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Antioxidative and antiinflammatory activities of quercetin-loaded silica nanoparticles

Ga Hyun Lee[a,b,1], Sung June Lee[a], Sang Won Jeong[a], Hyun-Chul Kim[a], Ga Young Park[c], Se Geun Lee[a,*], Jin Hyun Choi[b,c,**]

[a] Division of Nano & Bio Technology, Daegu Gyeongbuk Institute of Science and Technology, Daegu 42988, Republic of Korea
[b] Department of Advanced Organic Materials Science and Engineering, Kyungpook National University, Daegu 41566, Republic of Korea
[c] Department of Bio-fibers and materials Science, Kyungpook National University, Daegu 41566, Republic of Korea

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ABSTRACT

Utilizing the biological activities of compounds by encapsulating natural components in stable nanoparticles is an important strategy for a variety of biomedical and healthcare applications. In this study, quercetin-loaded silica nanoparticles were synthesized using an oil-in-water microemulsion method, which is a suitable system for producing functional nanoparticles of controlled size and shape. The resulting quercetin-loaded silica nanoparticles were spherical, highly monodispersed, and stable in an aqueous system. Superoxide radical scavenging effects were found for the quercetin-loaded silica nanoparticles as well as free quercetin. The quercetin-loaded silica nanoparticles showed cell viability comparable to that of the controls. The amounts of proinflammatory cytokines produced by macrophages, such as interleukin 1 beta, interleukin 6, and tumor necrosis factor alpha, were reduced significantly for the quercetin-loaded silica nanoparticles. These results suggest that the antioxidative and antiinflammatory activities of quercetin are maintained after encapsulation in silica. Silica nanoparticles can be used for the effective and stable incorporation of biologically active natural components into composite biomaterials.

1. Introduction

Antioxidants have been demonstrated to possess a myriad of biological activities that are considered to be beneficial to health, including antioxidative, free radical scavenging, anticancer, antiinflammatory, and antiviral activities. Biological compounds with antioxidant properties protect cells and tissues from the deleterious effects of reactive oxygen species (ROS) and other free radicals generated during inflammation. Among these biological compounds with antioxidant properties, quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one, Fig. 1), a widely distributed flavonoid in plants [1], has been shown to exhibit several biological properties, including antioxidant, antiinflammatory, antiviral, and antibacterial/antimicrobial effects [2,3]. In particular, quercetin has the highest antioxidant activity among flavonoids and is characterized by multiple mechanisms, including scavenging of oxygen radicals, inhibition of lipid peroxidation, and modulation of cell antioxidant responses [4,5].

Despite this wide spectrum of beneficial properties, the use of antioxidants in the commercial field is limited because of their low solubility in water and instability in thermal processes [6,7]. One way to circumvent these problems is to encapsulate these antioxidants into nanoparticles. Biodegradable polymers are used extensively for the encapsulation of many antioxidants because of their high hydrophobicity, biodegradability, biocompatibility, low toxicity, strong mechanical strength, and slow drug release [8]. Quercetin has been successfully encapsulated into liposomes, cyclodextrin, and chitosan nanoparticles [9,10].

Nanoencapsulation has become a technique of increasing interest because it offers numerous benefits, including ease of handling, enhanced stability, controlled release, and improved water solubility for hydrophobic ingredients, as well as enhanced bioavailability and efficacy [11,12]. However, current polymer nanoparticles, which are widely used, have some shortcomings. These polymer particles easily aggregate with each other by flocculation in water, are frequently damaged by friction, and have low heat tolerance in thermal processes. Using ceramic-based nanoparticles provides

* Corresponding author at: Division of Nano & Bio Technology, Daegu Gyeongbuk Institute of Science and Technology, Daegu 42988, Republic of Korea.
** Corresponding author at: Department of Bio-fibers and materials Science, Kyungpook National University, Daegu 41566, Republic of Korea.
E-mail addresses: sklee@dgist.ac.kr (S.G. Lee), jinhchoi@knu.ac.kr (J.H. Choi).
[1] Current address: Medical Textile Center, Gyeongbuk Technopark, Gyeongbuk 38412, Republic of Korea

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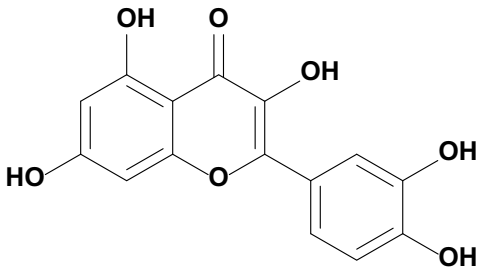

Fig. 1. Chemical structure of quercetin.

an approach that solves most of the problems associated with free or polymer-encapsulated, unstable bioactive materials. In addition, other advantages of inorganic encapsulation include high transparency and photostability. Furthermore, inorganic coatings have a controllable porosity, can release embedded agents, and are biologically inert. There are no swelling or porosity changes with changes in pH and temperature, and ceramic nanoparticles protect encapsulated components against microbial attack and denaturation. Silica, alumica, and titania nanoparticles are the typical examples known for their compatibility in biological systems [13–18].

