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Entrapping quercetin in silica/polyethylene glycol hybrid materials: Chemical characterization and biocompatibility

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ABSTRACT

Sol-gel synthesis was exploited to entrap quercetin, a natural occurring antioxidant polyphenol, in silica-based hybrid materials, which differed in their polyethylene glycol (PEG) content (6, 12, 24 and 50 wt%). The materials obtained, whose nano-composite nature was ascertained by Scanning Electron Microscopy (SEM), were chemically characterized by Fourier Transform InfraRed (FT-IR) and UV-Vis spectroscopies. The results prove that a reaction between the polymer and the drug occurred. Bioactivity tests showed their ability to induce hydroxyapatite nucleation on the sample surfaces. The direct contact method was applied to screen the cytotoxicity of the synthetized materials towards fibroblast NIH 3T3 cells, commonly used for in vitro biocompatibility studies, and three nervous system cell lines (neuroblastoma SH-SY5Y, glioma U251, and pheochromocytoma PC12 cell lines), adopted as models in oxidative stress related studies. Using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay NIH 3T3 proliferation was assessed and the morphology was not compromised by direct exposure to the materials. Analogously, PC-12, and U-251 cell lines were not affected by new materials. SH-SY5Y appeared to be the most sensitive cell line with cytotoxic effects of 20–35%.

1. Introduction

Dietary plants are a rich source of antioxidant polyphenols, whose regular intake has been claimed to be beneficial for human health. Cell culture-based assays and animal model studies provide growing evidence of the protective benefits of pure polyphenols against the occurrence of chronic degenerative diseases, such as cancer, cardiovascular and neurodegenerative disorders. Among the polyphenols, flavonoids, occurring ubiquitously in plant foods, are known to exhibit a wide range of physiological effects, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects [1].

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the most studied dietary flavonoids, belonging to the flavonol class. Its daily intake is estimated on average to be 10 mg, whereas the compound is available as a dietary supplement with daily doses between 200 and 1200 mg. Furthermore, quercetin may be used within the range 0.008–0.5% or 10–125 mg/serving as a nutraceutical for functional foods [2].

The poor solubility of the flavonol in aqueous media, as well as its promising therapeutic efficacy (as antioxidant, anti-inflammatory or anti-cancer agent), have aroused the interest of researchers wishing to incorporate it into pharmaceutical, cosmetic or food products [3]. Different methods have been applied using a wide array of carrier materials. Lipid microparticles loaded with the flavonoid were developed in order to enhance its stability in topical formulations [4]. Quercetin was efficiently encapsulated (to 96.7%) into polylactide nanoparticles using the solvent evaporation technique [5].

Recently, sol-gel materials have been investigated as biocompatible hosts of quercetin for controlled drug delivery applications. Nday et al. [6] proposed base-catalyzed silica gel matrices modified with PEG 3000 and the CTAB cationic surfactant as potential carrier materials for the controlled release of the antioxidant flavonoid under oxidative stress conditions in neurodegeneration. Hollow porous silica spheres, prepared by using a combined emulsion sol–gel process and triblock copolymer as a template, were also used to load quercetin [7]. Polyol-in-oil-in-water (P/O/W) emulsion and sol-gel methods were investigated for stabilizing quercetin [8]. Indeed, the chemical instability of the compound in aqueous alkaline and acidic media was broadly demonstrated. In such conditions, quercetin easily gives rise to oxidative derivatives [9–11], which can maintain or completely lose its typical bio-properties.

Recently, entrapping quercetin in a silica matrix, a new biocompatible and bioactive material was synthetized [12] by the sol-gel technique. That material proved able to provide antioxidant functionality and without exacerbating the body's normal oxidant and inflammatory response. The high encapsulation efficiency of the natural drug was accompanied by the weak release of an active quercetin aurone-like derivative, probably formed during the sol-gel process. These intrinsically antioxidant quercetin-based biomaterials were proposed in the dentistry and orthopedics fields, as components of glass ionomer

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cement and replacements for bone implants. The versatility of the sol-gel method, which allows glasses and ceramics to be made at low temperature, led also to organic–inorganic hybrid materials based on quercetin, poly(ε-caprolactone) and silica [13].

To further investigate the potential use of flavonol in dental and orthopedic applications, silica-PEG based materials, differing in their PEG amount (6, 12, 24, and 50 wt%) and entrapping quercetin, were synthetized in the present work. Polyethylene glycol (PEG) is a well-known degradable biopolymer which allowed highly biocompatible hybrid materials to be prepared [14,15] Moreover, PEG-functionalized silica nanoparticles (PSiNPs) demonstrated the capability to seal damaged cells and tissues providing neuroprotection subsequent to spinal cord trauma [16,17]. The addition of the flavonol was set at the weight percentage of 5 and 10 wt% to allow a comparison with the data obtained in previous work [12,13]. The new materials were characterized by FT-IR and UV-Vis spectroscopy. Biocompatibility was assessed by the MTT direct contact test using the murine fibroblast (NIH 3T3) cell line and three different nervous system (NS) cell lines (neuroblastoma SH-SY5Y, glioma U251, and pheochromocytoma PC12 cell lines). The choice of cell lines was deliberate. Fibroblasts are cell types that interact with proteins on biomaterials surfaces, playing important roles in biomaterials rejection and implant failure [18]. On the other hand, considering the potent antioxidant capability of quercetin, NS cells, particularly sensitive to oxidative stress onset, were also used. In fact, the brain is highly vulnerable to oxidative stress due to its high O_2 consumption, its modest antioxidant defenses and its lipid-rich constitution.

