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Sol-Gel Synthesis and Antibacterial Characterization of Bioactive Ferrous Citrate-Silica Hybrid Materials

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The aim of this work is the synthesis of therapeutic systems, iron (II) based, by sol-gel method. In a SiO_2 matrix, different weight percentages of ferrous citrate (Fe(II)C are embedded. Fourier Transform Infrared (FTIR) spectroscopy is used to study the interactions among different components in the hybrid materials. The bioactivity of the synthesized hybrid materials is evaluated by the formation of a layer of hydroxyapatite on the surface of samples soaked in SBF using FTIR spectroscopy. Finally, also, the potential antibacterial properties of the different materials against two different bacteria, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, are investigated.

1. Introduction

Iron is the transition element most abundant in the human body. It is involved in the process of the oxygen utilization and it is the component of several oxidase and oxygenase enzymes. Several ways have been developed by human body to conserve iron; in fact, it is strongly regulated by different molecular mechanisms, avoiding its accumulation to a toxic level or its deficiency.[1] The iron deficiency is considered the main cause of anemia worldwide, [2] and it is a major health problem that regard maternal and child mortality.[3] The use of oral iron supplementation for the treatment of the iron-deficiency is usually recommended, such as ferrous sulphate, anhydrous ferrous sulphate ferrous gluconate, and ferrous fumarate,[3] nevertheless the diagnosis and treatment of this disease could clearly be improved. In fact, oral iron supplementation is the treatment of choice for the majority of the patients because of its effectiveness and safety; the oral iron has disadvantages, including poor compliance, high incidence of adverse gastrointestinal effects, and high potential for interactions with other treatments.[4,5] In the last decade, the production of new biological systems able to release iron (II) in a controlled manner are an interesting alternative compared to systemic therapy. Therefore, the aims of this work was the development of therapeutic systems consisting of a SiO_2 matrix embedding different weight percentage of ferrous citrate (Fe(II)C). The SiO_2 + Fe(II)C) hybrid materials were synthetized by sol-gel technique, a versatile method used to produce glasses, ceramics and organic/inorganic hybrid materials at low temperature.[5,6] Fourier Transform Infrared (FTIR) spectroscopy was used to study the interactions among different components in the hybrid materials. A very important aspect as regards the long-term use of biomaterials is the risk of opportunistic bacterial species proliferation on their surface, which can lead to the biofilms formation and consequently to the failure of the therapeutic purpose. In the worst cases, these types of infections can lead to the patient's death. In this type of infection, there is an excessive stimulation of the host's immune defenses, with the formation of granular and fibrous tissue, caused by a strong inflammatory reaction localized in the site of the foreign body.[7] It is for this reason that these biomaterials must also be tested with regard to their antimicrobial capacities. In the present work, the potential antibacterial activity of the different materials has been tested on *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, respectively gram positive and gram negative, which are very often implicated in the pathogenesis of infections of implants with biofilm formation.[8,9]

2. Results and Discussion

Identification of the precipitate formed by the redox reaction between iron powder and citric acid as Fe(II)C, information on the structural organization of the synthesized materials, and confirmation of the presence of Fe(II)C in the silica matrix were obtained by FTIR spectroscopy. The FTIR spectrum of the precipitate contains all the typical bands of the citrate salts.[10] Moreover, all the typical bands of the silica sol-gel materials[10] are visible in the FTIR spectra of the hybrids SiO_2/Fe(II)C such as the strong band at 1080 cm^{-1} with a shoulder at 1200 cm^{-1}, as well as those at 800 and 465 cm^{-1} ascribable to asymmetric and symmetric Si-O-Si stretching and bending vibrations, respectively. The low-intensity band at 560 cm^{-1} was attributed to residual four-membered siloxane rings in the silica network.[10]

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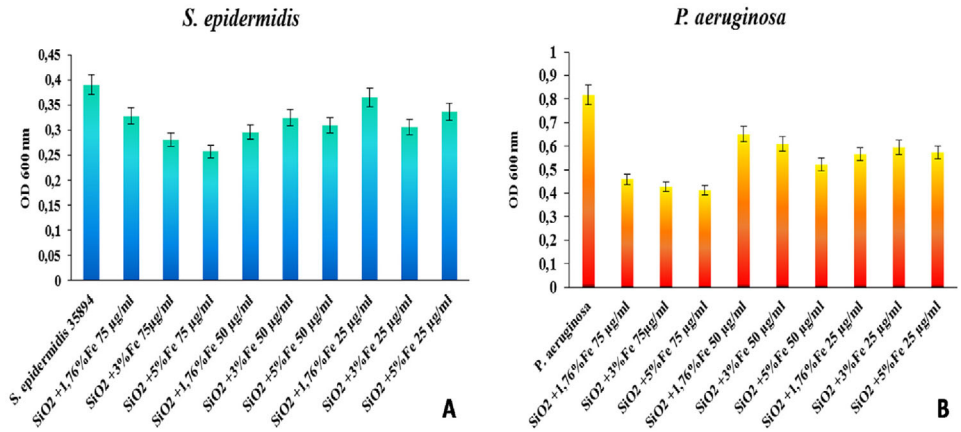

Figure 1. Optical density of A) *S. epidermidis* and B) *P. aeruginosa* after incubation with different concentrations of SiO_2/Fe(II)C for 24 h at 37°C. The data shown are representative of three different experiments ± S.D.