This study aimed to synthesize quercetin-loaded silica nanoparticles that can be used for effective and stable incorporation into composite biomaterials. The morphology and size of quercetin-loaded silica nanoparticles were investigated by transmission electron microscopy (TEM) and dynamic light scattering (DLS). It is well known that quercetin is a biologically active compound. Furthermore, the activity of quercetin should be maintained after encapsulation into nanoparticles. Thus, superoxide radical scavenging ability, proinflammatory cytokine levels, and cytotoxicity were estimated to confirm the antioxidative and antiinflammatory activities of quercetin-loaded silica nanoparticles.

2. Materials and methods

2.1. Materials

Quercetin hydrate, triethoxyvinylsilane (TEVS; 97%), and surfactant Aerosol OT (AOT; 98%) were purchased from Aldrich. Nitrotetrazolium blue chloride (NBT) and 3-aminopropyltriethoxysilane (APTES; 99%) were purchased from Sigma-Aldrich (USA). Xanthine (99%) was purchased from Sigma (USA). Xanthine oxidase from buttermilk was obtained from Fluka (USA). Ethanol (95%) and *n*-butyl alcohol (99%) were purchased from Duksan Pure Chemical (Korea).

2.2. Synthesis of quercetin-loaded silica nanoparticles

Quercetin-loaded silica nanoparticles were synthesized in the nonpolar core of water/surfactant/organic solvent micelles [18]. Quercetin was dissolved in *n*-butyl alcohol by magnetic stirring, and the resulting clear solution was adjusted to fixed concentrations (0.02, 0.04, 0.06, 0.08, and 1.0%). Next, 800 μL of *n*-butyl alcohol containing quercetin was dispersed in 20 mL of doubly distilled water, and the micelles were prepared by dissolving 0.44 g AOT in the dispersion by vigorous magnetic stirring. Next, 200 μL of TEVS was added to the micelle system. After 1 h, APTES (10 μL) was added, and the system was stirred for 24 h. The entire process was performed at room temperature. At the end of the process, a translucent solution was obtained, which indicated nanoparticle formation. After washing with excess of water by vigorous stirring and subsequent freeze-drying for 3 days, the nanoparticle powders were obtained.

2.3. Characterization of quercetin-loaded silica nanoparticles

The morphology of the nanoparticles was examined using TEM (H-7600, Hitachi, Japan) at an accelerating voltage of 100 kV. For TEM, one drop of the nanoparticle solution was placed onto a carbon-coated copper grid and dried at room temperature for 1 day. The mean hydrodynamic size of the silica nanoparticles was measured using a DLS spectrophotometer (Zetasizer Nano ZS, Malvern, UK). For each analysis, the freeze-dried nanoparticles were dispersed in water at the concentration of 0.05% and their homogenous solutions were used.

2.4. Superoxide radical scavenging activity of free quercetin and quercetin-loaded silica nanoparticles

Superoxide radicals were generated by xanthine/xanthine oxidase (XO) and quantified by the NBT reduction method [19]. Free quercetin and the quercetin-loaded silica nanoparticles were mixed in a 100 mmol/L phosphate buffer solution (pH 7.0) containing 1.65×10^{-2} units/mL XO and 133 μM NBT at 25 °C. The experiment was initiated with the addition of 164 μM xanthine. After 10 min, production of superoxide radicals was evaluated spectrophotometrically (560 nm) at 25 °C using a UV–vis spectrophotometer. Superoxide scavenging activity was calculated according to the following formula: Superoxide scavenging activity (%) = $[1-\{(A-B)/A)\}] \times 100$, where A and B represent the absorbance in the absence and presence of samples, respectively.

2.5. Cell culture

L-929 cells (murine fibroblast cell line) and RAW264.7 cells (murine macrophage cell line) were purchased from the Korean Cell Line Bank. The cells were grown in 90% Dulbecco's Modified Eagle's Medium (LONZA, USA) supplemented with 10% fetal bovine serum (LONZA, USA), 100 units/mL penicillin, and 100 units/mL streptomycin (GIBCO, USA). The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2. When the cells reached 80% confluence, they were trypsinized with 0.25% trypsin containing 1 mmol/L ethylene diamine tetraacetic acid (GIBCO, USA) and counted with a hemocytometer (Hausser Scientific, USA) prior to further use.

2.6. In vitro cytotoxicity of free quercetin and quercetin-loaded silica nanoparticles

Free quercetin and the quercetin-loaded silica nanoparticles were suspended in culture medium to serve as a stock solution. L-929 cells (1.0×10^5) were seeded with the growth medium (200 μL) in the individual wells of 96-well culture plates. The cells were then treated with free quercetin and the quercetin-loaded silica nanoparticles by adding a fixed volume of the stock solution for dilution to the required concentration followed by incubation in a humidified atmosphere containing 5% CO_2 at 37 °C for 24 h. The cells were then washed twice with phosphate-buffered saline to remove any remaining particles, and fresh culture medium was added. After replacing the old medium, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, USA) solution (5.0 mg ml^{-1}) was added to each well, and the cells were incubated for 4 h. Cell viability was evaluated using the degree of mitochondrial reduction of MTT to formazan by succinic dehydrogenase. The absorbance at 570 nm was measured using a microplate reader (Molecular Devices, USA). Cell viability (%) was expressed as the relative absorbance of the sample to that of the control.