Materials Science and Engineering C 63 (2016) 46–51

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Short communication

A new biocompatible nanocomposite as a promising constituent of sunscreens

Rehab M. Amin [a,*], Souad A. Elfeky [a,b], Thomas Verwanger [c], Barbara Krammer [c]

[a] Department of Laser Applications in Photochemistry, National Institute of Laser Enhanced Sciences, Cairo University, Egypt
[b] University of Bath, Department of Chemistry, Bath BA2 7AY, UK
[c] Department of Molecular Biology, University of Salzburg, Hellbrunnerstr. 34, 5020 Salzburg, Austria

ARTICLE INFO

Article history:
Received 27 October 2015
Received in revised form 9 February 2016
Accepted 15 February 2016
Available online 18 February 2016

Keywords:
Nanocomposite
Hydroxyapatite
Ascorbic acid
Sunscreens
Dermal fibroblasts
Keratinocytes

ABSTRACT

Skin naturally uses antioxidants to protect itself from the damaging effects of sunlight. If this is not sufficient, other measures have to be taken. Like this, hydroxyapatite has the potential to be applied as an active constituent of sunscreens since calcium phosphate absorbs in the ultraviolet region (UV). The objective of the present work was to synthesize a hydroxyapatite–ascorbic acid nanocomposite (HAp/AA-NC) as a new biocompatible constituent of sunscreens and to test its efficiency with skin cell models.

The synthesized HAp/AA-NC was characterized by Fourier transform infrared spectroscopy, transmission electron microscopy, absorption spectrophotometry and X-ray diffraction analysis. The protective effect of the construct was tested with respect to viability and intracellular reactive oxygen species (ROS) generation of primary human dermal fibroblasts (SKIN) and human epidermal keratinocytes (HaCaT). Both cell lines were irradiated with UV light, $\lambda_{max} = 254$ nm with a fluence of 25 mJ cm^{-2} to mimic the effect of UV radiation of sunlight on the skin. Results showed that HAp/AA-NC had a stimulating effect on the cell viability of both, HaCaT and SKIN cells, relative to the irradiated control. Intracellular ROS significantly decreased in UV irradiated cells when treated with HAp/AA-NC.

We conclude that the synthesized HAp/AA-NC have been validated *in vitro* as a skin protector against the harmful effect of UV-induced ROS.

1. Introduction

Concerns about skin cancer have been growing more and more [1]. The sun emits a wide spectrum of electromagnetic waves and ultraviolet (UV) light belongs to the most aggressive radiation for human tissues. Damaging effects of UV radiation on the skin are due to the generation of reactive oxygen species (ROS), which cause oxidation of nucleic acids, proteins, and lipids resulting in photoaging [2] and photocarcinogenesis [3].

The skin naturally uses antioxidants such as different vitamins to protect itself from the damaging effects of sunlight. If this is not sufficient, other protective measures have to be taken, such as the application of sunscreens.

Titanium dioxide (TiO_2) and zinc oxide (ZnO) are frequently used in sunscreens as inorganic, physical sun blockers because of their ability to filter UV radiation [4]. However, to solve the cosmetic drawback of these opaque sunscreens, microsized TiO_2 and ZnO have been increasingly replaced by TiO_2 and ZnO nanoparticles [5]. Titanium dioxide and zinc oxide are incorporated into sunscreens in the form of nanoparticles to optimize material delivery into the outer skin layers [6].

Sunscreens absorb or reflect the incident UV radiation protecting the individual [7]. However, many sunscreens penetrate to deeper skin layers causing photo-allergies, phototoxic reactions and skin irritations [8]. Therefore, there is an urgent need for developing a safer but still effective sunscreen. This can be achieved by using biocompatible materials with superficial skin penetration at most. Calcium phosphates were already investigated providing many possible applications in medicine [9], and, due to their chemical similarity with the mineral phase of bones and teeth, they showed no toxicity [10]. Since calcium phosphate absorbs in the UV region, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) particles may have the potential to be applied as active constituents of advanced sunscreens [7,11]. Moreover, hydroxyapatite as physical sunscreen agent has screening capability, high dermal tolerance and a lower whitening effect than other physical sunscreen agents [12].