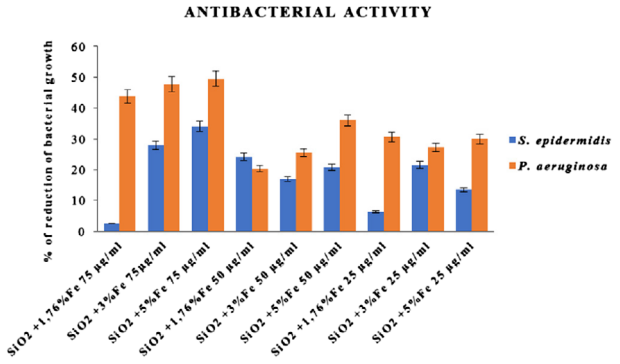

Figure 2. Percentage of growth reduction of *S. epidermidis* and *P. aeruginosa* after incubation with different concentrations of SiO_2/Fe(II)C for 24 h at 37°C. The data shown are representative of three different experiments ± S.D.

Furthermore, the bands in the SiO_2/Fe(II)C spectra related to Si-OH bond vibrations[10] and O-H stretching[10] appear slightly shifted toward lower wavenumbers compared to those of pure SiO_2 (from 960 to 955 cm^{-1} and from 3450 to 3425 cm^{-1}, respectively). This result suggests the formation of interactions between Fe(II)C and the inorganic matrix. Furthermore, when a high amount of Fe(II)C is present in the material, some bands related to citrate are visible as shoulder with respect to the silica signals. A preliminary evaluation of hydroxyapatite formation in the materials' surface was carried out by FTIR analysis. In the spectra recorded after SBF exposure 21 days, the samples show a doublet signal at 600 and 560 cm^{-1} and the band at 640 cm^{-1}, typical of the hydroxyapatite PO_4^{3-} groups.[10–12]

2.1. Antibacterial Properties

The results obtained indicate, as shown in **Figure 1** (A) and (B) and in **Figure 2** that the combination of SiO_2/Fe(II)C induces a reduction of bacterial growth at all tested concentrations, both for of *S. epidermidis* and *P. aeruginosa*. This inhibition is much more marked in both cases at the concentration SiO_2+ 5% Fe (II) C 75 μg mL^{-1}, and especially on *P. aeruginosa*.

3. Conclusions

In the present study, different amounts of pure Fe(II)C obtained were incorporated in a silica matrix by a sol-gel process to obtain therapeutic systems to use in iron-deficiency anemia therapy. FTIR investigation proved that Fe(II)C is embedded in the matrix and interacts with it, bioactivity test proves that the presence of Fe(II)C does not affect the bioactivity of the silica matrix. Antibacterial activity assay has shown that the combination SiO_2/Fe(II)C is active on the battery growth of both *S. epidermidis* and *P. aeruginosa,* although more effectively on *P. aeruginosa*. This behavior can be attributed to a greater affinity of the material towards the external membrane of the Gram-negative bacteria, absent in the structure of Gram-positive.[13]

4. Experimental Section

Sol-Gel Synthesis of the Hybrid Materials: The pure inorganic SiO_2 and the hybrids, consisting of the SiO_2 matrix in which 1.76%, 3% e 5%wt% of ferrous citrate was embedded (SiO_2+Fe(II)C), were synthesized by means of the sol-gel process. The inorganic silica gel was obtained by adding tetraethyl orthosilicate (TEOS; $Si(OC_2H_5)_4$; Sigma-Aldrich) to a solution of distilled water and 99.8% ethanol (EtOH, Sigma-Aldrich). Molar ratios equal to H_2O/TEOS = 23.5 and EtOH/TEOS = 6.2 were used in this study. As far as the synthesis of the hybrid materials, different amount of Fe(II)C were dissolved in distilled water under stirring at 38°C. After gelation, the wet gels were dried in an oven at 50°C for 24h to remove the residual solvent.

A Prestige 21 Shimadzu (Japan) FTIR instrument equipped with a deuterated tryglycine sulphate (with potassium bromide windows detector) was used to record FTIR transmittance spectra of all samples in the 400–4000 cm^{-1} region, with resolution of 4 cm^{-1} (45 scans). KBr pelletized disks containing 2 mg of each sample and 198 mg of KBr were made. The FTIR spectra were processed by Prestige software (IR solution).

The in vivo bioactivity test was carried out on synthesized hybrid materials by soaking the samples for 21 days in a simulated body fluid (SBF) solution. The dried gels were grinded in a mortar to obtain powders. A part of those powders was pressed by a hydraulic press (Specac, England) to obtain disks with diameter of 13 mm and thickness of 2 mm. The disks were soaked in SBF within polystyrene bottles and placed in a water bath at 37.0 ± 0.5°C. The solution was replaced every 2 days to avoid depletion of the ionic species in the SBF due to the nucleation of biominerals on the

samples. As the ratio between the total exposed surface and the volume solution influences the nucleation reaction, a constant ratio was chosen in agreement with the literature.[14] After 21 days of exposure, the disks were gently rinsed and dried in a glass desiccator. Afterwards, the sample powders (2 mg) were mixed with KBr (198 mg), compressed to make tablets and subjected to FTIR analysis.

Bacterial Strains and Culture media: *S. epidermidis* (ATCC® 35983) was cultured on Tryptic-soy broth (TSB – Difco Laboratories). *P. aeruginosa* (ATCC® 27853) was cultured on Luria-Bertani (Oxoid; Unipath, Basingstoke, UK). These strains were grown at 37°C for 18h.

Antibacterial Activity Assay: The SiO_2 powders added with different iron percentages (1.76%, 3%, and 5%) were dissolved in water and tested at three different final concentrations: 75 , 50 , and 25 μg mL^{-1}. The bacterial cultures prepared as previously described have been diluted up to 10×10^5 CFU mL^{-1}. The substances at different concentrations are added to the bacterial suspensions in a 96-well plates and then incubated at 37°C for 24 h.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

antibacterial properties, ferrous citrate, sol-gel method

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