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Phagocytosis of spherical and ellipsoidal micronetwork colloids from crosslinked poly(ε-caprolactone)

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Abstract

The effect of non-spherical particle shapes on cellular uptake has been reported as a general design parameter to control cellular recognition of particulate drug carriers. Beside shape, also size and cell-particle ratio should mutually effect phagocytosis. Here, the capability to control cellular uptake of poly(ε-caprolactone) (PCL) based polymer micronetwork colloids (MNC), a carrier system that can be transferred to various shapes, is explored in vitro at test conditions allowing multiple cell-particle contacts. PCL-based MNC were synthesized as spheres with a diameter of ~6, ~10, and 13 µm, loaded with a fluorescent dye by a specific technique of swelling, re-dispersion and drying, and transferred into different ellipsoidal shapes by a phantom stretching method. The boundaries of MNC deformability to prolate ellipsoid target shapes were systematically analyzed and found to be at an aspect ratio AR of ~4 as obtained by a phantom elongation εph of ~150%. Uptake studies with a murine macrophages cell line showed shape dependency of phagocytosis for selected conditions when varying particle sizes (~6 and 10 µm), and shapes (εph: 0,75 or 150%), cell-particle ratios (1:1, 1:2, 1:10, 1:50), and time points (1-24 h). For larger-sized MNC, there was no significant shape effect on phagocytosis as these particles may associate with more than one cell, thus increasing the possibility of phagocytosis by any of these cells. Accordingly, controlling shape effects on phagocytosis for carriers made from degradable polymers relevant for medical applications requires considering further parameters besides shape, such as kinetic aspects of the exposure and uptake by cells.
Keywords: Particle shape, phagocytosis, macrophage, polymer micronetwork colloids, poly(ε-caprolactone)

1. Introduction

The phagocytosis of particles by specialized cells such as macrophages or dendritic cells aims at removal of potentially pathogenic particulate matter from the organism. This process is highly relevant in pharmaceutics as it may, on the one hand, impede the targeting or long-term release function of micro-/nanoparticulate drug carriers [1] [2]. On the other hand, uptake can facilitate the delivery of proteins and/or adjuvants into antigen presenting cells, thereby allowing to initiate or modulate immune responses, such as for improved vaccination strategies [3] [4].

In the last decade, the role of particle shape on phagocytosis has been identified in model studies with isolated cells and particles, where non-spherical ellipsoidal polymer particles showed reduced engulfment [5] [6]. In model studies analyzing the interaction of single cells and single particles, particle surfaces with low curvatures at the contact point between cell and particle were identified to suppress phagocytosis, while larger curvatures enhance phagocytosis [5] [7] [8]. For prolate ellipsoidal particles, a cell-particle contact with the flat and less curved site is statistically more likely. Computational analysis of nanoparticle endocytosis based on curvature energy of lipid – bilayer membrane and particle-membrane contact adhesion energy suggest that a reorientation of adhered ellipsoids moving the long axis from parallel towards perpendicular (relative to the cell membrane) is possible but energetically not preferred [9]. Therefore, according to the current understanding, ellipsoids should be taken up by cells with reduced efficiency compared to spherically shaped particles. This relationship of macrophage uptake patterns is supported by studies with various types of particles including, e.g., spherical metal-organic framework (MOF) particles compared to rod-shaped MOFs of a different composition [10], L-cystine based composite particles loaded with CdTe quantum dots with preference of spheres over plates over needles [11], or poly(lactide-co-glycolide) (PLGA) nanoparticles and microparticles comparing spheres and prolate ellipsoids [12]. Accordingly, particle shape is expanding the list of particle features like size, surface properties and mechanical properties that influence cellular recognition and phagocytosis [13] [14] [15] [16].

