



Cytocompatibility of Caffeic Acid-Silica Hybrid Materials on NIH-3T3 Fibroblast Cells

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The hydroxycinnamoyl compound caffeic acid (CA), broadly occurring in plants, is receiving special attention in materials science thanks to its antioxidant, anti-inflammatory, and antimicrobial activities that make it promising for application use in various sectors. In this context, CA-based peptide biomaterials are recently developed as eco-friendly and multifunctional free radical scavengers useable in a wide range of consumer manufacture, ranging from cosmetics to household products, as well as clinical applications, including imaging, drug delivery, and disinfection. Furthermore, a water-soluble chitosan-caffeic acid conjugate, effective in delaying lipid oxidation, is also synthesized. Herein, exploiting sol-gel route versatility, CA/silica materials are synthesized. Hybrids, chemically characterized mainly through spectroscopic techniques, varied in their relative CA content, which represented 5%, 10%, 15%, or 20% of materials' weight. The synthesized materials are able to elicit anti-radical properties. The CA amount appeared to be determinant in anti-radical activity, as well as in biocompatibility assessment. To this latter purpose, mouse embryonic fibroblast cell line NIH-3T3 cells are utilized and directly exposed to hybrid materials. Redox mitochondrial activity is evaluated by means of the MTT test, whose results are in accordance with the materials' biocompatibility.

production of active films able to inhibit lipid oxidation in fish oil emulsion, or in the synthesis of chitosan derivatives in order to increase their antioxidant capabilities,^[2–5] or even in materials with antibacterial potential against *Escherichia coli* and *Enterococcus faecalis*.^[6] Also other natural compounds, containing a CA moiety in their molecular skeleton, have been previously entrapped in functionalized materials. It is the case, for example, of the depside chlorogenic acid (5-O-caffeoylquinic acid), which has been engaged in the synthesis of new organic-inorganic hybrid materials via sol-gel route together with silicon alkoxide and low molecular weight polyethylene glycol.^[7–9]

2. Synthesis and Spectroscopic Features of Hybrid Materials

Sol-gel technique was applied to the synthesis of four hybrid materials, in which ethanol solutions of CA at different

1. Introduction

3,4-dihydroxycinnamic acid, better known as caffeic acid (CA), is a plant-derived compound, present in several vegetables and fruits that are commonly consumed in our diet. A number of health-related properties have been ascribed to this molecule, demonstrated in vitro and in some cases in vivo.^[1] In the last decade, the interest towards CA led to its employment also in materials science, ranging from the food functional packaging to the biomedical field.^[2–3] Indeed, it has been employed in the

percentages (5, 10, 15, and 20% wt) were added to tetraethyl orthosilicate (TEOS) in a hydroalcoholic mixture acidified with nitric acid (Figure 1).

FTIR spectroscopy allowed us to investigate the phenol structural interactions with the silica network. To this purpose, FTIR spectra, which were recorded in the 400–4000 cm⁻¹ region using a Prestige 21 (Shimadzu, Japan) system, equipped with a deuterated tryglycine sulphate (DTGS) detector with potassium bromide windows, with a resolution of 4 cm⁻¹ (45 scans), were processed by Prestige software (IR solution). In Table 1, the assignment of the detected IR bands is reported, based on previous studies.^[10] When spectra of synthesized materials were interpreted in comparison with that of pure silica,^[11] the observed signals were very similar, likely due to a strong linkage between the phenol and the inorganic network. The only two bands that peculiarly characterized all the hybrids were those at 1725 and 1735 cm⁻¹, likely referring to an ester C = O stretching mode, whose intensity was getting stronger with the increase of CA wt%.

UV-Vis spectroscopy proved to be a useful tool to confirm the initial hypothesis about the formation of a new network in which CA was definitely merged in the SiO₂ matrix. To this aim, CA was solubilized in ethanol, whereas hybrids' powder underwent sonication using an ultrasound bath (Advantage Plus model ES, Germany) in a hydro-alcoholic solution, and UV-Vis spectra were

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Figure 1. Synthetic scheme of hybrid materials under study

Table 1. Caffeic acid IR absorption bands (s = strong; versus = very strong; m = medium).

Absorption bands (cm ⁻¹)	Interpretation
3440, 3240 (s)	OH stretching
1645 (vs)	C = O stretching
1625 (vs), 1530 (m), 1450 (vs)	C-C (aromatic and olefinic) stretching
1280 (vs), 1217 (s)	C-H (olefinic and aromatic) in-plane bending
< 1120 (m)	C-C-C (aromatic) in-plane and out-of-plane bending
815 (m), 648 (m)	C = O in-plane bending

acquired in the range 200–600 nm by a UV-1700 double beam spectrophotometer (Shimadzu, Japan). Some differences were detected, due to some structural modifications of embedded CA. Indeed, hypochromic shifts of carbonyl $n \rightarrow \pi^*$ electronic transitions (at 296 nm), as well as $\pi \rightarrow \pi^*$ ones of aromatic moiety (at 242 and 324 nm), occurred.

3. Antiradical Capacity and Biocompatibility Evaluation

Caffeic acid was found to exert a strong antiradical capacity, which was attributed to its catechol moiety and to its conjunction with the propenyl chain. The mechanism of the antiradical activity could be due to the abstraction of a hydrogen atom, with

the following formation of a stable aryloxy radical. This latter could undergo donation of another hydrogen atom to rearrange into *ortho*-quinone.^[12] Based on the scavenging capability of the embedded natural-derived compound, the capacity of the hybrid materials under study to act as radical scavengers was evaluated by DPPH and ABTS tests. They proved to counteract radical probes with respect to the material tested dose (0.5, 1 and 2 mg) and, within each series, in a CA amount-dependent manner, although the ABTS test sensitivity was higher (**Figure 2**).

