Process Biochemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

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

Cytotoxicity of methylcellulose-based films containing graphenes and curcumin on human lung fibroblasts

Marcela-Corina Roşu[a,*], Emöke Páll[b], Crina Socaci[a], Lidia Măgeruşan[a], Florina Pogăcean[a], Maria Coroş[a], Alexandru Turza[a], Stela Pruneanu[a]

[a] National Institute for Research and Development of Isotopic and Molecular Technologies, 67-103 Donat Street, 400293 Cluj-Napoca, Romania
[b] University of Agricultural Sciences and Veterinary Medicine, 3-5 Mănăştur Street, 400372 Cluj-Napoca, Romania

ARTICLE INFO

Article history:
Received 24 May 2016
Received in revised form
30 September 2016
Accepted 3 October 2016
Available online xxx

Keywords:
Methylcellulose
Graphene oxide
Reduced graphene oxide
Curcumin
Human lung fibroblasts
Cell viability

ABSTRACT

The present study focused on the preparation, physico-chemical, and biological characterization of several composite films containing graphene derivatives (graphene oxide or reduced graphene oxide) and curcumin as antioxidant in methylcellulose matrix. The morphostructural properties of the obtained composites were investigated by scanning electron microscopy (SEM), atomic force microscopy (AFM), Fourier transformed infrared spectroscopy (FTIR), and X-ray powder diffraction (XRD). The biological effects of composites were evaluated using the Cell Counting Kit-8 assay for cell viability and cytotoxicity determination on human lung fibroblast (HFL-1) cell line. Interestingly, the composite films containing graphene oxide showed higher cytotoxicity than those with reduced graphene oxide. In addition, the exposure of HFL-1 cell line to composites containing curcumin showed that the antioxidant caused a concentration-dependent reduction in the number of cells. The components and the physico-chemical properties of the composites create favorable environmental conditions for HFL-1 cell survival and provide bioactive substrates for HFL-1 cell growth.

1. Introduction

Tissue engineering, like many other biomedical fields, has experienced a spectacular progress, and its rapid expansion continues. One of the current efforts in tissue engineering focuses on the synthesis of novel biomaterials and their biological performance evaluation that includes cell adhesion, proliferation, and differentiation. On the basis of designing criteria related to the structure–property–performance relationship, a wide variety of polymeric materials from natural sources such as polysaccharides (alginate, cellulose, and chitosan) and proteins (collagen, keratin, and silk) or synthetic sources such as poly(lactic acid), poly(glycolic acid), and poly(-caprolactone) have been considered as scaffolds [1,2]. The most abundant natural biopolymers, namely cellulose and cellulose derivatives such as methylcellulose (MC), hydroxypropylmethyl cellulose (HPMC), hydroxyethyl cellulose (HEC), and carboxymethylcellulose (CMC), are used in various industrial and biomedical applications [3,4]. As an amphiphilic polymer, MC is used as a binder for different materials as it is nontoxic and nonallergenic. It is found as an additive in food (E461); cosmetics and personal care products (hair and body products and toothpastes); pharmaceuticals and tissue engineering scaffolds; ceramics and construction materials; pesticides and fertilizer powders; adhesives; and in the production of papers and textiles [4]. The combination of biocompatible/biodegradable polymers and nanomaterials is a great challenge to develop new composites with enhanced characteristics (good dispersion, strong interaction with the polymer matrix, and superior physical/mechanical properties) and improved performance for the intended purpose [5–9]. Carbon-based nanomaterials (e.g., graphene, carbon nanotubes, and nanodiamonds) have been already used for energy, environment, and electronics as well as for biomedical fields [10–12]. Graphene, a two-dimensional structure composed of sp^2-bonded carbon atoms arranged in a honeycomb network, shows excellent electronic, optical, thermal, and mechanical characteristics, which promote it as a promising material for various applications such as nanoelectronics, sensors, energy storage, and nanocomposites [10,13,14]. The exploitation of physico-chemical and biological properties of graphene for biomedical applications is relatively new, although it covers a wide range of topics including photother-

* Corresponding author.
E-mail address: marcela.rosu@itim-cj.ro (M.-C. Roşu).

http://dx.doi.org/10.1016/j.procbio.2016.10.002
1359-5113/

mal therapies, stem cell/tissue engineering, drug/gene delivery, biosensing, and bioimaging [13,14–16]. The literature reported different studies related to biological effects of graphene and its derivatives on various cell types, for example, on myoblasts [17], osteoblasts [18], neuronal, and stem cells [19]. To date, the experimental data regarding the biological effects of graphene and its derivatives on fibroblasts are inconsistent [20–22]. As connective tissue cells, fibroblasts play a key role in the support and repair processes of damaged tissue and their controlled accumulation at the injury sites is essential for tissue regeneration [23,24]. Therefore, the present study investigates the ability of graphene oxide (GO) and reduced graphene oxide (GR) embedded in MC matrix to act as bioactive substrates in the survival of human lung fibroblasts (HFL-1). The second aim is to assess the biological influence of curcumin (CR, a natural antioxidant) on fibroblast growth. CR, derived from the plant *Curcuma longa*, is known to have antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and anticancer properties [25–27]. Despite its proven therapeutic efficacy on different diseases, CR exhibits poor solubility and rapid degradation. Numerous materials were developed (nanoparticles and crystals, micelles, nanoemulsions or microemulsions, liposomes, cyclodextrins, dendrimers, etc.) to improve its biopharmaceutical properties such as absorption, distribution, metabolism, and excretion [28–32].