However, the cellular uptake of particles is a kinetic process typically approaching a distinct maximum level, which theoretically can be either identical or different for specific particle
types/shapes and may be independent from the respective uptake velocities. For different types of latex beads, microscopic analysis of single uptake events after cell contact suggested slower and more broadly distributed uptake rates for ellipsoids (5 to 55 min; mean 25 min) compared to spheres (2 to 10 min; mean 3 min) [17]; still those values are on a similar time scale and make the time point of observation in \textit{in vitro} studies highly relevant. Studies with both nanoparticles and microparticles of ellipsoidal and spherical shapes revealed a practically identical uptake by dendritic cells within each size class (150 nm; 2 µm) as examined after overnight incubation [18]. For PLGA nanospheres vs. -ellipsoids with or without surface functionalization with an invasive protein, the uptake by an epithelial cell line was not influenced by particle shape when observed at different time points over 5 hours [19]. In some studies, sharp tipped particles were even preferentially engulfed compared to particles of more compact shapes [20] [21]. It also needs to be considered that, at a given size distribution of particles, individual size fractions may behave differently since major effects of small size changes on phagocytosis efficiency are well known at least for spherical particles. Often, emulsion techniques applied, e.g. for polylactide-based degradable particles lead to relatively broad size distributions. For such polylactide-based particles, a higher uptake of spheres compared to prolate ellipsoids was observed after 4 hours, the time point of data analysis used in this study [6]. Furthermore, one has to note that mechanistic studies exploring the interaction of single cells and particles may not represent the conditions as present \textit{in vivo}. In some experimental conditions that enable multiple cell-particle contacts, it was observed that prolate ellipsoidal model particles fabricated from polystyrene were initially recognized/attached more efficiently than spheres [22], which is a relevant factor contributing to the probability of overall engulfment particularly at a dense particle/cell packing. Therefore, beside shape, cell-particle ratio, time-point of observation and size should mutually affect phagocytosis rates. As additional parameters, e.g. the protein adsorption that depends on polymer properties [23], can contribute to biological interaction and recognition [24], it may not be possible to predict shape dependency of phagocytosis for all types of particle materials without studying each specific carrier system.

A degradable material with relevance for biomedical applications is poly(\(\varepsilon\)-caprolactone) (PCL). Using oligo(\(\varepsilon\)-caprolactone) [oCL] based precursors, polymer micronetwork colloids (MNC) were recently reported, which allow the deformation from spheres to ellipsoids with memory of the original shape [25], thus enabling to create different shapes from otherwise identical particles. Here, the maximum aspect ratios (\(AR\)) that can be created from identical stock particles as well as the role of shape and size on MNC phagocytosis were investigated.
Conditions were applied allowing multiple particle-cell contacts by systematically altering cell-particle ratios and time points of observation. Out of the prepared MNC with narrow size distributions, MNC with a diameter of ~6 and ~10 µm were selected for phagocytosis studies, which provide a high internal volume for future payloads. These particles are in the mean and upper range of sizes accepted by phagocytic cells, thus enabling to explore shape, size and kinetic aspects of phagocytosis with expected distinction between phagocytizing and non-phagocytizing cells. By loading with a fluorescent model compound for particle visualization, cellular uptake of spheres and ellipsoids of different prolate ellipsoidal shapes by macrophages could be explored.

2. Materials and Methods

2.1 Synthesis of MNC

oCL-diol ($M_n = 8 \text{ kDa}$, Perstop, UK Ltd., United Kingdom) was functionalized with 2-isocyanatoethylmethacrylate to oCL-IEMA (97% functionalization, melting temperature $T_m = 56 ^\circ\text{C}$). Spherical oCL-IEMA particles prepared by microfluidic emulsification and solvent evaporation were photocrosslinked internally to MNC in the molten state in aqueous dispersion by UV irradiation (308 nm; XeCl-Excimer, Heraeus, Germany). The semi-crystalline MNC 6 (volume-moment mean $D[4,3]$ main peak 6.3 µm; Uniformity $U = 0.688$), MNC 10 (9.7 µm; $U = 0.338$) and MNC 13 (D[4,3] 12.5 µm; $U = 0.02$) had $T_m$'s of 53, 48, and 45 °C, respectively (DSC 204 F1, Netzsch, Germany; nitrogen atmosphere, second heating cycle from -100 to 150 °C, 10 K-min$^{-1}$). MNC-powder was obtained by washing with water on 0.45 µm nylon filters (NL17, Whatman®, United Kingdom) and lyophilization (Alpha 1-2 LD plus, Martin Christ, Osterode, Germany; 0.08 mbar, ice condensor -55 °C).

