mV)
Nile red-loaded NPs	165 ± 9	0.080 ± 0.002	−39.0 ± 3.8
TMR-labeled NPs	164 ± 2	0.140 ± 0.002	−40.4 ± 0.7

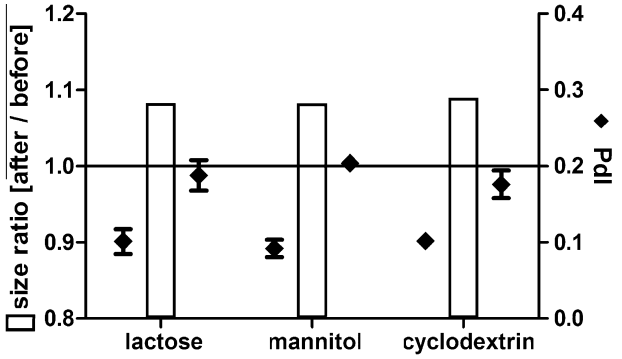

Fig. 1. Nanoparticle size distribution before and after atomization of nanosuspensions. All suspensions show a size increase of 9% and monomodal distributions.

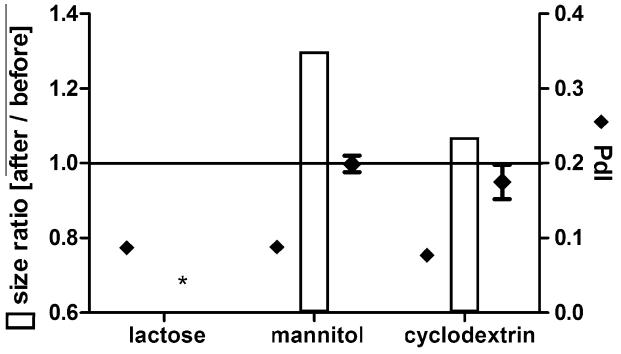

Fig. 2. Nanoparticle size distribution before and after spray-drying. No resuspension was possible with lactose as stabilizer. Drying with α-CD retained the size of sprayed NP.

In the 20:80 ratio, microparticles were obtained for all stabilizers. Spray-drying yielded particles in a size range suitable for inhalation of $D(v,0.5)$ 3.2 ± 0.2 μm only for α-CD microparticles (Table 2). For the other stabilizers, the initial concentration or the feed flow can be reduced to obtain the desired size range for inhalation [9].

3.3. Particle characterization

Preservation of NP integrity was important to maintain the colloidal characteristics of these formulations. This was analyzed by dissolving the MC-matrix in ultrapurified water by gently shaking. Size distributions were measured 5 min after reconstitution. Lactose showed no effect on the protection of NP properties during the drying process, as NP were not redispersible in water even after application of ultrasound. With the use of mannitol as a stabilizer, the *Z*-Ave of the resuspended NP significantly increased by 130% (Fig. 2).

From the tested carbohydrates, only α-CD maintained NP size in a dry state. The NP could be easily resuspended by gently shaking within 5 min in water without the use of any surfactants. For the 20:80 ratio, the *Z*-Ave was still below the 200 nm limit, even though the NP size distribution slightly increased to 187 ± 13 nm (Fig. 2). After the spray-drying process and reconstitution in water, the nanosuspension was stable showing a ζ-potential of −33.4 ± 1.4 mV. The particle size increased by 5.5% during spray-drying when the ratio was changed to 40:60 (NP/α-CD) ratio. At a ratio of 10:90, the increase in size was negligible with 0.6% compared to the original suspension.

Several attempts have been reported concerning spray-drying of nanocarriers. Sprayed nanocapsules with colloidal silica yielded a dry MC product. Various parameters were investigated such as MC size, density and residual moisture, but redispersibility of the nanocapsules was not communicated [13,14]. Also spray-dried poly (caprolactone)/miglyol nanocapsules using mannitol, lactose or K30PVP as stabilizers were reported to generate redispersible formulations [26]. Solid lipid NP (SLN) were spray-dried with a variety of carbohydrates (mannitol, lactose, trehalose, sorbitol, glucose and mannose) [10]. All these formulations contained surfactants to ensure redispersibility.

Table 2
Microparticle size distribution determined by laser diffraction.

Size in μm	$D(v, 0.1)$	$D(v, 0.5)$	$D(v, 0.9)$
Stabilizer			
Lactose	3.99 ± 1.54	20.6 ± 1.7	67.7 ± 1.0
Mannitol	0.88 ± 0.02	9.3 ± 0.0	35.1 ± 3.7
Cyclodextrin	0.91 ± 0.06	3.2 ± 0.2	10.4 ± 0.8

Sham et al. described the preparation of disintegrating MP as carriers for NP under avoidance of surfactants. High drug loading and a potent drug is needed with this formulation because the stabilizer to nanosphere ratio is 100:1 [15] and therefore 20-fold higher than in our experiments leading to osmotic effects and irritation of lung epithelia [27].

