LIPID MICROPARTICLES AS QUERCETIN CARRIERS FOR THE TREATMENT OF BURN WOUNDS: MANUFACTURING, PROPERTIES, AND *IN VITRO* STUDIES ON MACROPHAGES POLARIZATION

Julia Stępień[1]*, Anna Kusibab[1], Ana Beatriz Sousa[2,3], Judite Novais Barbosa[2,3], Elżbieta Pamuła[1]

[1] AGH University of Krakow,
Faculty of Materials Science and Ceramics,
Department of Biomaterials and Composites,
al. A. Mickiewicza 30, 30-059 Krakow, Poland
[2] Instituto de Investigação e Inovação em Saúde da Universidade do Porto,
Rua Alfredo Allen, 208, 4200-125 Porto, Portugal
[3] ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto,
R. Jorge de Viterbo Ferreira 228,
4050-313 Porto, Portugal
*e-mail: stepienjulia98@gmail.com

Abstract

Burn wounds are a unique type of injury that can affect the entire body and cause irreversible damage. They are characterized by significant morbidity and mortality due to the pathophysiology of the healing process manifested by unremitting inflammation, leading to a critical need to search for new treatments. This study focuses on the development of drug delivery systems in the form of lipid microparticles loaded with quercetin, as an agent to combat acute inflammation in burn wounds. We aimed to explore the effect of quercetin in modulating macrophage polarization from proinflammatory (M1) to anti-inflammatory (M2) phenotype. The absence of a cytotoxic effect of the produced particles on macrophages, as well as the lack of negative effects on their morphology was proven. The study confirmed the ability of quercetin and quercetin-loaded lipid microparticles to modulate macrophage polarization in an anti-inflammatory direction, based on the analysis of their surface markers expression performed with the use of flow cytometry. With the use of quercetin, the expression of M2 specific marker increased. Furthermore, better results were obtained for encapsulated quercetin, confirming the necessity of encapsulation to increase the therapeutic potential.

***Keywords**: lauric acid, quercetin, encapsulation, cytotoxicity, macrophage polarization, burn wounds*

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Introduction

Wound healing is a complex process regulated chemically, by molecules such as growth factors or cytokines, and mechanically, by tension of the skin or cell contraction forces. This process consists of overlapping phases, namely homeostasis, inflammation, proliferation, maturation, and remodeling [1].

The inflammatory phase (4-6 days) involves clot formation, platelet activation, and neutrophil and monocyte-driven debris removal. Macrophages are known to clean the wound site and release growth factors and cytokines inducing inflammation. During the proliferation phase (up to 3 weeks), the most important phenomena is related to granulation tissue formation, deposition of extracellular matrix (ECM), angiogenesis, and epithelialization. Guided by growth factors and ECM proteins, fibroblasts and endothelial cells proliferate, to cover the wound site and achieve wound closure. In the maturation and remodeling phase (from day 8 to ~1 year) collagen is reorganized and ECM is remodeled by metalloproteinases. Collagen type III is replaced with collagen type I, angiogenesis stabilizes, and the scar matures, resulting in a lower strength of the healed tissue. Cellular and molecular interactions drive wound closure and tissue repair [1-6].

In the burn wound healing process, these three phases can also be distinguished; however, in this type of injuries, recovery is more complicated, it takes longer to move from one phase to the next, and the inflammatory response is stronger. Burn wounds can be classified according to their total body surface area (TBSA) into major and minor (>10% of TBSA) wounds [7-11]. In addition, it is also important to classify the injury according to its depth and size. Skin burn injuries can be classified as partial or full thickness. If the injury affects the upper layer of the skin (only the epidermis), it is classified as a superficial burn (first-degree). Superficial partial thickness (second-degree) burns involve the epidermis and part of the dermis. In superficial and superficial partial-thickness burns, most of the adnexal structures (hair follicles, sweat, and sebaceous glands) are preserved. Deep partial-thickness (second-degree) burns destroy a larger proportion of the dermis, some adnexal structures, and partially pain receptors. A full-thickness burn (third-degree) destroys the whole epidermis, dermis, and all adnexal structures. It is not typically painful due to damage of the nerve endings. Fourth-degree burns involve damage of deeper tissues, such as muscle or bone, and often lead to loss of the burden part [7].

In the case of extensive burn injuries, there is an unprecedented inflammatory response, which distinguishes this type of harm from other traumas. The cellular defense mechanisms begin with the proinflammatory phase, known as the systemic inflammatory response syndrome. Macrophages produce proinflammatory mediators and biochemical cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). Thermal injury results in prolonged and profound hypermetabolism, which leads to an increased production of proinflammatory cytokines, as well as the formation of reactive oxygen species (ROS). Free radicals are known to have a beneficial effect on antimicrobial action and wound healing; however, in the case of burns, they are produced in enormous amounts, leading to infection and sepsis, tissue damage, and multiple organ failure [7,10-12].

This response of the body makes it very important to fight inflammation in an early stage of treatment. This applies mainly to superficial partial thickness burns, which do not require surgical intervention, and when treating them, the most important step is covering the wound at the early stage. Topical antimicrobials are fundamental to non-surgical approaches. These dressings are applied to cover burn wounds, promote epithelialization, prevent infection and mechanical trauma, keeping wounds moist and reducing pain. The ideal dressing for burn wounds should also have the ability to fight inflammation and do not require frequent changes, which is especially painful in the case of burns. However, none of the dressings currently clinically used possess all of these features. Drug delivery systems are expected to be beneficial for the treatment of this type of burn wounds, due to their ability to meet all or most of the requirements for ideal burn wound dressings [7,10,11,13].

One of the compounds that show the potential to reduce inflammation is quercetin. Quercetin is a plant-derived flavonoid that can be found in fruits (e.g., apples, grapes, berries), vegetables (e.g., capers, brassica vegetables, onions, tomatoes), and in herbs and medical plants (e.g., tea, elderberry). It has many desirable properties to be explored in medicine, such as various bactericidal mechanisms and biofilm destruction ability. Furthermore, it is recognized to have antioxidant and anti-inflammatory properties. Quercetin is known to scavenge free radicals and interact with cellular molecules influencing their antioxidant activity. Supporting cellular antioxidant capacity is mainly based on upregulating the expression of specific molecules, for example, glutathione (GSH), which are able to neutralize free radicals and transfer their harmful effects to less sensitive cell structures (e.g., from the lipid membrane to the cytosol). Direct actions include, for example, antilipoperoxidative activity and hydrogen donating ability [14-18].

The main goal of this study was to develop a drug delivery system in the form of lipid microparticles loaded with quercetin and to evaluate their potential to influence the polarization of human macrophages towards a M2 anti-inflammatory phenotype. The use of macrophages for *in vitro* studies is related to their crucial role in modulating inflammation in the body. The microparticles, manufactured by a hot emulsification method, were evaluated in terms of the morphology, size, and encapsulation efficiency of quercetin. *In vitro* studies focused on evaluating the cytotoxic effect of microparticles on human macrophages, the influence of quercetin on cell morphology, and the ability of the particles to modulate macrophage polarization toward an anti-inflammatory phenotype.