2.2 MNC loading with model compound

1 ml dichloromethane (DCM, 5 mL, $\rho=1.33 \text{ g·mL}^{-1}$) supplemented with Rhodamin B (Rb, 0.05 wt.%, 3.3 mg) was added to dry MNC powder (35 mg) in 50 ml vials at room temperature. MNC swollen in DCM were dispersed in water by sonication (Sonoplus HD 3200, MS 72 Sonotrode, Bandelin, Germany, 30% amplitude, 30 s) after careful overlaying the organic phase with 10 mL of 5 wt.% polyvinylalcohol in water (PVA; Mowiol®, 4-88, $M_n = 12.6 \text{ kDa}$;
Three of such aliquots were added to 60 mL PVA-solution (2.5 wt.%) for solvent evaporation (7 h, 500 rpm overhead stirrer), followed by washing with water and lyophilization.

### 2.3 Preparation of ellipsoids by MNC deformation

Suspensions of 10 mg·mL⁻¹ MNC in 22.5 wt.% PVA solution (Mowiol® 3-85, $M_n = 5.6$ kDa; $PD = 2.5$) were casted in molds and dried (24 h RT, 4 h 60 °C) to obtain flat phantoms. Phantoms were uniaxially stretched (7.5 mm·min⁻¹) up to 290 % in a tensile tester (ZP 20, Zwick, Germany) at 80 °C with subsequent cooling to 20 °C (heating/cooling rates 10 K·min⁻¹). Prolate ellipsoid MNC were harvested by phantom dissolution and MNC washing with water (Ampuwa® Plastipur, Fresenius, Germany).

### 2.4 Particle size characterization

The size distributions of MNC were determined by static light scattering (SLS, Hydro 2000 µP-sample unit, Multiple Narrow Mode, Mastersizer 2000, Malvern Panalytica, Kassel, Germany) and given sizes correspond to the observed main peak of $D_{[4,3]}$. Particle suspensions were dropwise added to ~20 ml degassed water as measurement medium until a laser obscuration of 1-10% was reached. Typically, three analysis runs were performed for each sample.

Automated light microscopy analysis and determination of $AR$ was performed for 180-1300 single particles of each aqueous MNC-suspension, which were observed between a microscope slide and a cover slip with a Morphology G3 instrument (Malvern, United Kingdom).

Ir-coated (4 nm; Sputter coater Q150TES, LOT Quantum Design, Darmstadt, Germany) samples were prepared on thin glass plates and analyzed by scanning electron microscopy (SEM, Gemini Supra™ 40 VP, Carl Zeiss NTS, Oberkochen, Germany) at acceleration voltages of 2 kV with an Everhart-Thornley (SE2) detector in high vacuum at room temperature.

### 2.5 Phagocytosis studies

Contaminations of particles with endotoxins were investigated by an established protocol with HEK-Blue™ reporter cell lines [26]. Briefly, $5 \times 10^4$ HEK-Blue™-hTLR4 or HEKBlue™-Null2 cells (InvivoGen, San Diego, USA) were cultured directly in 100 µL VLE-RPMI
(Biochrom®, Berlin, Germany) in the presence of the MNC (4x10^4) for 24 hours at 37 °C (5% atmospheric CO₂). Cell culture supernatants were subjected to the QuantiBlue™ assay (InvivoGen) with determination of absorbance at 620 nm (Tecan plate reader) after 3 h of incubation with the reagent at 37 °C (5% atmospheric CO₂). Pure medium and lipopolysaccharide (LPS) from E.coli strain O111:B4 served as negative and positive controls, respectively.

