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Thermoresponsive mesoporous silica nanoparticles as a carrier for skin delivery of quercetin

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ABSTRACT

Recently, mesoporous silica nanoparticles (MSNs) have emerged as promising drug delivery systems able to preserve the integrity of the carried substance and/or to selectively reach a target site; however, they have rarely been explored for skin application. In this study, thermoresponsive MSNs, designed to work at physiologic cutaneous temperature, are proposed as innovative topical carriers for quercetin (Q), a well-known antioxidant. The thermosensitive nanoparticles were prepared by functionalizing two different types of matrices, with pore size of 3.5 nm (MSN_{small}) and 5.0 nm (MSN_{big}), carrying out a free radical copolymerization of *N*-isopropylacrylamide (NIPAM) and 3-(methacryloxypropyl)trimethoxysilane (MPS) inside the mesopores. The obtained copolymer-grafted MSNs (copoly-MSNs) were physicochemically characterized and their biocompatibility was attested on a human keratinocyte cell line (HaCaT). The release profiles were assessed and the functional activity of Q, free or loaded, was evaluated in terms of antiradical and metal chelating activities. *Ex vivo* accumulation and permeation through porcine skin were also investigated. The characterization confirmed the copolymer functionalization of the MSNs. In addition, both the bare and functionalized silica matrices were found to be biocompatible. Among the copolymer-grafted complexes, Q/copoly-MSN_{big} exhibited more evident thermoresponsive behavior proving the potential of these thermosensitive systems for advanced dermal delivery.

1. Introduction

The biological role of flavonoids, a large class of secondary plant metabolites, is well established. Flavonoids are abundantly present in fruit, vegetable, cocoa, wine, tea, and soy. They share a common carbon skeleton of two benzene rings joined by a 3-carbon bridge (C6–C3–C6), comprising many subclasses, such as flavonols, flavones, anthocyanidins, flavanones, and isoflavones (Middleton et al., 2000; Miyake and Shibamoto, 1997). Flavonoids are helpful in the treatment of many diseases and are important antioxidant, anticancer and neuroprotective agents (Maalik et al., 2014). Notably, they have been proposed as an interesting cost-effective therapeutic tool in inflammatory diseases and represent an alternative to immunosuppressive agents, which are known to have multiple side effects (Ribeiro et al., 2015). In addition flavonoids, besides being efficient free radical scavengers (Roubalova et al., 2015), can up-regulate the endogenous antioxidant system (Ehren and Maher, 2013), suppress oxidative and nitrosative stress, decrease macrophage oxidative stress through cellular oxygenase inhibition as well as through interaction with several signal transduction pathways. Moreover, they also show therapeutic effects against atherosclerosis (Siasos et al., 2013).

Interestingly, several studies have demonstrated the efficacy of flavonoids in reducing the symptoms of chronic venous insufficiency. Indeed, flavonoids have been widely used to manage the symptoms of venous diseases because they can address certain microcirculatory deficiencies involved in ulcer pathophysiology (Rabe et al., 2015). These include decreasing leukocyte adhesion and free radical formation, decreasing permeability and fragility of

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vein valves and venous wall (and therefore decreasing abnormal leakage in the lower limbs), and increasing venous flow (Werner et al., 2015). Nevertheless, they cannot attack the underlying cause of venous hypertension (Scallon et al., 2013).

Most flavonoids are administered orally, but they can also be applied topically in the form of creams, gels, lotions, etc. The topical use of flavonoids is very desirable since it allows a synergic combination of three important benefits: the skin hydration of the affected area, the massage action that takes place during the application, the active compound release. Accordingly, flavonoid extract, having antioxidant, anti-inflammatory and sun protection properties, can enrich skin care products, adding valuable benefits to the formulation (Saija et al., 1998).

Cutaneous application of flavonoids, however, is limited by their low solubility, low stability and low release after application (Kim et al., 2004; Kumar and Pandey, 2013). Moreover, flavonoid chemical changes resulting from degradation may decrease the effectiveness and safety of skin care products. Thus, to ensure that topically administered antioxidants are effective upon skin application, a crucial point in the product formulation is their stabilization. Under this point of view, antioxidants are very unstable and they may be converted to inactive forms before reaching the target; on the other hand, antioxidants must be properly adsorbed into the skin, reach their target tissue in the active form and remain there long enough to exert the desired effects. Taking into account these considerations, we designed a thermoresponsive delivery system able to release bioactive compounds when in contact with the skin.

Recently, MSNs have been proposed in the dermocosmetic field as carriers of active ingredients (Ambrogi et al., 2007; Berlier et al., 2013a; Gastaldi et al., 2012; Sapino et al., 2015). They are characterized by an ordered mesoporous structure and high biocompatibility; moreover, by grafting functional moieties on their surface, it is possible to modulate the delivery of a bioactive agent in response to different *stimuli* including light, temperature, pH, electric fields, or chemicals (*e.g.* enzymes). Functional moieties used for this purpose are often smart polymers which can protect the drug until it reaches the site of action and can then modulate its release to obtain the desired release profile (Wang, 2009).