Materials and Methods

Manufacturing of quercetin carriers

Lauric acid (MKBR440v – SIGMA-ALDRICH) was chosen as a lipid matrix for the production of particles. The hot emulsification method was used to produce microparticles (microparticle production scheme shown in FIG. 1). The water phase was a solution of 10% poly(vinyl alcohol) (Mowiol® 4-88 – SIGMA ALDRICH), and the oil phase was lauric acid – for the unloaded microparticles, or lauric acid and quercetin (QE) (Q4951-10G – SIGMA-ALDRICH) – for the loaded microparticles. Briefly, the oil phase was melted in a water bath at 65°C. After homogenization, the oil phase was combined with the water phase, then the mixture was poured into liquid nitrogen and left to thaw. Microparticles were manufactured with different QE concentrations: 5%, 10%, and 20% (w/w).

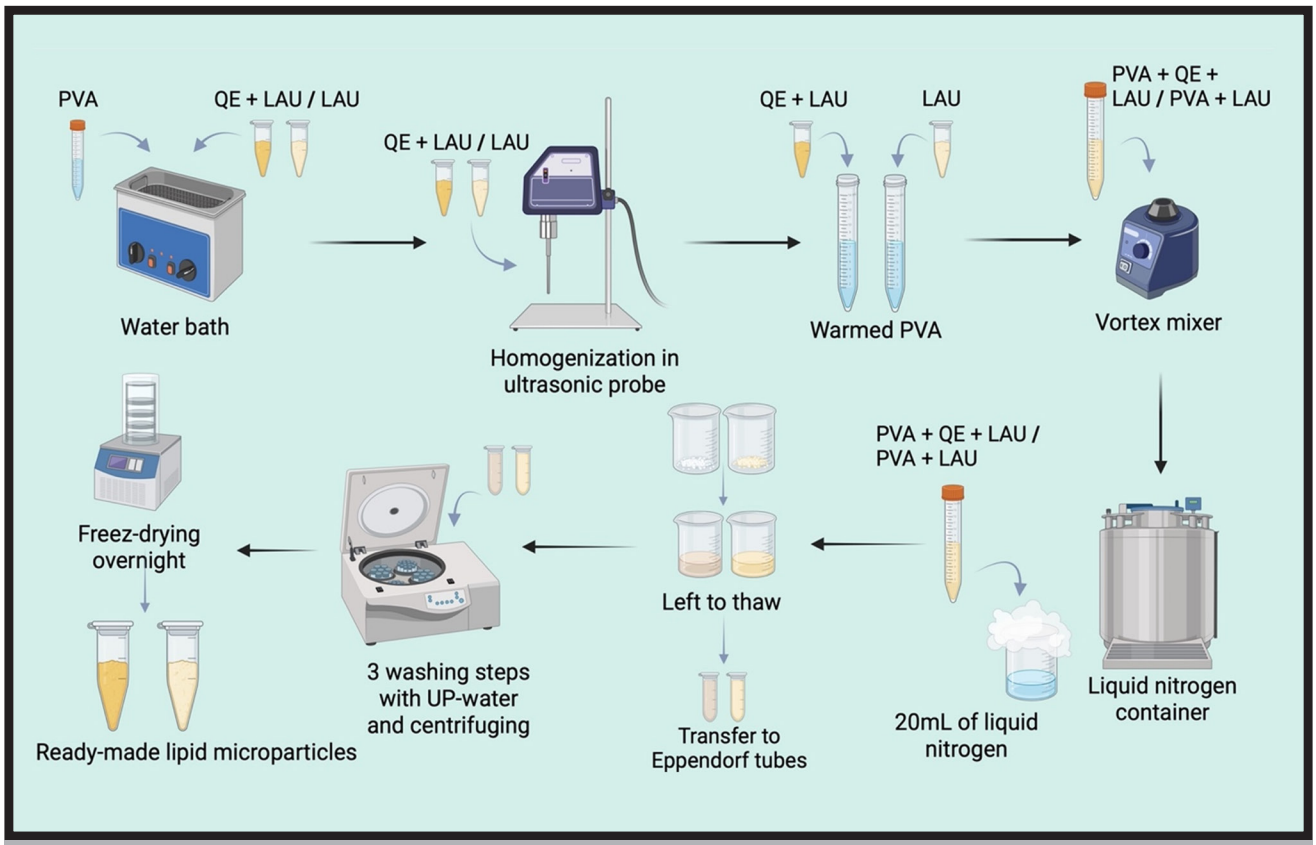

FIG. 1. Scheme of production of lipid microparticles by the hot emulsification method.

Particles size and morphology

In order to evaluate particles morphology, pictures were taken under an optical microscope (ZEISS, Axiovert 40 CFL). On their basis, the shape and size of the particles were assessed. The particle size distribution was also evaluated using the program ImageJ.

Fourier-transform infrared spectroscopy (FTIR)

FTIR was used to confirm the presence of QE in the lipid microparticles. Before FTIR examination, the samples were placed in a vacuum oven, at room temperature, for 2 days for thorough drying. The particles and QE powder were then mixed in a mortar with KBr in a 2:200 ratio. In the case of the QE powder, less quantity was used, due to its intensive color.

Before testing the samples, background measurement was made (without a sample) to calibrate the device. Then the samples were sequentially placed in the FTIR device (PerkinElmer) for measurements with the same parameters for all the samples (initial energy: 5624, resolution 4 cm^{-1}, OPD: 0.2, detector: MIR TGS and scan range at: 4000-400 cm^{-1}).

Monocytes isolation and differentiation into macrophages

To evaluate the anti-inflammatory potential of the QE loaded particles, primary human macrophages were selected for *in vitro* tests, due to their significant contribution to maintaining inflammation in burn wounds. Human monocytes were isolated from the peripheral blood of healthy donors, by negative selection, using the RosetteSep Monocyte Enrichment antibody cocktail (STEMCELL Technologies, Inc.). The samples were obtained from the Centro Hospitalar Universitário de São João (CHUSJ) in Porto. The use of human blood samples for research was approved by the CHUSJ Ethics Committee for Health (references 90/19 and 260/11). All procedures were performed in agreement with the Helsinki Declaration and the Oviedo Convention and all donors gave their written consent before blood collection.

Briefly, after buffy coats were centrifuged, peripheral blood mononuclear cells (PBMC) were separated, treated with a cocktail of specific antibodies, and diluted with a fetal bovine serum (FBS) solution. This mixture was then layered over a density gradient medium, Histopaque-1077, and centrifuged to isolate monocytes. Monocytes were washed, counted and cultured on coverslips in a 24-well plate.