In phagocytosis studies, several specific cell-to-MNC ratios (1:1, 1:5, 1:10, 1:50) were applied as ensured by particle counting using a Neubauer chamber and adjusting MNC stock suspensions (10^8 MNC·mL⁻¹; 1 wt.% PVA; Mowiol® 4-88). A final PVA concentration of 0.25 wt.% allowed reproducible MNC aliquotation and was shown to be non-toxic (Supp. Information, Supp. Fig. 2 and 3). RAW264.7 macrophages (1·10^5/well) were fluorescently labeled (eFluor670, eBiosciences, Germany, 10 µM) before MNC addition to clearly discriminate cells and MNC when analyzed by flow cytometry. The cells were cultivated in DMEM-medium (150 µL, Biochrom AG, Germany; 10% FBS, Sigma) in 96 well plates (Corning, Sigma-Aldrich, MO, USA) at 37 °C and 5% CO₂, to which 50 µL MNC suspensions were added. Gentle centrifugation (400·g, 5 min at room temperature) of the cell culture plate facilitated instantaneous cell–MNC contact.

The viability (staining with 1 µg·ml⁻¹ 4,6-diamidino-2-phenylindole (DAPI) directly before measurement) and particle uptake (R₆ signal) were analyzed by flow cytometry (MACSQuant flow cytometer, Miltenyi Biotec, Bergisch-Gladbach, Germany) at different time points. Confocal laser scanning microscopy (CLSM; LSM 510, Zeiss, Germany) was applied after cell fixation (paraformaldehyde), membrane permeabilization (Triton-X100) and staining of actin (5 U·ml⁻¹ Alexa 488 phalloidin, Invitrogen, CA, USA) and nuclei (0.5 µg·ml⁻¹ DAPI, Invitrogen).

2.6 Statistical evaluation

Statistical analysis was conducted by one-way ANOVA analysis with post-Tukey’s multiple comparison test, comparing for each MNC type the groups with different shapes (εₚ) and cell-to-MNC ratio at a given time point (GraphPad Prism software v6.01). Experimental errors of analytical techniques are typically < 3%.

3. Results and Discussion
Preparation of MNC and creation of ellipsoidal shapes

Polymer MNC based on oCL were synthesized from oCL-IEMA precursor as previously described [25], with the exception that a microfluidic particle templating was applied to produce MNC with a more narrow size distribution and that linear precursors were crosslinked by exposure to UV irradiation (308 nm) in the molten state. During the crosslinking step, IEMA units are converted (FTIR data, see Supp. Fig. 1) and oligomethacrylate chains were formed acting as covalent netpoints [27], which define the permanent shape of the MNC. These netpoints are connected by coiled oCL chain segments. The obtained spherical MNC showed sizes of ~6 µm (MNC 6), ~10 µm (MNC 10) and ~13 µm (MNC 13) as determined by static light scattering (SLS) in aqueous dispersion.

Based on their composition of covalent netpoints and elastic chain segments, MNC can undergo an elastic deformation upon application of external forces at least at temperatures above the melting temperature $T_m$ of PCL crystalline domains (e.g. $T_m,\text{MNC 13} = 45 ^\circ\text{C}$). For macroscopic materials, such deformation experiments can be performed by directly clamping the material in a tensile tester. In contrast, the systematic and reproducible deformation of numerous MNC required the use of phantom matrices. The MNC-loaded phantoms (films) were prepared by dispersing MNC in aqueous poly(vinyl alcohol) (PVA) solution, casting the dispersion into molds, and drying by evaporation of water (Fig. 1). These PVA phantoms can subsequently be subjected to uniaxial stretching, e.g. at 80 °C ($T > T_m,\text{MNC}$) to the desired degrees of phantom elongation $\varepsilon_{\text{ph}}$ [5] [25], enabling elastic deformation of numerous MNC in parallel within the phantoms. By subsequent cooling below $T_m,\text{MNC}$, the MNC adapted a semi-crystalline morphology with PCL crystallites fixing the ellipsoidal shape. Subsequently, MNC can be collected by phantom dissolution in water, while their ellipsoidal shape remains stable.