To explain the surfactant-free stabilization by CD, we postulate a decoration of the NP with small crowns of CD-molecules acting as spacers between the particles. Probably due to its rigid structure and fixed positions of the OH-groups for hydrogen bonding, the interaction between α-CD and NP surface is more stable.

Because of its small cavity (0.57 nm for α-CD compared to 0.78 nm for β-CD) [24], inclusion complex formation of α-CD with drugs may be neglected and cholesterol is reported not to form inclusion complexes with the smallest of the cyclodextrins. Nevertheless, toxicity of α-CD has to be evaluated for lung delivery before in vivo tests with this formulation can be performed.

A critical parameter for the deposition of particles in the lung is their aerodynamic behavior. The mass median aerodynamic diameter (MMAD) was analyzed using a next generation cascade impactor (NGI) where dry-powder formulations were investigated in a commercially available dry-powder inhaler. Apart from the size distribution of the dry-powder formulations (Fig. 3), also their desagglomeration into individual nanoparticles can be tested. The mass median aerodynamic diameter (MMAD) was in the ideal range for bronchio/alveolar targeting with 4.3 ± 0.2 μm [1]. The geometric standard deviation (GSD) with 1.62 ± 0.04 is narrow.

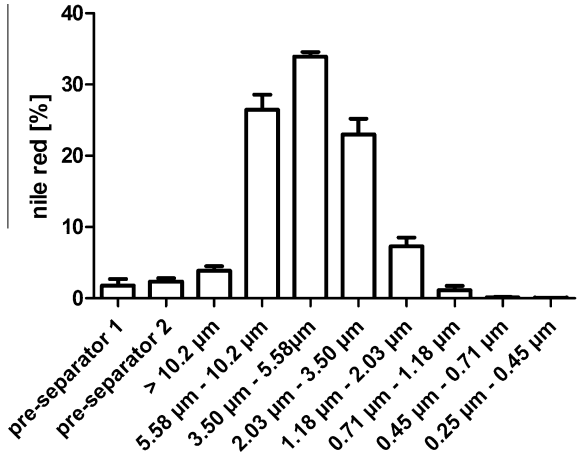

Fig. 3. Cascade impaction of cyclodextrin microcarriers. The aerodynamic size distribution of nile red-labeled nanoparticles in microcarriers is in the size range for pulmonal application with a MMAD of 4.3 ± 0.2 μm.

Ca. 60% of the applied dose from each α-CD formulation (20:80) was collected in the impactor. More than 50% of these particles were smaller than 5 μm (fine particle fraction). This value indicates highly efficient drug delivery to the lungs compared to other formulations applied with the Handihaler® device [28].

The scanning electron micrographs showed partially collapsed spherical particles with a rough surface (Fig. 4 A and B). These deformations are probably caused by the spray-drying process as well as by orientation of colloidal particles in drying droplets as suggested by Tsapis et al. [29]. The size of the small bulges at the surface of MC corresponds roughly to that of NP after dissolving the cyclodextrin matrix of the carrier particles. The individual NP after reconstitution can be seen in Fig. 4 C representing a narrow size distribution.