2. Materials and methods

2.1. Sol-gel synthesis of the materials

Organic-inorganic hybrid nanocomposites were synthesized by means of a sol-gel route. The obtained materials consisted of a SiO_2 inorganic matrix in which the antioxidant quercetin and poly(ethylene glycol) (PEG) were entrapped. Several formulations of the hybrids were synthesized, which differed in both their polymer and drug content (Table 1).

The inorganic silica sol was first obtained by adding tetraethyl orthosilicate (TEOS; $Si(OC_2H_5)_4$; Sigma-Aldrich), the metal alkoxide precursor, to a solution of HNO_3 (≥65%, Sigma-Aldrich) and distilled water in pure ethanol (99.8% Sigma-Aldrich). The water allows the hydrolysis of TEOS. The condensation of the obtained species leads to the transition from the colloidal solution (called sol) to the rigid gel. An acidic environment was used to favor the kinetics of hydrolysis and condensation reactions. The choice of the used pH, as well as the H_2O/alkoxide molar ratio, is very important because those parameters affect the microstructural properties of the obtained inorganic matrix [19]. To synthesize the silica sol the following molar ratios of the reagents were used: $TEOS:HNO_3:EtOH:H_2O = 1:1.7:6:2$.

To prepare the SiO_2/PEG/Que materials, PEG (MW = 400, Sigma-Aldrich) was dissolved in ethanol and added to the silica sol under stirring. Subsequently, a previously prepared solution of quercetin in pure ethanol (99.8%, Sigma-Aldrich) was added under stirring to the silica/PEG mixture. After gelation, the products were air-dried at 50 °C for 24 h to remove the residual solvent. Higher temperatures were avoided to prevent the thermal degradation of both polymer and drug.

The sol-gel procedure used is schematized in Fig. 1. The materials were crushed to a powder with size <75 μm using an agate mortar.

Table 1
Composition and label of the synthesized systems.

LABEL	SYSTEM COMPOSITION	
	PEG content (wt%) in SiO_2 matrix	Que content (wt%) in SiO_2 matrix
SiO_2/PEG6/Que5	6%	5%
SiO_2/PEG6/Que10	6%	10%
SiO_2/PEG12/Que5	12%	5%
SiO_2/PEG12/Que10	12%	10%
SiO_2/PEG24/Que5	24%	5%
SiO_2/PEG24/Que10	24%	10%
SiO_2/PEG50/Que5	50%	5%
SiO_2/PEG50/Que10	50%	10%

2.2. Study of the material structure

Instrumental analyses allowed us to investigate the structure of all synthesized materials as a function of both polymer and drug content. The microstructure of the synthesized gels was studied by Scanning Electron Microscope (SEM) (Quanta 200, FEI, the Netherlands) equipped with a EDAX silicon-drift detector for energy-dispersive X-ray analysis (EDX). The chemical composition of the materials and the interaction between their components (SiO_2 inorganic matrix, PEG and Quercetin) were investigated by Fourier transform infrared spectroscopy (FT-IR). Transmittance spectra were recorded in the 400–4000 cm^{-1} region using a Prestige 21 (Shimadzu, Japan) system, equipped with a DTGS KBr (Deuterated Tryglycine Sulphate with potassium bromide windows) detector, with a resolution of 4 cm^{-1} (45 scans). Disks with a diameter of 13 mm, a thickness of 2 mm, a weight of 200 mg and containing 1 wt% of sample in KBr, were obtained by pressing sample powders into a cylindrical holder using a Specac manual hydraulic press. The FT-IR spectra were analyzed by the Prestige software (IR solution). UV-Vis spectra of extracts from powders (2.0 mg) of each different synthesized material, previously soaked in Dulbecco's Phosphate Buffer Saline (1.0 mL) or methanol (1.0 mL), were acquired in the range 200–600 nm using a Shimadzu 1700 spectrophotometer.

2.3. Bioactivity test

The ability of the synthesized materials to bond to living bones, and thus the materials' bioactivity, was evaluated by an in vitro apatite forming-ability test, carried out following the procedure of Kokubo [20]. Disks with a diameter of 13 mm and a thickness of 2 mm were obtained by pressing 200 mg of sample powders into a cylindrical holder using a Specac manual hydraulic press. The pelletized disks of all SiO_2/

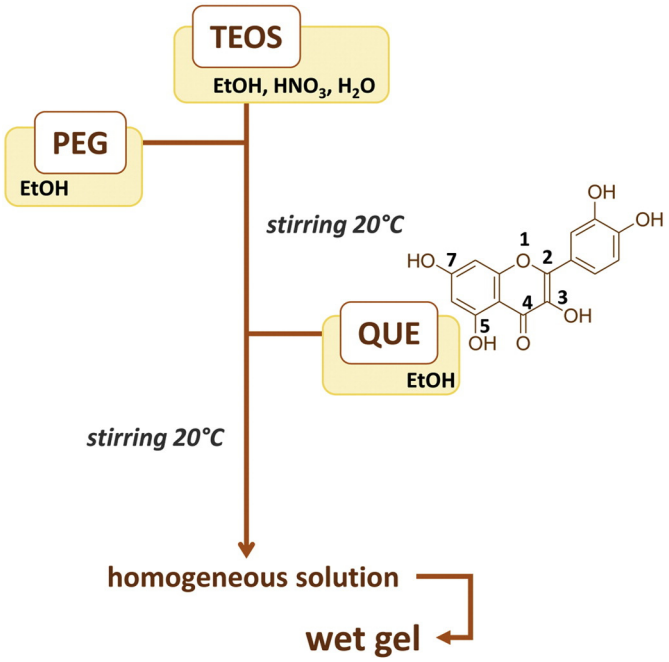

Fig. 1. Flow chart of the used sol-gel procedure.