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Cytocompatibility and in vivo biodegradation of graphene-modified chitosan 3D porous scaffold

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ABSTRACT

Interconnected porous chitosan scaffolds coated by chemically modified graphene (such as graphene oxide, GO) film have previously been prepared. In the present study, the attachment and proliferation of L929 on the surface of the composite scaffolds were reported and their in vivo biocompatible and biodegradability were evaluated. L929 cell culture on GO-coated chitosan scaffold surface with lower GO content promoted cell viability, but at higher content (1.0 mg/mL) a slight cytotoxicity appeared. The biodegradation rate of the composite scaffolds exhibited a content dependent behavior, decreased of remaining area from pure chitosan scaffold to the composite of 1.0 mg/mL GO content. The results demonstrated the high potential applications of GO-coating in fields of tissue engineering and drug delivery.

1. Introduction

Chemically modified graphene, i.e. graphene oxide (GO) and reduced graphene oxide (rGO) consisting of a carbon monolayer has been intensively and widely investigated to form a composite or interact with biomolecules, cells and tissues because of their significant surface activity caused by oxygen-containing functional groups. Recent studies have indicated that GO nanosheets dispersing in chitosan (CS) solution outstandingly enhanced the mechanical properties of the composite materials due to the interfacial interaction between the oxygen-containing groups of GO sheets and amino groups of CS [1]. Furthermore, a synergistic effect between the nanofillers and pristine CS on biological functions makes the composite a promising candidate for applications in tissue engineering and drug delivery [2,3].

Biocompatibility and biodegradability of a biomaterial are the two crucial parameters for the successful applications both in biomedical systems and tissue engineering scaffolds. Chitosan, the deacetylated derivative of chitin, has been shown to degrade in vivo and the degradation rate of pure CS is dependent on the degree of deacetylation [4]. Some inorganic or organic constituent were studied to overcome the rapid degradation of CS to meet various requirement of tissue growth or drug release [5]. Like many other carbon-based materials, pristine graphene is considered to be non-biodegradable. However, after being chemically modified, the GO might degrade in the circumstance of hydrogen peroxide and horseradish peroxidase [6]. Recently, Loeblein et al. [7] studied the biodegradation mechanism of three-dimensional graphene after being treated by O_2-plasma. The modified 3D porous graphene scaffold with —OH and —COOH groups as active sites degraded into single layers over 16 weeks in vitro in peroxidase solution. As nanofillers in CS, GO exhibited ability to tune the degradation rate of the system [8], i.e. resist to enzymatic degradation partly due to covalent or non-covalent linkage between CS and GO which restricts the movement of CS molecular chains, about 45% of remaining weight for composite scaffolds compared to its pure counterpart (lower than 20%) after 2 weeks of degradation [8].

In previous study, we reported for the first time on the rGO film-coated 3D porous chitosan scaffold and the surface morphology and mechanical properties of the scaffold could be modified [9]. However, the in vitro and in vivo biocompatible properties of this type of composite scaffolds have not yet been studied, although GO and rGO modified scaffolds have been proved to modulate cell adhesion, proliferation and differentiation [10]. Therefore, this work aims to demonstrate the in vitro cytocompatible property of GO-coated CS scaffold. To investigate the in vivo degradation behavior, the scaffolds were implanted in rats for various prescheduled periods.

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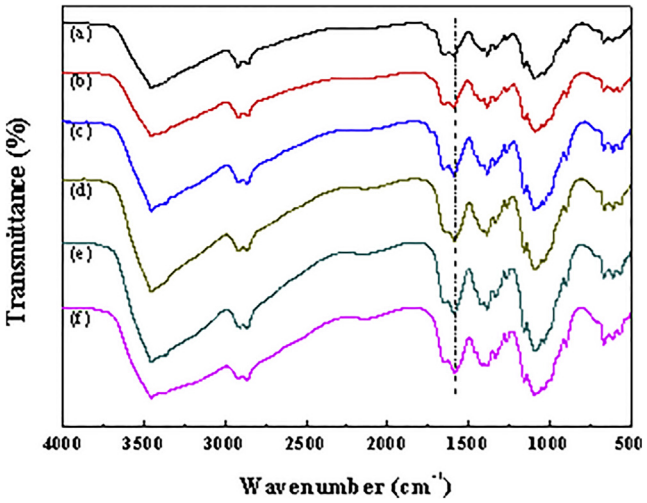

Fig. 1. FTIR spectra for samples of pure CS-150 (a), 0.02 mg GO/CS-150 (b), 1.0 mg GO/CS-150 (c), CS-60 (d), 0.02 mg GO/CS-60 (e), and 1.0 mg GO/CS-60 (f).

2. Experimental section

2.1. Preparation and characterization of scaffolds

Materials used for preparing GO, chitosan scaffold, and GO/chitosan composites have been described previously, including the structural characterization of the samples [9]. Fourier Transform infrared spectroscopy (FTIR) measurement was performed on a FTS-60A infrared spectrometer. All composites were cut into slices, sterilized by ultraviolet for 2 h and dipped in DMEM medium overnight.