For macrophage differentiation, $0.5 \cdot 10^6$ monocytes/mL were seeded on glass coverslips in 24-well plates (500 µL/well) and cultured for 7 days. Monocytes were cultured in complete Roswell Park Memorial Institute (RPMI) 1640 media (Corning) containing L-glutamine and supplemented with 10% FBS (Sigma-Aldrich). After 7 days, cell culture medium was replaced.

To activate monocytes toward an M1-like phenotype, monocytes/macrophages were stimulated with 10 ng/mL lipopolysaccharide (LPS derived from *Escherichia coli* O55:B5, Sigma-Aldrich Co., St Louis, MO, USA) and 50 ng/mL interferon gamma (IFN-γ, ImmunoTools, Friesoythe, Germany). The experimental protocol is shown in FIG. 2.

The developed microparticles and pure quercetin (resuspended in cell culture medium) were added to the culture on day 8 and left in contact with the cells for 24 h. The following samples were studied: unloaded particles at a concentration of 0.15 mg/ml, quercetin-loaded particles at 0.15 mg/ml concentration (theoretical quercetin content 0.0075 mg/ml), quercetin-loaded particles at 0.30 mg/ml concentration (theoretical quercetin content 0.015 mg/ml) and pure quercetin at a concentration of 0.15 mg/ml. Production efficiency was calculated from the formula:

$$P_f = \frac{m_f}{m_i} \cdot 100\%$$

where:
m_i – initial mass of ingredients,
m_f – final mass of the obtained microparticles.

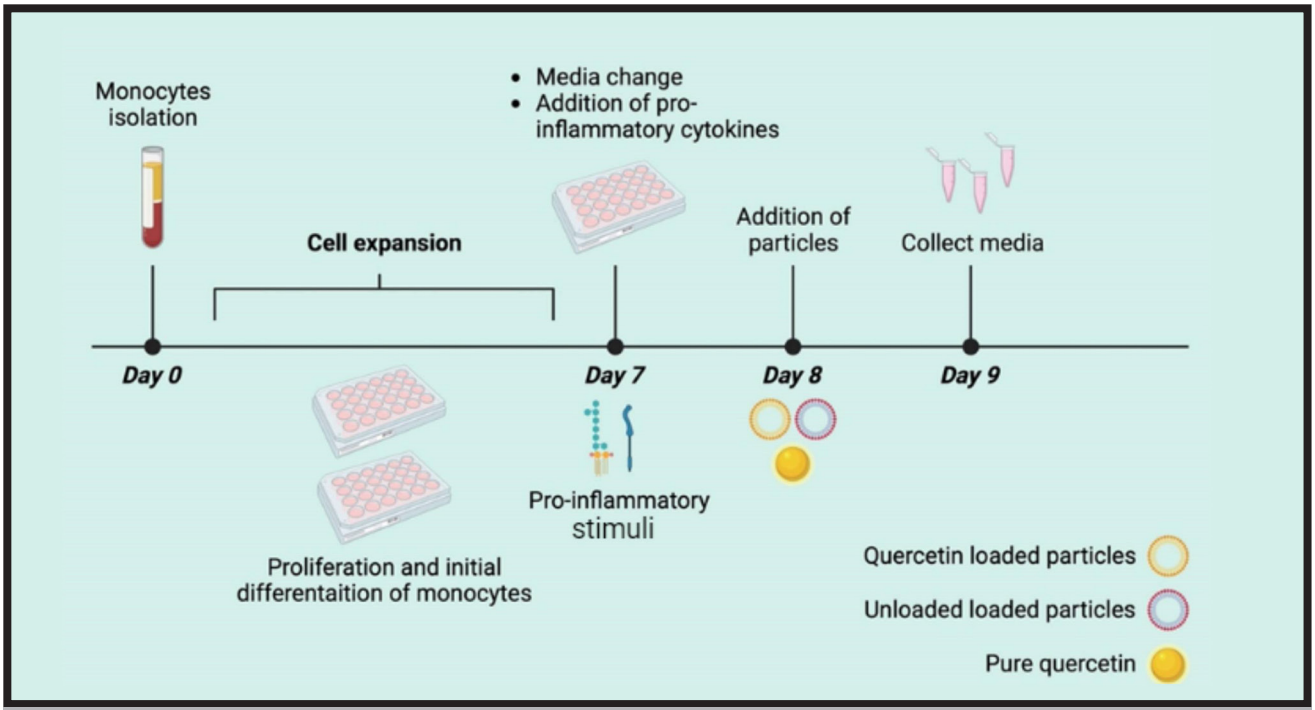

FIG. 2. Experimental design used for the establishment of proinflammatory conditions. Cells were left to differentiate for 7 days. On day 7, proinflammatory stimuli (LPS and IFN-γ) were added to the culture. On day 8, manufactured particles were added, and on day 9 the cells were fixed and the cell culture media was collected for further analysis.

Cytotoxicity test

The cytotoxic effect of the particles on macrophages was evaluated by measuring lactate dehydrogenase (LDH) levels, which is an enzyme present inside cells. During the death of a cell, LDH escapes into the medium, and by measuring its level, it is possible to determine the number of cells that have died. On this basis, the cytotoxicity of the material is determined. The LDH assay was performed according to the manufacturer's protocol (CytoTox 96® Non-Radioactive Cytotoxicity Assay, Promega Corporation, Madison, WI, USA). Briefly, 50 µL of culture medium was added to 50 µL of Substrate Mix. Following a 30 min incubation at room temperature, the reaction was stopped with 50 µL of the Stop Solution. The optical density of the plate was read with a microplate reader (Synergy Mx, BioTek, Vernusky, VT, USA) set at 490 nm. The cytotoxicity threshold was calculated by dividing the negative control (100% live cells) by the positive control (100% dead cells) multiplied by 100%. The value obtained determines the number above which the molecules show cytotoxicity. Positive control was obtained with the use of lysis buffer provided by the manufacturer.

Influence of quercetin on macrophages morphology

Nuclei and actin staining were performed to assess macrophage morphology. Phalloidin red (diluted in a ratio of 1:200, A12381; ThermoFisher Scientific, Invitrogen) and 4′,6-diamidino-2-phenylindole (DAPI diluted in a ratio of 1:1000, D3571; Alfagene) were used for staining.

Influence of quercetin on macrophages polarization

To evaluate the influence of quercetin encapsulated in microparticles and of pure quercetin on macrophages polarization, flow cytometry assays were performed. Human CD14 antibody (21279146; ImmunoTools) was used as a pan macrophage marker. Cells after 48 h of incubation with microparticles or pure quercetin were stained with antibody and examined with a flow cytometer (BD FACSCanto IITM flow cytometer, Becton Dickinson). For antibody staining, cells were transferred to a 96-well plate. Staining solutions were prepared separately for each condition, by combining 2 µL of CD14 antibody and completed with FACS buffer to achieve a final volume of 50 µL. Cells were incubated with staining solutions for 30 min and then cleaned with FACS buffer.