Furthermore, CA was proved to be antiproliferative against different cancer cells,^[13,14] whereas it was able to reduce the levels of lipid peroxidation, and DNA damage in UVB-irradiated lymphocytes, preserving normal cells antioxidant status.^[15] Thus, in order to preliminarily assess biocompatibility of the synthesized materials, mouse embryonic fibroblasts (NIH-3T3 cell line) were employed, as they are commonly considered a suitable model for in vitro studies.^[16] MTT test was carried out after direct exposure of cells to 0.5 mg of hybrid materials for 48 h, as previously reported.^[12] Results were expressed as cell viability percentage and demonstrated a weak cytotoxicity (never exceeding 20%) or even absent. Furthermore, cell images, acquired with a phase-contrast microscope, highlighted their ability to grow in the presence of materials (**Figure 3**).

4. Conclusion

Embedding antioxidant natural compounds augments the versatility of benefits that could be provided by new materials for different purposes. Herein, sol-gel route proved once again to be a successful strategy in the synthesis of silica-based hybrid materials, and the use of CA as organic component is prompt to realize a material that is able to preserve the antiradical properties, showing also a good cytocompatibility towards fibroblast cells. In particular, the antiradical capability of the new materials appeared to be strictly related to the CA rate, whereas the augmentation of the CA percentage in hybrids, favored a clear increase in biocompatibility towards fibroblast cells.

Conflict of Interest

The authors declare no conflict of interest.

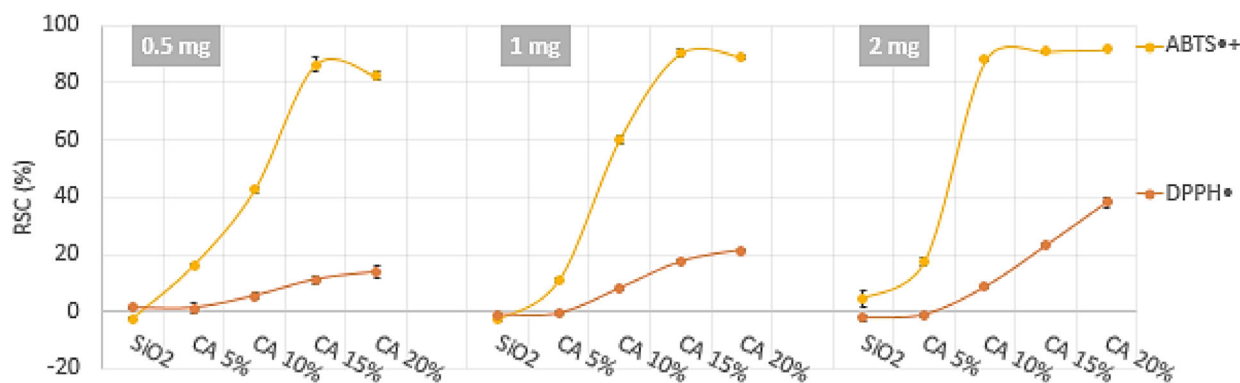


Figure 2. Radical scavenging capacity (RSC %) by DPPH and ABTS tests. All the chemicals and solvents for antiradical assays were from Sigma-Aldrich (Buchs, Switzerland). The absorbances at 515 nm (DPPH test) and 734 nm (ABTS test) were measured in reference to a blank, using a Perkin-Elmer Victor³ multi-label reader (Shelton, USA).

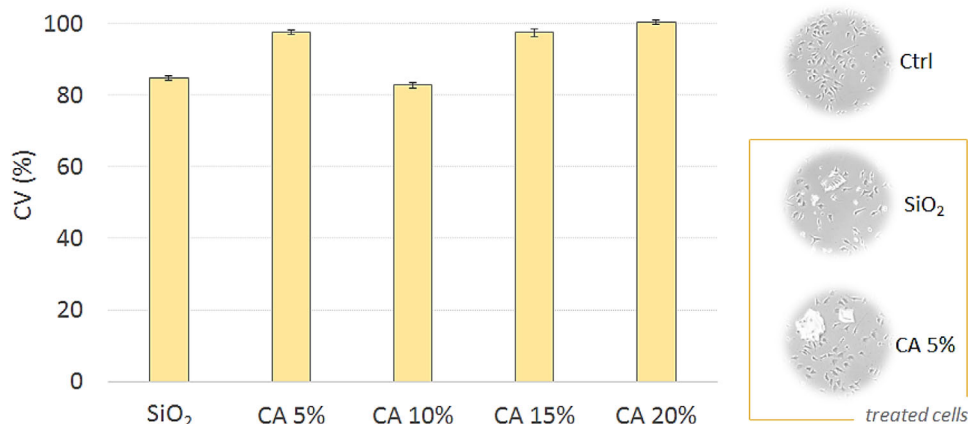


Figure 3. Cell viability (CV %) of NIH-3T3 cell line treated with pure silica and hybrid materials (0.5 mg each) after 48-h exposure time by means of MTT data. MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was from Sigma-Aldrich (Buchs, Switzerland), and cell culture media were obtained from Invitrogen (Paisley, Scotland, UK). Representative images of untreated/treated cells, acquired with a phase-contrast microscope (Inverted Phase Contrast Brightfield Zeiss Primo Vert Microscope, Carl Zeiss Microscopy, NY, USA) are also shown.

Keywords

antiradical capability, biocompatibility, caffeic acid, fibroblast cells, sol-gel route

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