2.2. Cell viability

L929 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco) containing 10% FBS (Gibco), 50 U/mL penicillin and 50 U/mL streptomycin (Sigma), and then incubated at 37 °C with 5% CO_2. Cell viability was measured using CCK-8 (SAB) assay at 24 h, 48 h and 72 h. Absorbance was measured at the wavelength of 450 nm with a microplate reader (Model 680; Bio-RAD, USA).

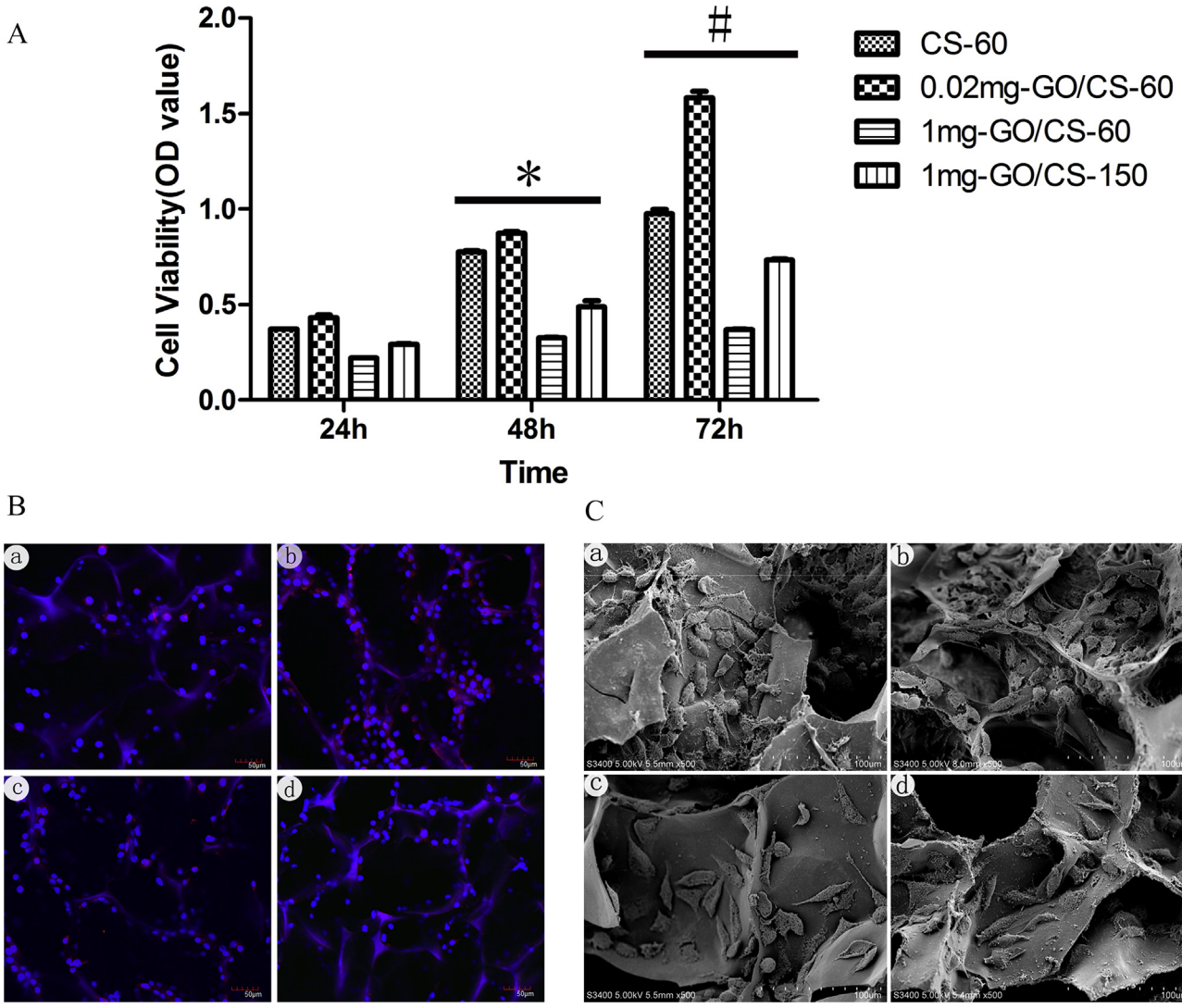

Fig. 2. Results of CCK-8 assay (A) of L929 cells incubated with GO/CS at 24 h, 48 h and 72 h; CLSM micrographs (B) and SEM images (C) of L929 fibroblasts cultured for 48 h on the CS-60 (a), 0.02 mg-GO/CS-60 (b), 1.0 mg-GO/CS-60 (c), and 1.0 mg-GO/CS-150 (d) scaffolds. Statistical significance: $^{*}P < 0.05$, $^{\#}P < 0.05$ versus 24 h.