In order to allow identification of samples originating from a distinct phantom elongation, $\varepsilon_{\text{ph}}$ is indicated in the sample code, e.g., MNC 6(150) for $\varepsilon_{\text{ph}} = 150%$. In order to exclude any unknown handling effects and thus allow a direct comparison of MNCs in phagocytosis studies, particles indicated e.g. as MNC6 (0) were also subjected to phantom embedding and stretching, while only a marginal deformation of $\varepsilon_{\text{ph}} = 0.1%$ was applied that practically did not change the spherical particle shape.

As the particles are composed of a covalent network structure limiting infinite deformation, the possible range of aspect ratios $AR$, i.e. the ratio of longest and smallest particle axis, should be determined for MNC in a first instance. Based on their monodispersity ($U < 0.02$), MNC 13 were employed in this set of experiments. In a simplified model (Fig. 1B), the stretching of MNC by a factor $f$ in one spatial direction results in a reduction in the other two spatial
directions by the factor $f^{-0.5}$. When assuming full displacement of $\varepsilon_{\text{ph}}$ to the individual incorporated MNC at $T > T_{m,MNC}$, then $f = (\varepsilon_{\text{ph}}/(100\%))^{-1} + 1$. Under these conditions and within the elastic deformation regime, the experimentally observed $AR$ should correspond to the theoretical $AR_{\text{th}}$ predicted by the model ($AR = AR_{\text{th}}$). In contrast, $AR < AR_{\text{th}}$ may be observed when the network resists deformation, e.g. by being maximally stretched. When MNC13 were subjected to $\varepsilon_{\text{ph}}$ ranging from 0% to 290% and subsequently studied by automated light microscopy/shape analysis, a mean $AR$ could be determined for the isolated particle populations. As expected, prolate ellipsoidal MNC shapes of increasing $AR$ were observed (Fig. 1C). By plotting the experimentally determined $AR$ over the phantom elongation (Fig. 1D), an excellent agreement with $AR_{\text{th}}$ was illustrated in the low deformation range. As expected, at large $\varepsilon_{\text{ph}}$, $AR$ adapted a maximum value when the elastic chain segments were fully stretched. Based on the here observed boundary of MNC deformability at $\varepsilon_{\text{ph}}$ of ~150% resulting in $AR_{\text{th}}$ of ~4, this condition was determined as maximum $\varepsilon_{\text{ph}}$ applied for samples used in phagocytosis studies.

**Fig 1:** Preparation of MNC in spherical shape and transformation into prolate ellipsoidal shapes. (A) Principle of preparation. (B) Deformation model. (C) Microscopic images of MNC 13 isolated from phantoms prepared at different $\varepsilon_{\text{ph}}$. (D) Effect of phantom elongation on $AR$ for MNC 13 compared to theoretical $AR_{\text{th}}$ as predicted according to panel (B).

**Loading of MNC**

For their exploration as potential drug carriers, a suitable strategy for compound incorporation is required. Since the presence of drug may not be preferred during MNC synthesis to avoid drug alteration at crosslinking conditions, which comprise radical polymerization of IEMA moieties under UV-irradiation, a subsequent loading by swelling was
anticipated (Fig. 2A). For such MNC, degrees of swelling in the range of 300 vol.% were observed in DCM. Importantly, due to the covalent MNC structure, the integrity of the colloidal particles is preserved. The swelling approach conceptually allows introducing various types of substances, as here exemplarily shown for Rhodamin B (Rb), which should allow intracellular visualization of the particles in subsequent cell experiments. While rapid uptake of Rb was observed during MNC swelling in DCM, a critical technical step was the subsequent removal of the swelling agent, since aggregation of swollen particles may occur.
**Fig 2:** Dye loading and characterization of obtained prolate ellipsoid MNC. (A) Principle of $R_B$ loading of MNC by swelling in dried MNC in organic dye solution, followed by MNC redispersion and drying. (B) Procedure of dispensing swollen MNC by sonication in a two phase system: (left) organic phase of swollen MNC covered by aqueous top-layer, (middle) sonication for separation of swollen MNC into aqueous dispersion, (right) powder of dye-loaded MNC after washing and lyophilization. (C) Size-distribution analysis of MNC before and after loading. (E) Exemplary SEM and light microscopy images of loaded spherical and deformed MNC 6 ($\varepsilon_{ph} = 150\%$).
By adding a PVA solution to the swollen MNC and applying sonication, an aqueous dispersion of swollen MNC was obtained (Fig. 2B). The internal polymer network structure allowed for MNC integrity during this process. Subsequent extraction/evaporation of DCM resulted in RB-loaded, semi-crystalline MNC. SLS showed identical size distribution plots compared to non-loaded MNC (Fig. 2C). Light microscopy of aqueous dispersions confirmed the absence of aggregates or ultrasound-induced fragments/debris formed in this step. The minor fractions of smaller or larger particles can be assigned to the microfluidic preparation process, where syringe pumps show occasional pulses in flow as known from literature [28]. Overall, the suitability of the applied swelling process to create successfully loaded MNC samples without major aggregation was confirmed.