The present study aimed to obtain composite substrates based on biocompatible polymer (e.g., MC) containing graphene derivatives and CR antioxidant with suitable physico-chemical and biological properties that promote fibroblast cell–biomaterial interactions allowing cell survival.

2. Materials and methods

2.1. Materials

MC (Loba, Austria) was used as matrix for film formulations. CR was purchased from Alfa Aesar, Germany. Ethanol (Riedel-de Haen, Germany) and double distilled water were used as solvents. GO and GR were prepared in our laboratory.

2.2. Preparation of films

Polymer solution with a mass fraction of 2% was separately prepared by dissolving MC in ethanol:double distilled water (1:4 vol. ratio). GO (0.1 mg/ml) and GR (0.1 mg/ml) were dispersed in 20 ml of polymer solution. GO was synthesized from natural graphite powder (from a graphite rod, Pierce Eurochemie bv, Netherlands) using a modified Hummers' method [33]. Furthermore, GO was heated at 190 °C in argon atmosphere to obtain the GR. For comparison, similar formulations containing CR were prepared by adding CR (2.5 or 10 μM) to the initial ethanol:water mixture. All polymer-based suspensions were sonicated for 30 min. Subsequently, they were mechanically stirred at 150 rpm in a thermo bath (40 °C for 2 h) until complete dissolution and homogenization. The final viscous suspensions (2.5 ml) were poured into polystyrene Petri plates (5 cm diameter) and placed in an oven at 50 °C for drying (24 h). After cooling to room temperature, the MC-based films were removed from the plates. The samples were denoted according to their individual components: methyl-cellulose as control sample (MC); methyl-cellulose/graphene oxide (MC–GO); methyl-cellulose/reduced graphene oxide (MC–GR); methyl-cellulose/curcumin (MC–CR); methyl-cellulose/graphene oxide/curcumin (MC–GO–CR); and methyl-cellulose/reduced graphene oxide/curcumin (MC–GR–CR). The numerical marks indicate the molar concentrations of CR solutions that were used (2.5 and 10 μM), for example, MC–CR 2.5 and MC–CR 10.

2.3. Structural and morphological characterization of the films

The polymer-based films were characterized by infrared spectroscopy (FTIR), X-ray powder diffraction (XRD), scanning electron microscopy (SEM), and atomic force microscopy (AFM). FTIR analysis was performed with a JASCO FTIR-6100 (Japan) within a range of 400–4000 cm^{-1}. The XRD data were collected with a BRUKER D8 Advance X-ray powder diffractometer (Germany) in the 2θ scattering range of 5°–80° using CuKα radiation. SEM analysis was performed with an SU-8230 STEM instrument (Hitachi, Japan). The AFM images were acquired in the tapping mode using an NTEGRA Spectra scanning probe microscope (NT-MDT, Russia).

2.4. Biological assays

2.4.1. Cell lines

HFL-1 cells (ATCC® CCL-153™, passages 27; Romania) were cultured in HyClone DMEM/F12- (Sigma Aldrich, UK) as support for cell culture growth. The standard cell culture media were supplemented with additional components to sustain normal cell metabolism and protect them from bacterial and fungal contaminants: 10% (v/v) fetal bovine serum (HyClone), L-glutamine (2 mM), 1% nonessential amino acids (NEAs), and 1% antibiotics and antimycotics. The cell cultures were maintained in a humid atmosphere containing 5% CO_2 at 37 °C. The films were cut into 3 × 3 cm^2 strips and then sterilized with ethylene oxide (EtO) gas, before the cell seeding procedure. After ethylene oxide exposure for 72 h, the films were moved in 24-well plates. HFL-1 cells were seeded directly on the samples at a density of 2×10^4 cells/ml and incubated for 24 h. The controls were represented by HFL-1 cells cultivated without films, in the normal expansion medium.

2.4.2. Cytotoxicity assays

A sensitive colorimetric method, Cell Counting Kit-8 (CCK-8, Sigma-Aldrich, USA), was used for the determination of viable cell number. CCK-8 is based on the bioreduction of tetrazolium salt, WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) by cellular dehydrogenases, in the presence of an electron mediator (1-methoxy-PMS; Patent No. WO97/38985, Dojindo Laboratories Co. Ltd, Japan). The complex cellular mechanism occurs primarily at the surface and is mostly dependent on the generation of NADPH by the viable cells. Thus, the amount of WST-8 formazan product is directly correlated to the number of metabolically active cells in the culture. CCK-8 solution (10 μl) was added to each well, and the cell cultures were incubated for 3 h at 37 °C. The concentration of the formazan generated by dehydrogenases in cells was quantified using a microplate reader (Bio-Rad, Hercules, CA, USA) by measuring the absorbance at 450 nm. Cell viability (%) was expressed as a percentage to the control cells by using the following equation: cell viability (%) = OD test × 100/OD control. Statistical analysis was performed using the results from three experiments.

3. Results and discussion

3.1. Structural and morphological characterization of films

3.1.1. FTIR analysis

The characteristic vibrations in the infrared spectra of GO and GR reveal several differences (Fig. 1A). The FTIR spectrum of GO shows the characteristic absorption bands of oxygen-containing functional groups. Vibrational modes can be seen at 3428 cm^{-1}

Please cite this article in press as: M.-C. Roşu, et al., Cytotoxicity of methylcellulose-based films containing graphenes and curcumin on human lung fibroblasts, Process Biochem (2016), http://dx.doi.org/10.1016/j.procbio.2016.10.002