Results

Evaluation of particles morphology

Microparticles were characterized based on microscopic images (FIG. 3) and their size was measured in the ImageJ program (FIG. 4). The microparticles have a round, regular shape, and those loaded with quercetin had a yellow-greenish color. The coloration of the particles may be due to the presence of quercetin inside. It can be observed that particles do not have a tendency to aggregate; they exist as individual entities. Aggregation occurs only in particular locations and can be caused by a large number of particles in the sample.

According to the size distribution histograms of all analyzed samples, microparticles with a size lower than 2 µm predominate (FIG. 3). Unloaded (FIG. 3A) and 5% QE samples (FIG. 3B) consist of microparticles up to 12 µm in size, while for 10% QE (FIG. 3C) and 20% QE samples (FIG. 3d) microparticles with a size up to 18 µm were present. The median particle size for all samples varied between 1.52 µm and 2.16 µm.

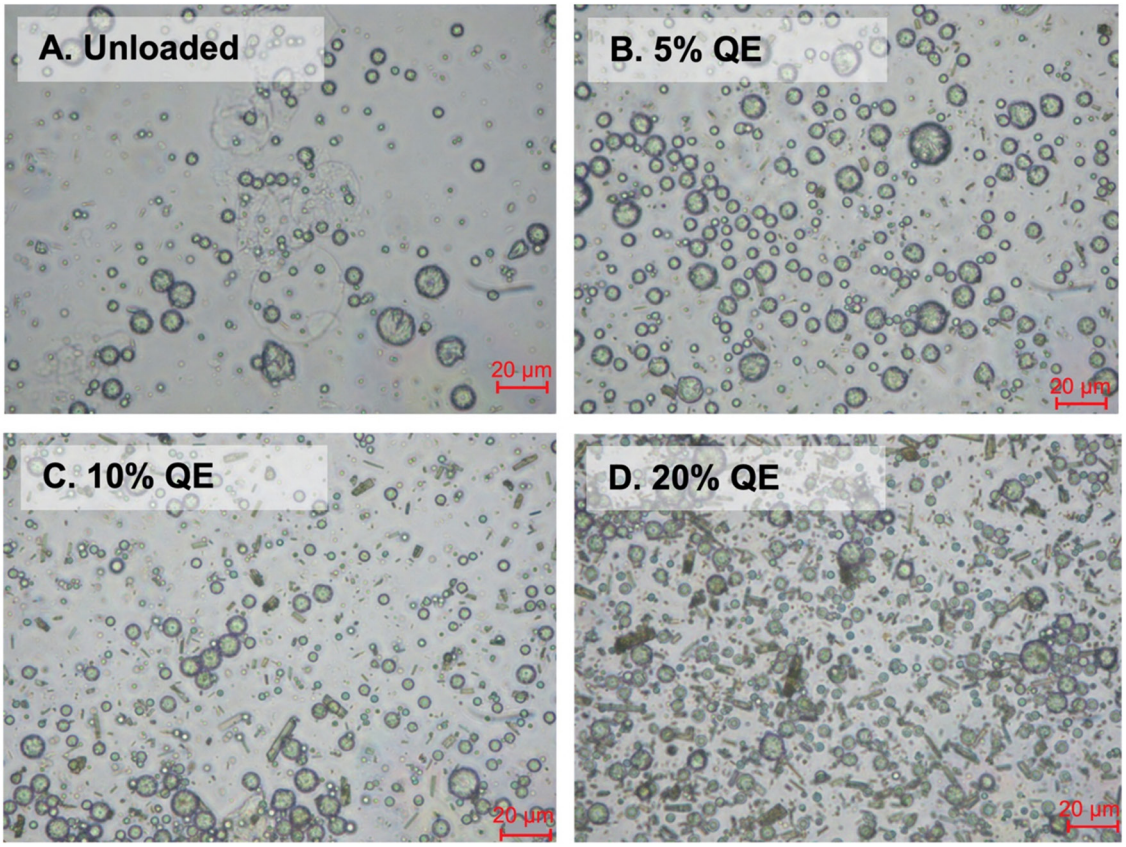

FIG. 3. Microscopic image of manufactured particles: A. unloaded, B. loaded with 5% QE, C. loaded with 10% QE, and D. loaded with 20% QE; scale bar = 20 µm.