The RB loaded particles were incorporated in phantoms, transferred to their target shapes ($\varepsilon_{\text{ph}} = 0.1$, 75, or 150%) and studied by electron microscopy, again confirming the desired shapes and illustrating the presence of intact particles with smooth surfaces (Fig. 2D). As determined with a Neubauer counting chamber, ~250 million MNC were programmed in a single programming step in case of MNC 6. Importantly, MNC remained fluorescent, suggesting suitable persistence of RB in the MNC.

Phagocytosis of MNC depending on size, shape, cell-particle ratio and time point

Prior to conducting phagocytosis experiments, the presence of MNC bound and soluble endotoxins should be excluded. For this, the HEK-BlueTM reporter cell lines was used according to an established protocol [26]. The HEK-BlueTM-hTLR4 are genetically engineered to express toll-like receptor 4 (TLR4), which binds endotoxins and more specifically LPS. After TLR4 engagements, the HEK-BlueTM-hTLR4 secrete an alkaline phosphatase (AP), which is proportional to the amount of LPS contaminations. The AP can be quantified by using QUANTI-BlueTM medium containing a substrate for the AP. The HEKBlueTM-Null2 cells serve as control since they bear the same genetic modifications allowing the secretion of AP, but lack the capacity to express TLR4. As positive control, the HEK-BlueTM-hTLR4 were treated with LPS. When both cells types were cultivated for 24 hours in the presence of the different MNC, neither of them secreted AP indicating that the MNC were not contaminated with endotoxins (Fig 3).
Fig 3: Investigation of endotoxin levels of exemplary MNC with reporter cell lines using the QuantiBlue™ assay. Control: HEKBlue™-Null2 cells lacking TLR4 expression; Test cells: HEK Blue™ hTLR4 cells expressing TLR4 responsive to lipopolysaccharides.

The shape-dependency of particle uptake of MNC 6 and MNC 10 ($\varepsilon_{ph} = 0.1, 75, \text{ or } 150\%$) was studied with RAW264.7 macrophages by systematically altering the cell-to-MNC ratios as well as the time point of observation. This should allow identifying potential concentration effects and understanding if distinct shapes are preferentially phagocytized in case of multiple cell-particle contacts.

Microscopic analysis illustrated that a complete coverage of cell-culture plates by MNC was given at a ratio of 1:10 for MNC 10 (1:50 for MNC 6) (Supp. Fig. 4). As quantitatively analyzed by flow cytometry (Fig. 4A), the viability of macrophages was not substantially influenced by the presence and concentration of MNC. Therefore, cytotoxicity as interfering factor in phagocytosis experiments could be excluded.