Incorporation of antioxidants to the UV absorber nanoparticles could provide additional benefit by scavenging free radicals [13]. Previously, it was reported that L-ascorbic acid applied topically to the skin reduces the photo injury produced by both UVB (290–320 nm) and UVA (320–400 nm) irradiation [14,15]. Combining vitamin C with sunscreens is important to maximize photo protection against UV damage [16].

* Corresponding author at: National Institute of Laser Enhanced Sciences, Cairo University, Egypt.
E-mail address: rehabamin@niles.edu.eg (R.M. Amin).

http://dx.doi.org/10.1016/j.msec.2016.02.044
0928-4931/

Ascorbic acid (vitamin C) has been widely used in the pharmaceutical, chemical, cosmetic and food industries because of its bioactive and antioxidant properties. This substance promotes collagen biosynthesis, provides photo-protection and scavenges free radicals [17]. The problem is that ascorbic acid easily decomposes into biologically inactive compounds making its use very limited in the above mentioned fields [18].

In order to overcome the chemical instability of ascorbic acid numerous studies were carried out concerning its encapsulation [19,20].

Nanoparticles are used for a number of biomedical applications. Nanosized hydroxyapatite (HAp) enhances bioavailability, therapeutic response, efficacy, safety, and prolonged release of a drug when applied as a carrier for drug delivery [21,22].

Polyvinylpyrrolidone (PVP) is used in targeted drug delivery systems as a binder, stabilizer or solubility enhancer because it is generally considered to be non-toxic and non-immunogenic. It may be coated on the nanoparticle surface to disperse it. PVP impedes nanoparticle aggregation, prevents the particle surface from oxidation and makes it more biocompatible [22].

Therefore the aim of this study was the synthesis of nanosized HAp modified by ascorbic acid (AA) and stabilized with PVP. HAp was combined with the antioxidant drug AA, then dispersed and stabilized with PVP. The characterization of the prepared composite was performed by Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), absorption spectroscopy and X-ray diffraction (XRD). The effect of the construct was tested with respect to viability of skin cell models and to ROS generation.

2. Material & methods

2.1. Synthesis and characterization of hydroxyapatite–ascorbic acid nanocomposite (HAp/AA-NC)

Preparation of HAp/AA-NC was carried out as follows: an aqueous solution of H_3PO_4 (Sigma Aldrich, USA, purity 85%) (0.6 M, 25 ml) was added into an aqueous suspension of $Ca(OH)_2$ (Acros Organics, USA, purity 98%) (0.025 M, 25 ml) including ascorbic acid (0.025 M) (Alfa Aesar, USA, purity, 99%) to make a solution (1) [23]. Dispersion or stabilization of HAp/AA-NC was done directly by PVP (VWR Chemicals, USA). Typically, 20 mg of PVP was added into double distilled water to form a 2.0 mg ml^{-1} aqueous solution, which was added into solution (1) followed by 30 min ultra sonication. The obtained HAp/AA-NC mixture was then stirred for 18 h [24]. Afterwards the dispersion was then centrifuged for 20 min at 5000 rpm, the solid phase was rinsed with double-distilled water and dried in air for 24 h.

2.2. Characterization of hydroxyapatite–ascorbic acid nanocomposite

Shape and size of the prepared particles were determined by TEM and selected area diffraction pattern (SAED) studies. Analyses were performed using a JEOL2010F transmission electron microscope (JEOL, Germany) with a field-emission gun operating at 120 kV. A drop of strongly diluted sample solution was deposited on an amorphous carbon–copper grid and left to evaporate at room temperature.

Absorption spectra were recorded with a Perkin-Elmer Lambda 40 spectrometer (Perkin-Elmer, USA) using quartz cuvettes (3.5 ml) containing 3 ml of the sample that was dissolved in distilled water.