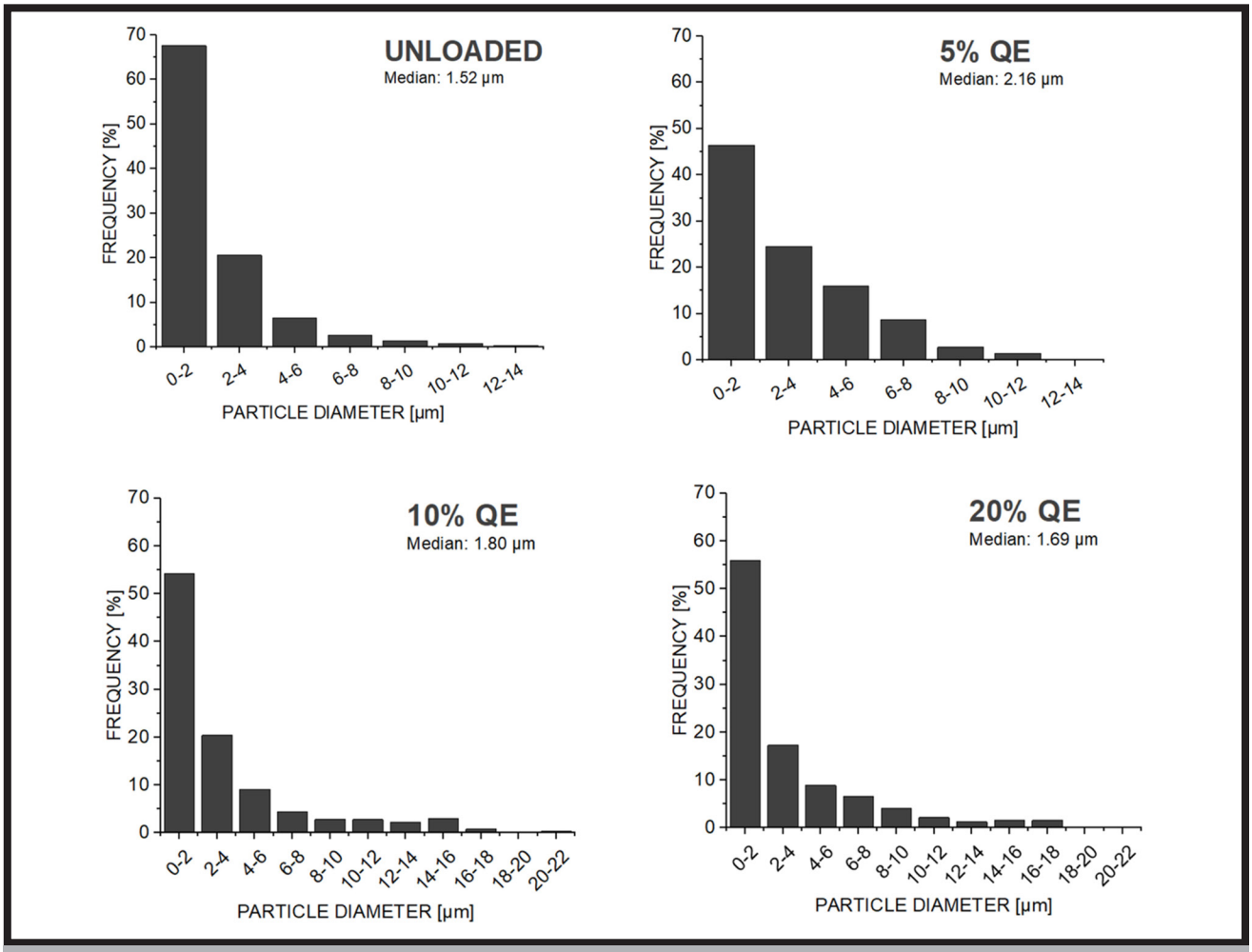

FIG. 4. Size distribution of manufactured particles: A. unloaded, B. loaded with 5% QE, C. loaded with 10% QE, and D. loaded with 20% QE. $\bar{x}_n$ = 384 of measured particles per condition.

Fourier-transform infrared spectroscopy (FTIR)

For FTIR analysis, characteristic bonds in quercetin were found: hydroxyl group (–OH) and carbonyl group (C=O) bonds were selected for analysis (marked in FIG. 5A). The analysis of lauric acid involved peaks originating from the carboxyl group (–COOH) and a methylene group ($–CH_2$), which were chosen for analysis (as marked in FIG. 5B).

The bonds were marked on the spectra shown in FIG. 6 with letters: A – OH stretching vibrations in quercetin, B – COOH stretching vibrations in lauric acids, C – C=O stretching vibrations in quercetin, and D – CH_2 bending vibrations in lauric acid.

In order to analyze the spectra, the ratio of B to D peak area was calculated for the unloaded particles, 5% QE, 10% QE, and 20% QE. The ratio changed slightly, so it was concluded that the presence of quercetin does not affect the formation of new bonds in lauric acid.

In the next step, the ratio of B to C peak area was calculated for 5% QE, 10% QE, and 20% QE. This allowed us to determine whether the amount of quercetin in the particles is increasing. The ratio decreased with increasing quercetin concentration in the particles, so it was found that, as expected, the amount of encapsulated quercetin increases with increasing concentration in reaction mixture during microparticles preparation. Calculated ratios are shown in TABLE 1.

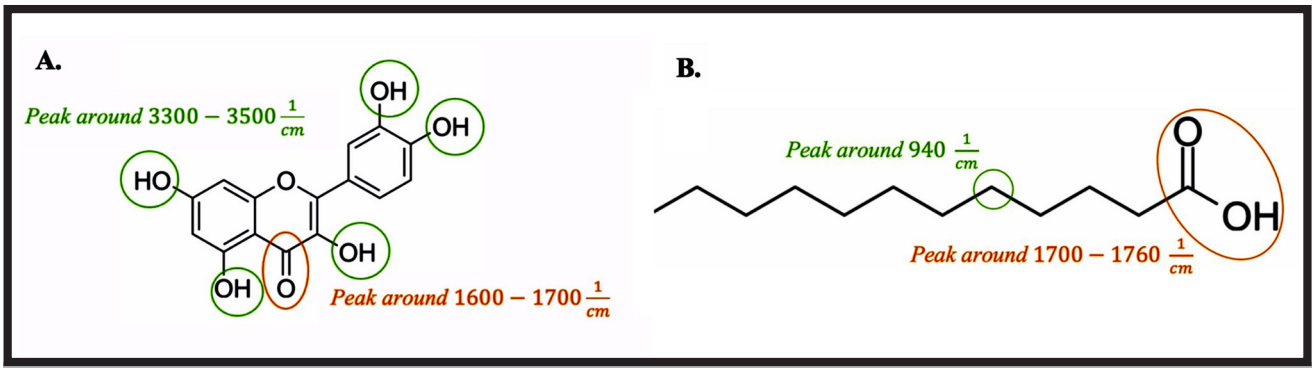

FIG. 5. The most characteristic bonds of A. quercetin and B. lauric acid and their FTIR peak position.