When comparing the extent of phagocytosis for different particle concentrations, an increasing uptake of MNC at higher particle concentrations (decreasing cell-to-MNC ratios) was observed after 24 h of incubation (Fig. 4B). This was true for both particle sizes and all particle shapes, suggesting that defined cell-to-MNC ratios are of significance for comparability of literature reports on shape dependent phagocytosis. Generally, the extent of phagocytosis after 24 h was lower for MNC 10 compared to MNC 6, which may be assigned to steric reasons considering the cell size of about 10 µm. CLSM analysis after 24 h suggested that numerous MNC 6 were engulfed by individual cells, while other macrophages did not incorporate a single MNC (Fig. 5). Interestingly, more than one phagocytized MNC 10 per cell was observed only very seldom as may be rationalized by the large volume of MNC 10 compared to cells.
Fig 4: Phagocytosis study with RAW264.7 macrophages enabling multiple cell-particle contacts and employing different MNC-sizes (6 or 10 µm sphere diameter), degrees of elongation ($\varepsilon_{\text{ph}} = 0, 75, 150\%$) and cell–to–MNC ratios (1:1, 1:5, 1:10, 1:50). (A) Viability, (B) MNC uptake after 24 h, and (C) kinetics of uptake, all determined by flow cytometry. Data from three independent experiments are shown (mean; standard error of the mean; * $p < 0.1$; ** $p < 0.01$).

As phagocytosis is a time-dependent process, also the uptake kinetics were studied for selected cell–to–MNC ratios. A significantly higher phagocytosis was detected for spherical MNC 6(0) compared to ellipsoids, particularly after 1 h as assumed (Fig. 4C). At later time
points, the differences in engulfment for MNC 6 spheres compared to ellipsoids were limited to selected conditions only (1:5 cell–to–MNC ratio). Beyond this, for MNC 10, the effect of shape on uptake was less clear, as data unexpectedly suggested a higher extent of phagocytosis of non-spherical compared to spherical particles after 24 h as well as at intermediate time points.

Conceptually, phagocytosis of ellipsoids may need a more extensive rearrangement of the cells’ cytoskeleton than for spheres. The time required for this reorganization might justify the presence of shape effects on MNC 6 phagocytosis at early time points, while being only limited at later time points. This is in line with models describing first order kinetics for the internalization process [29]. Accordingly, conclusions on shape-dependent particle engulfment reported at distinct (early) time points may not in all cases be representative for a terminal state, at least for the PCL-based MNC studied here.

It should be noted that in a setting with an excess of particles, i.e. multiple particles available for uptake per cell, elongated and larger MNC (e.g. MNC 10) may bridge between several cells, thus increasing the possibility of phagocytosis by any of these cells due to the higher number of cell contacts. This suggests that, in the boundaries of parameters and particles tested in this study, ellipsoidal particles may not necessarily show a reduced uptake in application relevant conditions of multiple cell-particle contacts. Subsequent studies with several types of particle materials may need to continue exploring this subject, whereby an important step will be to investigate the particles in tissue models.

4. Conclusions

A methodology was applied to investigate phagocytosis of oCL-derived MNC as an application-relevant polymer depending on shape, size and exposure time, while allowing multiple cell-particle contacts. Boundaries of MNC ellipsoidal shapes were determined and a
swelling/drying technique for compound loading was successfully employed. For the PCL-based particles investigated, this study illustrated a limited effect of particle shape on phagocytosis by a macrophage cell line *in vitro* at least at later time points and for larger particles. Based on the different uptake pattern upon slight size shifts from MNC 6 to MNC 10, the size distribution of particulate carrier systems, which typically is broad for particles prepared by conventional emulsion techniques, may need to be considered in the fabrication of particles for shape-selective uptake.

Overall, controlling shape dependent phagocytosis requires evaluating further parameters besides particle shape, including, e.g. kinetic aspects of cell exposure, tissue models, and particle size distribution. By applying the methodology reported here, a variety of further materials may be studied in the future to gain a comprehensive view on opportunities and boundaries of shape-dependent uptake.

**Author contributions**

FF: planning of experiments/setups, preparation of samples, data acquisition, analysis, interpretation, article drafting and approval

TR: design of study, data acquisition, analysis, interpretation, article drafting and approval

BS: data acquisition, analysis, article drafting and approval

AL: design of study, supervision, data interpretation, article revision and approval, funding acquisition

CW: design of study, supervision, data analysis, interpretation, article revision and approval, funding acquisition

**Declaration of Competing Interest**

AL is an inventor on patents/patent applications in the field of shape-memory polymers and drug delivery systems based on these.

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