FTIR measurements have been acquired at room temperature using FT/IR-4600 spectrometer (JASCO, Japan). The samples were prepared by mixing the pure hydroxyapatite–ascorbic acid nanocomposite powder with potassium bromide and then pressing the mixture into transparent disks for FTIR measurement. The spectral resolution was 4 cm^{-1} and the number of scans was 64.

The crystal structure of the synthesized HAp/AA-NC was established by powder XRD analysis. XRD was done using the X-ray diffractometer system X'Pert^{3}Powder (PANalytical, The Netherlands) at room temperature (25 °C) with continuous scan type. The X-ray anode source utilized was Cu target (Cu Kα1) with settings of 30 kV and 30 MA.

2.3. Cell cultures

Primary human dermal fibroblasts (SKIN) and human epidermal keratinocytes (HaCaT) were used as cell models. All cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, Austria) supplemented with 10 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl] ethane sulfonic acid (HEPES), 4 mM L-glutamine, 1 mM Na-pyruvate, 100 U ml^{-1} penicillin, 0.1 mg ml^{-1} streptomycin and 10% fetal calf serum (FCS) (all from PAA Laboratories, Austria), in a humid atmosphere at 37 °C and 7.5% CO_2. For experimental purposes, cells were cultured in 96-well plates (100 μl of cell suspension per well). Cells were allowed to attach for 24 h before treatment.

2.4. Cytotoxicity of hydroxyapatite–ascorbic acid nanocomposite

Cell monolayers were washed with PBS and HAp/AA-NC was applied in growth media in different concentrations. Cytotoxicity was studied using concentrations ranging from 10 up to 50 μM of the compound. After 24 h the nanocomposite was removed by washing the cells with PBS and incubating them in growth medium for further 24 h. Then, cell proliferation was determined by using the MTT assay.

2.5. MTT assay

Mitochondrial dehydrogenase activity is an indication for the metabolic and therefore vital state of cells. MTT assay was performed as described previously [25], but slightly modified. Briefly, cells were incubated for 45 min with 0.5 mg ml^{-1} MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide, Sigma-Aldrich, Austria) added to the culture medium. After that, the supernatant was aspirated and the resultant formazan was dissolved by addition of 100 μl DMSO–glycine (6 vol. dimethylsulfoxide + 1 vol. 100 mM glycine, pH 10, both Sigma-Aldrich, Austria) per well. Absorbance was read at 565 nm on a Spectrafluor microplate reader (Tecan, Austria). All assays were carried out in triplicate. After subtraction of the blank values the mean values and standard deviations were calculated. Data are presented as percent activity of untreated control cells.

2.6. UV irradiation of cells

After 24 h incubation with HAp/AA-NC, UV irradiation was carried out on both cell lines. HAp/AA-NC was removed by washing the cells with PBS and new DMEM media was added. Then cells were irradiated with an UV illuminator (Stratalinker 2400, Stratagene, USA; λ_{max} = 254 nm) with a fluence of 25 mJ cm^{-2}. 24 h later the MTT assay was performed as described above.

2.7. Measurement of intracellular ROS

The measurement of intracellular ROS is based on the oxidation of carboxy-H_2-DCF-DA (2′,7′-dichlorodihydrofluorescein diacetate), which is quantified by flow cytometry. Both cell lines were incubated with different concentrations of HAp/AA-NC for 23 h, before 50 μM carboxy-H_2-DCF-DA (Life Tech., Austria) was applied for 1 h. Subsequently, cells were UV irradiated with 25 mJ cm^{-2}. Then, cells including the supernatant were detached using accutase (PAA Laboratories, Austria); all following steps were performed on ice. The cells were spun down at 840 ×g and washed once with PBS; after another centrifugation step, the pellet was resuspended in 500 μl PBS and the resulting green fluorescing DCF (2′-7′-dichlorofluorescein) was analyzed by flow cytometry (FACSCanto II, Becton Dickinson, USA). For each sample, the green fluorescent signal of DCF in 10,000 events was recorded.