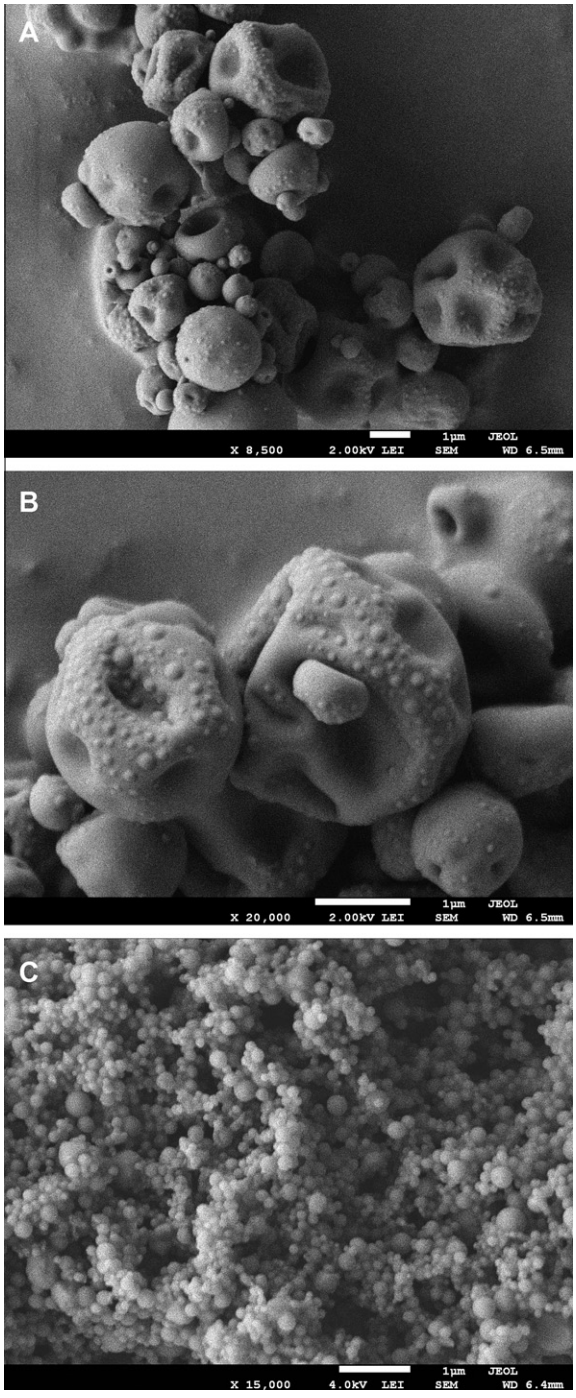

Fig. 4. SEM of cyclodextrin microcarriers: (A) and (B) on the surface of the spray-dried particles individual nanoparticles in the sub 200 nm range are observed. (C) Spherical individual nanoparticles after resuspension in a small amount of water.

The spray-dried material can be directly filled in hard gelatin or polyethylene capsules suitable for use in commercially available handheld dry-powder inhalers such the Handihaler®, allowing a straight forward pulmonary administration of NP preparations. This route of application is more convenient and patient friendly, compared to the use of nebulizers [2]. Biodegradable NP must be stored under dry conditions to prevent both polymer degradation of the NP by hydrolysis and to avoid agglomeration during storage. Therefore, freeze-dried products must be resuspended in water before nebulization. The proposed application form is ready for use and can be expected to reduce polymer degradation. Lung deposition is mainly controlled by the aerodynamic diameter of the carrier particles (MC). Larger particles will be deposited preferentially in the bronchial region, whereas smaller particles could penetrate deeper into the lung reaching the alveolar space. The size of MC can be controlled by spray-drying process by fine-tuning process parameters, such as concentration of the solid, spraying pressure and opening of the orifice [9]. Drug release from polymeric MP depends on the diffusion distance of the drug. Larger particles show more sustained release in comparison with smaller ones, so the drug release is not evenly distributed throughout the lung. By embedding NP into fast disintegrating MC, the drug release can be decoupled from particle size of the carrier particles leading to a uniform drug release pattern in the lung.

3.4. Particle clearance by macrophages

Another interesting aspect of the "nano in micro"-principle could be that MP are thought to be more readily opsonized and hence taken up by macrophages in the alveolar and bronchial space depending on their particle size and surface properties. On the other hand, NP might escape from macrophage uptake, although this issue is discussed somewhat controversially in the literature [6,30,31]. To elucidate the cell interaction between NP as well as MC in a cell culture system based on activated macrophages, PLGA was fluorescently labeled. For uptake studies, it is important that the polymer is covalently labeled with the fluorescent dye otherwise the dye may mimic uptake by migration into cell membranes [32]. The detection of uptake into macrophages was performed on a semi-quantitative level using confocal scanning microscopy. During the uptake study, all settings were kept constant for all time points and images. Therefore, an increase in fluorescence inside macrophages could be an indicator for enhanced cellular uptake. More detailed quantitative information can be expected from FACS studies currently under way. The results of a time series (0.5–8 h) of uptake experiments in U 937 cells using both MP and NP are shown in Fig. 5. *Z*-stacks were performed to allow localization of particles in cells.

The left row in Fig. 5 depicts the uptake of PLGA reference microparticles (TMR-MP) by U 937 cells. After 30 min, a large fraction of the particles is, despite extensive washing, associated with the macrophages and/or already taken up. This is in agreement with observations from other groups [1,33]. The TMR-NP show lower affinity to the macrophages and a smaller fraction seems to be internalized into U 937 cells (Fig. 5 *right column*). Based on these results, one could speculate that macrophage clearance of NP could be reduced compared to the microparticles.

