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Biocompatible silica-gelatin hybrid aerogels covalently labeled with fluorescein

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ABSTRACT

Silica-gelatin hybrid aerogels, introduced recently as drug delivery devices, were investigated for biocompatibility with SCC tumor cells by a time-lapse video-microscopy technique. The particles proved to be not only non-toxic in nature, but the cells migrated towards them. Fluorescently labeled hybrid aerogels were synthesized by covalently binding fluorescein isothiocyanate either to the amino groups of gelatin molecules, or to the amine-functionalized silica backbone prior to sol-gel polycondensation and co-gelation steps. Scanning electron microscopy and fluorescence spectroscopy characterization of pristine and labeled aerogels showed identical structures and spectra, while fluorescence lifetimes were 10% shorter than that of the free fluorescein, due to covalent attachment. Migration plots based on time-lapse video recordings showed that the cells exhibited a directive migration nearby the aerogel particles. No signs of apoptosis or necrosis were observed at any phases of the cell cycle in the presence of the silica-gelatin aerogel particles, indicating that the hybrid aerogels are biocompatible materials, and may be used as drug delivery matrices in living organisms.

1. Introduction

Aerogels are excellent candidates for developing drug-delivery systems (DDS) due to their extraordinary properties, such as their huge specific surface area, open mesoporous structure and easily tunable surface characteristics [1]. Numerous papers have been published related to the pharmaceutical applications of aerogels. It was shown that with the help of these porous matrices both immediate and sustained drug release is feasible [2–4].

The lack of the biodegradability of silica aerogel raised an ever-growing interest in fully biobased and hybrid materials in order to broaden the area of use and ensure safe applications [5–7]. Along with this, new delivery routes came into view. Besides oral and nasal delivery, subcutaneous, intraperitoneal and many other administration routes gained significance. Using aerogels as DDS in these routes also requires the monitoring of the particles in the living system. Tracking the movement and possible accumulation of aerogel particles in different organs are crucial to ensure their safe usability and to gather more information for developing targeted delivery. The most convenient and the most widely used monitoring technique in animal studies is fluorescence imaging [8,9]. Some fluorescent labeling and doping strategies targeting aerogels were investigated before, but most of them are not suitable for biological applications. Several reports can be found on the fluorescence properties of lanthanide-doped silica or silica-alumina/titania aerogels, but the preparation of those types of aerogels are not feasible for the synthesis of hybrid or fully biobased materials, because the procedure requires high temperature heat treatment [10–12]. Entrapment of fluorescein in silica matrices was also thoroughly investigated, yet the entrapped fluorescein is easily removed by soaking the labeled matrix in water, which is undesirable in biological applications. The explanation is that only secondary bonds (e.g. dispersion forces, H-bonds) bind the dye in the aerogel matrix, which can easily break during the hydration of the dye and the matrix [13].

In this study we focus on silica-gelatin hybrid aerogel which is an excellent DDS candidate [7,14]. For biocompatibility investigations, a potential method is the in vitro time-lapse video-microscopy, which enables long-term dynamical observations [15]. The well characterized murine SCC cell line (SCC VII) was chosen for the in vitro studies [16–18]. This squamous carcinoma cell line arose spontaneously and was isolated from the C3H mouse abdominal wall [19]. SCC VII cells have the ability to penetrate Matrigel basement membrane model due to their collagenase activity [20]. The collagen content of the silica-gelatin aerogel particle can be an appealing signal for the tumor cells

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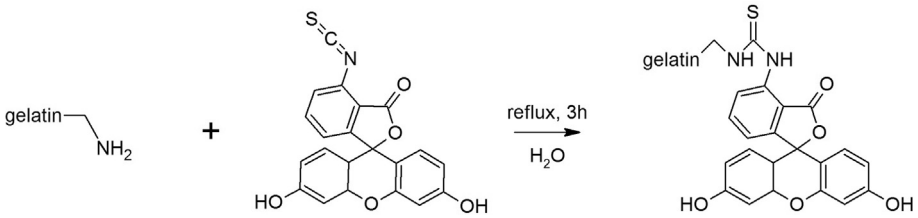


Scheme 1. The chemical reaction leading to the formation of the labeled gelatin. The free $-NH_2$ groups of the peptide chains directly react with fluorescein isothiocyanate (FTIC).

[21]. Accordingly, the silica-gelatin hybrid might be successfully developed into a carrier of anti-tumor drugs. After proving by in vitro tests, that this material is biocompatible, it was used as a model to develop a robust fluorescence labeling technique for silica based aerogels for biological applications. A good labeling technique should not alter either the structure of the aerogel matrix or the fluorescence properties of the dye applied in order to obtain representative results in subsequent biological experiments. To reach this goal we investigated the feasibility of two types of strategies for the covalent attachment of fluorescein to the backbone of hybrid silica-gelatin aerogel. After studying the effect of the modification on the structural properties of the aerogel, we examined the steady state spectrum and the fluorescence lifetime of the immobilized fluorescein.