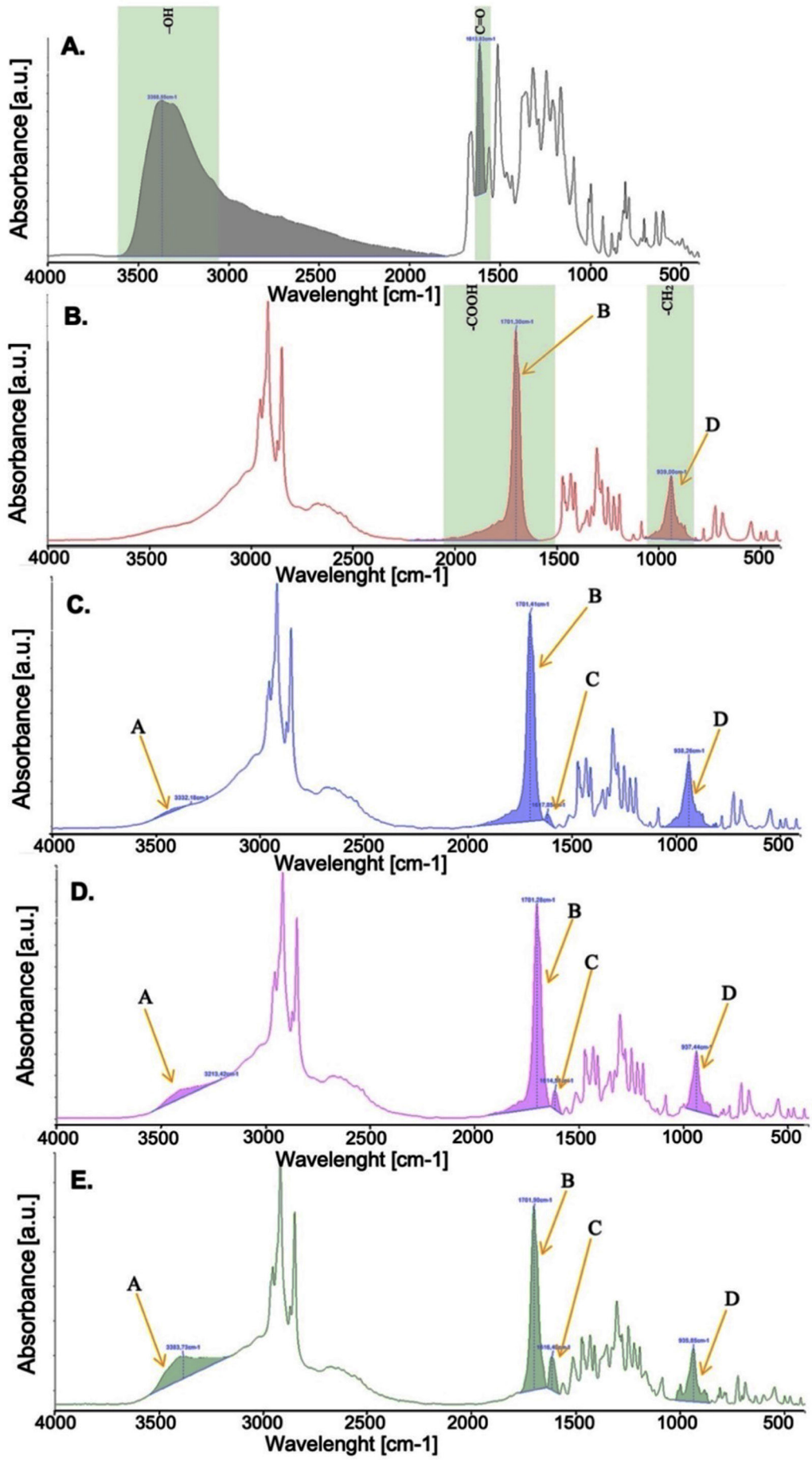

FIG. 6. FTIR spectra of manufactured particles and pure quercetin: A. unloaded particles, B. pure quercetin, C. loaded with 5% QE, D. loaded with 10% QE, E. loaded with 20% QE.

TABLE 1. The ratio of quercetin to lauric acid peaks indicating increasing encapsulation efficiency and ratio of lauric acid to lauric acid peaks as a validation of the absence of significant reactions between quercetin and lauric acid.

Concentration of QE in particles	Ratio of LAU : QE peaks	Ratio of LAU : LAU peaks
Unloaded	-	3.3:1
5%	24.5:1	3.2:1
10%	11.1:1	3.4:1
20%	5.6:1	3.5:1

Cytotoxicity test

To assess the ability of the particles to affect macrophage cytotoxicity, the LDH assay was performed.

The assay was performed for unloaded particles at 0.15 mg/ml concentration, quercetin powder at a concentration of 0.15 mg/ml, and quercetin-loaded microparticles at a concentration of 0.15 mg/ml and 0.30 mg/ml. The results from three independent donors are presented in FIG. 7.

The result showed that incubation with developed QE-loaded particles resulted in a higher percentage of LDH release for cells than unloaded particles; however, these values are distributed below the cytotoxicity threshold, which proves that QE-loaded particles were not cytotoxic for human macrophages.

To validate the significance of our findings, a statistical analysis was conducted. This analysis involved performing a t-test to compare the concentrations of quercetin (QE) within microparticles (MPs_QE0.15 and MPs_QE0.30) against microparticles without quercetin (MPs), as well as comparing QE alone against MPs. The results, as presented in TABLE 2, detail the t-test values and corresponding p-values for each comparison. Our analysis reveals a statistically significant distinction between both types of quercetin-loaded microparticles and unloaded microparticles. However, no significant differences were observed between the presence of QE alone and the unloaded microparticles.

Influence of quercetin on macrophage morphology

To assess macrophage morphology, cell nuclei and f-actin filaments were stained using DAPI and fluorescently labeled phalloidin-594, respectively. Fluorescence microscopy pictures were taken to analyze cells in terms of changes in morphology (FIG. 8).

Morphological differences were observed between cells incubated with quercetin (both encapsulated and pure substance) and unloaded particles when compared to the control group (primary human cells without stimulus – in the form of particles, pure active substance, or LPS). Macrophages incubated with unloaded particles and the control group had a small round-shaped morphology. However, cells exposed to quercetin were more spread and flattened. It can suggest that quercetin has an effect on macrophage morphology, causing their activation. Nonetheless, some research proved that M2 macrophages demonstrate a more spread morphology and have a larger size than the M1 phenotype [19]. Further research is needed to get a better insight into this matter. In this case, immunohistochemistry with the use of antibodies specific to M1 and M2 phenotypes could be advantageous.

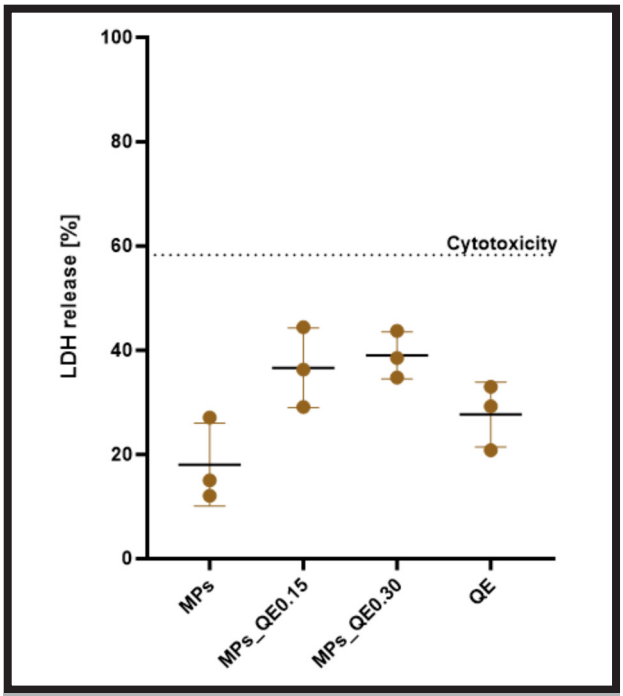

FIG. 7. Percentage of LDH released by macrophages from three different donors for: unloaded particles at a concentration of 0.15 mg/ml (MPs), particles loaded with quercetin at 0.15 mg/ml concentration (MPs_QE0.15), particles loaded with quercetin at 0.30 mg/ml concentration (MPs_QE0.30), and pure quercetin at a concentration 0.15 mg/ml (QE) after a 24 h incubation time. $n_{samples}$ = 3 per condition, p = 0.043 (one-way ANOVA). Statistical analysis including mean value and standard deviation.

TABLE 2. Statistical analysis of LDH release results including t-test, and corresponding p values, aimed to state the significance of the difference between the tested conditions.

	MPs_QE0.15	MPs_QE0.30	QE
MPs	t = 2.912, df = 4 p = 0.0436	t = 3.972, df = 4 p = 0.0165	t = 1.647, df = 4 p = 0.175

Visual inspection of nuclei staining has not shown significant differences in cells exposed to quercetin. The cell nuclei were round, of a similar shape, and stained with a similar intensity. These features indicate a lack of harmful effect of quercetin on nuclei morphology. The most significant differences in cytoskeleton staining can be observed between cells without stimuli (FIG. 8A) and MPs_QE0.30 (FIG. 8D). Cells in contact with a higher amount of QE-loaded microparticles were more spread and had more developed cytoskeleton fibers, similar to those cultured in contact with pure QU powder (FIG. 8E).