Macrophages seem to show two thresholds for particle uptake as currently discussed in the literature. The lower limit observed here was described previously by several groups. Particles <200 nm are not actively taken up by macrophages [6,34,35]. The upper size limit of macrophage uptake is defined by the volume ratio of particle to macrophage. Large particles cannot be

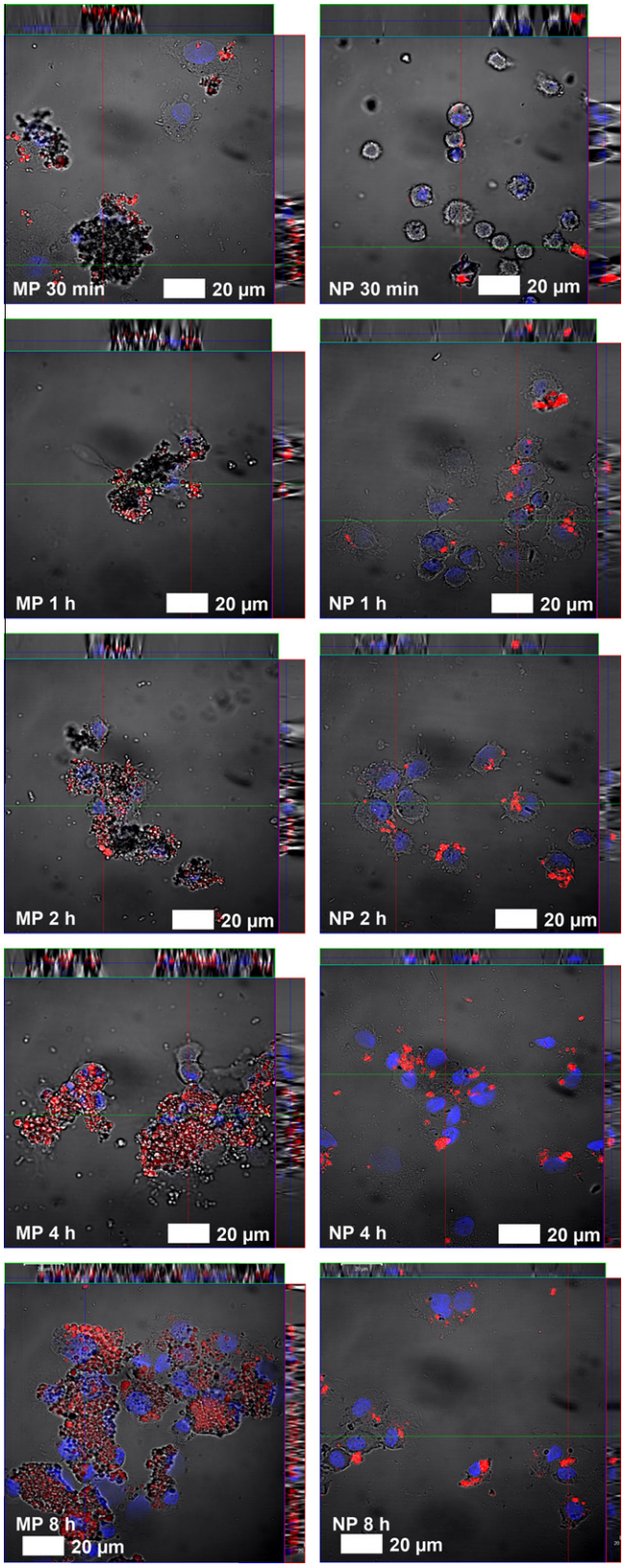

Fig. 5. U 937 cells incubated for 30 min to 8 h with reference TMR-MP (*left column*) and TMR-NP from α-CD-MC (*right column*). The microparticles show higher affinity to the macrophages and are more subject to clearance than sub 200 nm nanoparticles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

phagocytised because of their large volume. This effect is exploited in the large porous particle approach [36,37]. Obviously many factors apart from size and surface properties may affect phagocytosis [31]; hence, uptake studies under *in vivo* conditions would be desirable to confirm these results.

4. Conclusion

Surfactant-free stabilization of NP was achieved by co-spraying with α-CD. The particles are easily and quickly resuspendable in water and no increase in NP size was observed compared to the original suspensions. Three main problems in pulmonary delivery of biodegradable NP were addressed successfully: (1) NP are stabilized against hydrolytic degradation by transformation into the dry state using spray-drying. (2) NP can be stored and resuspended without change in particle size. (3) NP delivery could be facilitated using dry-powder inhalers avoiding inconvenient nebulizers. Pulmonary deposition of carrier particles is controlled by particle size in the air stream (MMAD). Therefore, targeting to specific segments of the lung could be fulfilled achieved by preparation of spray-dried MC with narrow size distributions without affecting NP size-dependent release properties.

The obtained MC open a door for surfactant-free delivery of nano-devices in a high mass ratio to the lungs. The use of α-CD in this formulation is an important step forward to convenient and patient friendly delivery systems of nanoparticles to the lungs via conventional dry-powder inhalers such as the Handihaler®.

The results are encouraging for further investigations under *in vivo* conditions to elucidate size effects on the deposition and uptake of nanoparticles in the lung.

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