2. Materials and methods

2.1. Chemicals

Tetramethyl orthosilicate (TMOS) and household gelatin were obtained from Fluka and Dr. Oetker, respectively. Methanol and acetone (both 99.99%) were purchased from Molar Chemicals (Hungary). $(NH_4)_2CO_3$ was provided by Merck. Fluorescein sodium salt, fluorescein 5(6)-isothiocyanate and (3-aminopropyl)trimethoxy silane were purchased from Sigma Aldrich. Supercritical CO_2 was produced from min. 99.5% pure gas (Linde). All aqueous solutions were prepared with Milli-Q water (Millipore). Other chemicals (HCl, NaOH, NaH_2PO_4, Na_2HPO_4) were ACS reagent grade (Sigma-Aldrich).

2.2. Aerogel preparation

The sol-gel synthesis of silica-gelatin hybrid aerogels of different compositions is given in our previous publications [7,14]. These recipes were not modified for producing samples for the in vitro biological experiments.

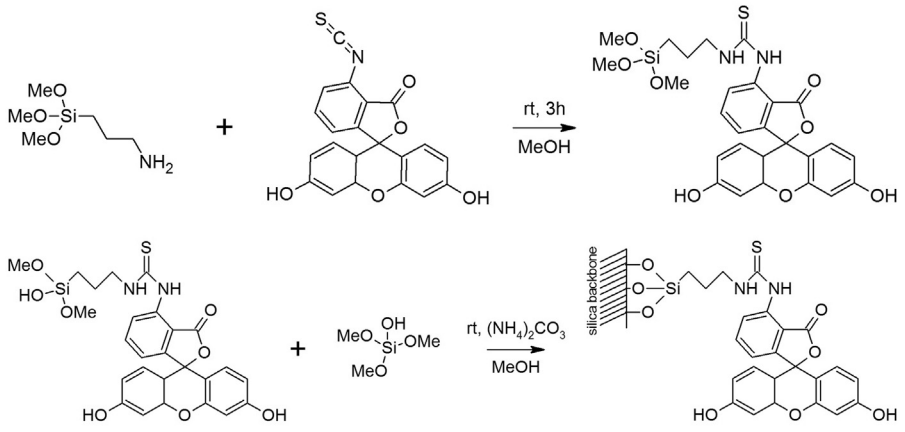
In order to prepare hybrid silica-gelatin aerogels covalently labeled with fluorescein, previously published labeling strategies [9,22] were adapted. Using these methods, both gelatin and silica can be labeled via linking fluorescein moieties to them with covalent binding. Two different synthetic strategies were implemented. First, gelatin was modified before the sol-gel synthesis of the hybrid backbone. Second, the hybrid backbone was homogeneously labeled during the sol-gel synthesis and before the supercritical drying.

The first route is the following. Proteins (e.g. gelatin) can be covalently labeled with fluorescein via a simple reaction. 20.00 g aqueous solution containing 0.10 g gelatin was mixed with 1.8 mg fluorescein isothiocyanate (FITC) under moderate stirring and reflux for 3 h. After this, 70.0 mg solid ammonium carbonate was dissolved in the mixture, as the catalyst for the upcoming sol-gel synthesis. A second solution of 3.00 mL tetramethyl orthosilicate (TMOS) in 7.00 mL methanol was also prepared. After cooling the aqueous solution near to room temperature, the second solution was added under vigorous stirring. Then the mixture was poured into a cylindrical plastic mold for gelation. Excess FITC does not react with silica moieties under these conditions, thus only gelatin is labeled during the first process.

The second strategy is the following. In order to covalently link fluorescein to the silica backbone, a two-step synthesis route was implemented. First 10.0 mg FITC and 16.25 µL (3-aminopropyl)trimethoxy silane (APTMS) was mixed at room temperature for 3 h in 7.00 mL methanol in order to attach fluorescein to the silane reagent, which will later incorporate into the silica backbone during gelation. Next, 3.00 mL TMOS was introduced into the mixture. A second aqueous solution (20.00 g) containing 0.10 g gelatin and 70.0 mg $(NH_4)_2CO_3$ was also prepared. These two solutions were mixed under intense stirring and were poured into a plastic mold for gelation. In this case, the unreacted FITC from the first solution reacts with the gelatin introduced in the second solution. Thus, a higher fluorescein content and the homogenous labeling of the hybrid backbone can be reached in the second process.

The chemical reaction leading to the production of labeled gelatin is given in Scheme 1. Scheme 2 details the chemical reactions leading to the formation of the labeled silica backbone.

The last steps of preparing aerogels from the alcogels are the same for the two processes. After 24 h, the alcogels were removed from the mold and placed into a perforated aluminum container for multiple step solvent exchange. First, the samples were soaked in methanol for 24 h to remove water. Next, methanol was replaced by acetone in four 24 h soaking steps, and acetone was replaced 2 more times after 24 h soaking. Finally, acetone was extracted with supercritical CO_2 at



Scheme 2. The chemical reaction steps leading to the formation of the labeled silica backbone. The first process is the reaction of fluorescein isothiocyanate (FTIC) with (3-aminopropyl)-trimethoxy silane (APTMS). The second process is the co-hydrolysis of the labeled product with trimethoxy silane (TMOS). TMOS does not react directly with FTIC.