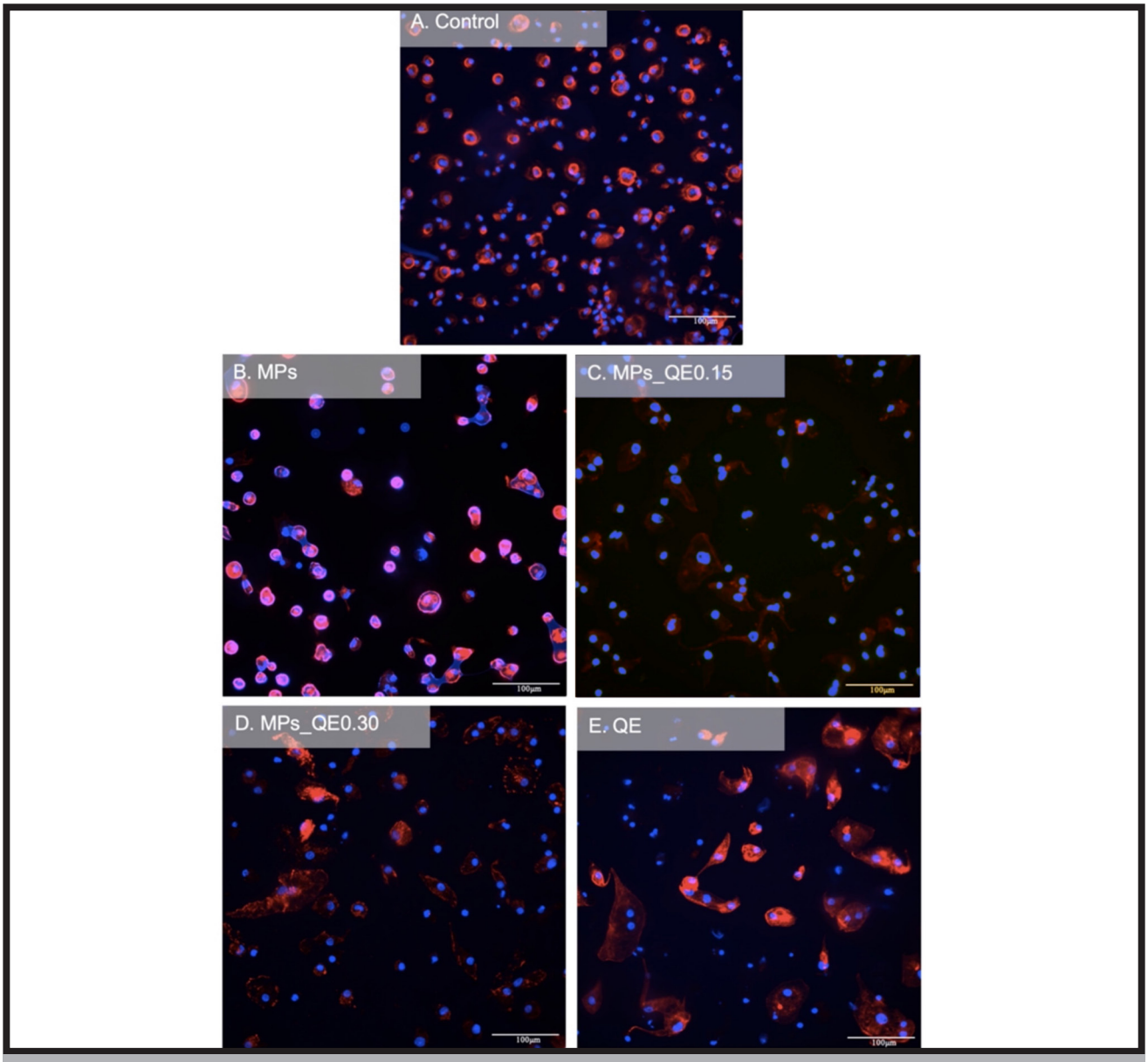

FIG. 8. Fluorescence microscopy images of DAPI/phalloidin staining of macrophages incubated for 24 h with the microparticles: cells without stimuli (Control) (A) and with: unloaded particles at a concentration of 0.15 mg/ml (MPs) (B), QE loaded particles at 0.15 mg/ml concentration (corresponding to 0.0075 mg/ml QE) (C), QE loaded particles at 0.30 mg/ml concentration (corresponding to 0.015 mg/ml QE) (D), and pure quercetin at 0.15 mg/ml concentration (E). Scale bar = 100 µm.

Influence of quercetin on macrophage polarization

Macrophages play a crucial role in directing inflammation due to their plasticity. Therefore, the ability of the particles to affect macrophage polarization was explored by flow cytometry for cells obtained from two donors. It was hypothesized that incubation with QE-loaded particles will influence the polarization state of macrophages. In FIG. 9, differences in dot plots can be observed between different conditions. Forward Scatter (FSC) and Side Scatter (SSC) in flow cytometry are providing information about the size and granularity of cells. However, activated or apoptotic cells might exhibit alterations in size or granularity, leading to shifts in their positions on the dot plot. Based on that, differences between dot plots in FIG. 9 might suggest that macrophages in different conditions exhibit different polarization states. Nevertheless, further research is necessary to study the influence of quercetin on the polarization state of macrophages.

Discussion

Quercetin is a naturally occurring flavonoid compound that has anti-inflammatory and anti-oxidant properties, dependent on numerous mechanisms. Taking into account the ability of quercetin to fight ROS and modulate inflammatory responses, it can be considered beneficial in the treatment of various diseases. Apart from its own anti-inflammatory mechanisms, this flavonoid can modulate actions of various immune cells, such as, e.g., macrophages, by influencing RNA transcription, antioxidant enzyme activity, and upregulating mitochondrial membrane potential. All of these actions result in a phenotypic change in macrophages, from an inflammatory phenotype to an anti-inflammatory phenotype [20,21]. Other research also shows quercetin suppressing effect on macrophage apoptosis pathways [22].