Smart polymers are materials responding in a fast and substantial way to very slight changes in the environment. Poly (*N*-isopropylacrylamide) (poly-NIPAM) is a temperature-responsive polymer that was first synthesized in the 1950s (Schild, 1992). At approximately 33 °C, it exhibits a lower critical solution temperature (LCST). Above this temperature, the swollen hydrated polymer chains shrink to dehydrated ones, losing about 90% of their total mass. As this coil-to-globule transition occurs at a temperature close to human body temperature, poly-NIPAM has recently been proposed as a smart polymeric carrier suitable to deliver therapeutic molecules (Gandhi et al., 2015; Natalia et al., 2015). Generally, to be of interest for potential *in vivo* applications, carriers must retain active molecules tightly before usage, but they have to release them in the application site according to individual requirements. Recently, variable stimuli-responsive systems based on MSNs have been designed for drug delivery. However, to date, very few reports have focused on the controlled dermal release of active substances, while most research and development activities are aimed at encapsulating drugs for systemic applications.

Thus, in order to maintain the effectiveness of antioxidants in skin care products during shelf-life and the period-after-opening, we developed MSNs functionalized with a thermoresponsive copolymer of NIPAM and we successfully loaded them with the flavonoid quercetin as a model compound characterized by poor water solubility, low stability and a short half-life (Berlier et al., 2013b; Li et al., 2013). Taking advantage of the attractive tunable structural features of MSNs, nanoparticles with two different pore sizes (3.5 and 5.0 nm) were synthesized in the attempt to find the best affinity for the guest molecule. After a physico-chemical characterization and a biocompatibility test of these delivery systems, preliminary experiments were performed to verify their capability of triggering the flavonoid release upon skin contact. Hence, the antioxidant efficacy of the most promising complexes was assessed by *in vitro* studies, and the permeation and accumulation of quercetin through the skin were tested by Franz diffusion cells.

This proof-of-concept study is expected to address the challenges related to the application of copolymer-functionalized MSNs as carriers for the loading and thermosensitive release of active molecules on the skin.

2. Materials and methods

2.1. Materials

3,3′,4′,5,7-Pentahydroxyflavone (quercetin anhydrous, Q), 1,3,5-trimethylbenzene (TMB), 2,2′-azobis(2-methylpropionitrile) 98% (AIBN), 3-(methacryloxypropyl)trimethoxysilane (MPS), *N*-isopropylacrylamide (NIPAM), absolute ethanol, dimethyl sulfoxide (DMSO), hexadecyltrimethylammonium bromide (CTAB), methanol, tetraethyl orthosilicate (TEOS), sodium phosphate dibasic, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-*p*,*p*′-disulfonic acid monosodium salt hydrate (ferrozine), iron(II) sulfate ($FeSO_4$), 2 2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) sodium azide, sulforhodamine B (SRB), Dulbecco's modified Eagle's medium (DMEM), fetal calf serum, Tris buffered saline, trichloroacetic acid and antibiotics for cell cultures were purchased from Sigma–Aldrich. Acetic acid was from Carlo Erba. Sodium chloride, hydroxyethylcellulose (HEC) and propylene glycol were purchased from ACEF. Potassium dihydrogen phosphate dehydrate and sodium hydroxide were from Fluka. Human immortalized keratinocyte cell line, HaCaT, was kindly provided by Dr. Carlotta Castagnoli (Dipartimento di Chirurgia Generale e Specialistica, Banca della Cute, AOU Città della Salute e della Scienza di Torino, Italy).

2.2. Synthesis of MSNs

MCM-41-like nanoparticles were synthesized by optimizing a literature procedure, employing CTAB as the structure directing agent (SDA) and TEOS as the silica source (Kresge et al., 1992). In particular, two types of MSNs were prepared: conventional MSNs with small pore size (*i.e.* 3.5 nm) and MSNs with big pore size (*i.e.* 5.0 nm), hereafter labelled MSN_{small} and MSN_{big}, respectively.

In a typical reaction to obtain MSN_{small}, CTAB (1 g) was dissolved in distilled water (480 mL); the solution was heated at 80 °C and then NaOH (3.5 mL, 2 M) was added, so the mixture was stirred for 30 min. TEOS (5 mL) was added dropwise. The mixture was then stirred for 2 h at 80 °C. After cooling to room temperature (RT), the powder product was isolated by filtration, washed with distilled water (750 mL) and methanol (500 mL), and air-dried for 24 h.

The synthesis of MSN_{big} was obtained employing TMB as the micelle core swelling agent. In particular, in this case, differently from MSN_{small} synthesis, after NaOH addition and the stabilization of the temperature, a proper amount of TMB was added to the system. The mixture was stirred for 30 min for the formation of a stable white emulsion. Then TEOS was added following the same procedure as reported for MSN_{small}.

In both cases, the as-synthesized materials were calcined by flowing nitrogen from RT to 550 °C (2 °C/min ramp) and then switching the flow to oxygen, for a 6 h isotherm at 550 °C.