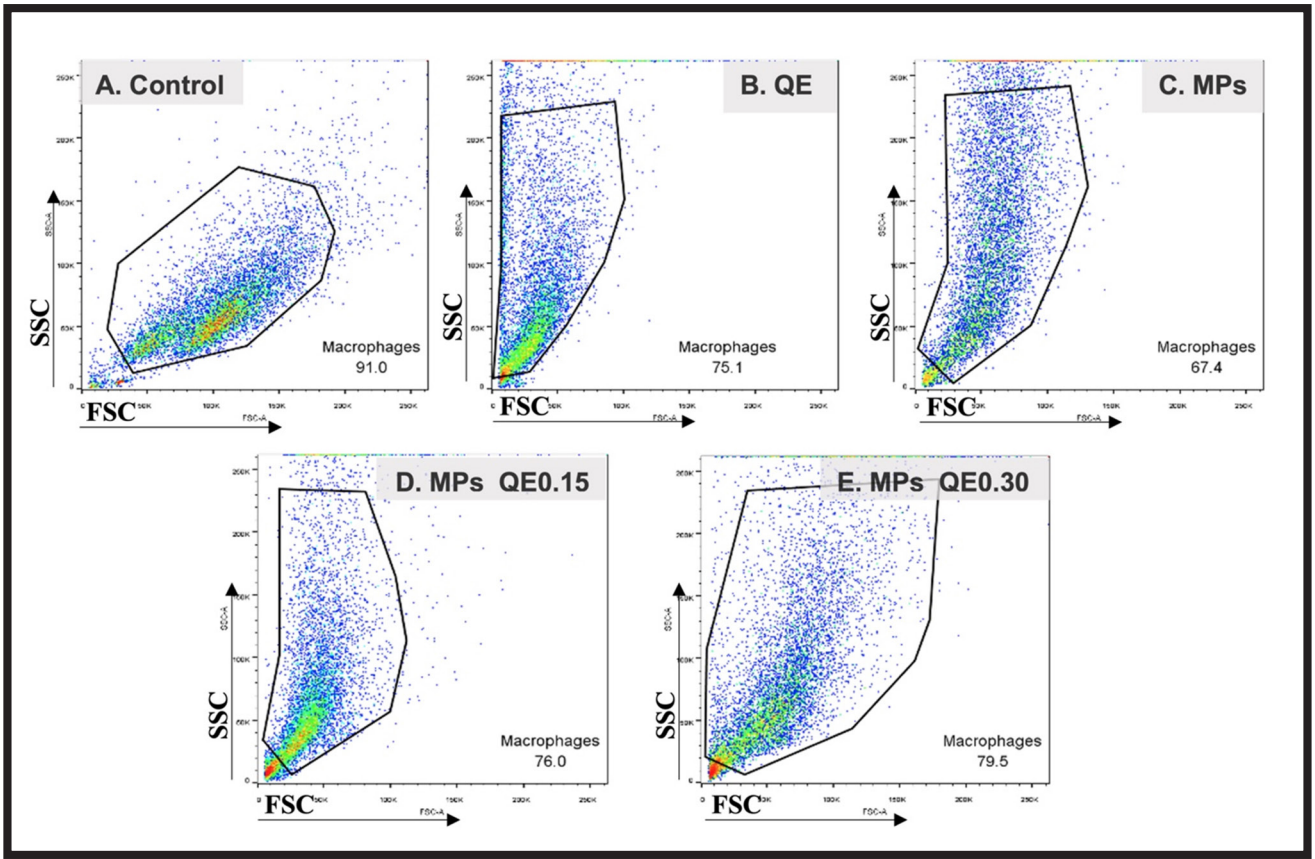

FIG. 9. Size and granularity of macrophages after 48 h of incubation with particles and pure active substance. Representative dot plots showing the aspect ratio.

In order to find agents supporting the burn wound healing process, lipid quercetin-loaded particles were produced and evaluated in terms of morphology, physicochemical properties, and biological capacity to affect macrophage viability and polarization. The manufacture of particles by the hot emulsification method guaranteed an average production efficiency of 46.5%. Moreover, the obtained particles presented a rounded shape and a defined size in a homogeneous final solution. On average, almost 60% of the particles had diameters less than 2 μm, which is advantageous due to the targeted use of the particles on skin wounds.

FTIR analysis confirmed the presence of quercetin and encapsulation efficiency in the lipid microparticles. It has been proven that no reactions occur between quercetin and lauric acid, by analyzing the area ratios between lauric acid:lauric acid characteristic peaks, which have retained their value. Furthermore, the are ratios between lauric acid:quercetin characteristic peaks allowed us to conclude that there was a decrease in these ratios when the concentration of quercetin increased, meaning that loading of quercetin in the particles is also increasing. To obtain the percentage values of loading efficiency, nuclear magnetic resonance (NMR) can be used in the future.

Lack of cytotoxicity of the manufactured particles and the necessity of encapsulation QE were proven by conducting LDH release assay. Adjusted concentrations of encapsulated and nonencapsulated active substance did not show a cytotoxic effect on macrophages after 24 h. Statistical analysis of our results confirmed a significant difference between encapsulated quercetin and unloaded microparticles. However, such a distinction was not observed between microparticles and quercetin alone. Nevertheless, encapsulation of the substance is essential to regulate the rate, time, and place of active substance release in drug delivery systems.

Macrophage morphology after incubation with microparticles and QE alone was also assessed through the staining of cells cytoskeleton and nuclei. The nuclei were round, with a similar shape, under all conditions. Moreover, differences between cells exposed to QE and incubated without QE were observed. Macrophages not exposed to QE can be described as small and round-shaped, while those exposed to QE non-encapsulated and encapsulated were more spread, flattened, and larger. These changes may be due to cell activation toward M2 macrophage phenotype as shown by others [23]. Further research would be needed to get a deeper insight into this observation. In this case, immunohistochemistry with the use of antibodies specific to M1 and M2 phenotypes could be advantageous.

The influence of QE on macrophage polarization was explored by flow cytometry. An evaluation was made for two healthy donors. As expected, a high number of CD14 expression levels were observed (data not shown). The inclusion of CD14 staining serves as a quality control measure for the experiment. Changes in size and granularity of macrophages under different conditions were observed, which might be due to differences in the activation state of the studied cells. However, to determine whether QE-loaded particles modulate polarization of macrophages, flow cytometry with the use of specific for macrophages subpopulations antibodies would have to be performed. To test the usefulness in fighting inflammation of the encapsulated material, further tests are needed to be performed, with particular emphasis on studying the release of quercetin from the particles and correlating them with macrophages polarization.

Conclusions

The production of lipid microparticles by the hot emulsification method allowed us for the encapsulation of quercetin, with increasing loading of the active substance by increasing the concentration of quercetin (5%, 10% and 20%) in the system during preparation. The presence of quercetin in the microparticles was confirmed by FTIR spectroscopy. The carriers were produced with a sufficient production efficiency of favorable shape and a median size in the range of 1.62-2.16 μm. Moreover, *in vitro* macrophage response for quercetin-loaded lipid microparticles was assessed. The particles did not show negative effects on macrophage morphology, as observed by the analysis of fluorescence images of DAPI-phalloidin red stained cells, although the cells in contact with pure quercetin and quercetin-loaded microparticles were more spread, which suggests their activations or polarization toward the anti-inflammatory (M2) phenotype. The carriers were found to be nontoxic to the cells, as shown by the LDH assay. The study used flow cytometry to examine the influence of quercetin on macrophage polarization, indicating potential changes in macrophage activation states. Further flow cytometry with specific antibodies for macrophage subpopulations is necessary to confirm the modulatory effect.

Overall, the obtained results show the potential of quercetin loaded lipid microparticles to combat inflammation and modulate burned skin regeneration.

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ORCID iD

J. Stępień: https://orcid.org/0009-0002-4002-3373
A. Kusibab: https://orcid.org/0000-0002-5580-7392
A.B. Sousa: https://orcid.org/0000-0002-3460-0499
J. Novais Barbosa: https://orcid.org/0000-0003-1852-2790
E. Pamuła: https://orcid.org/0000-0002-0464-6189

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