Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 5 (2009) 93–100
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Cell proliferation and controlled drug release studies of nanohybrids based on chitosan-g-lactic acid and montmorillonite

Dilip Depan, Annamalai Pratheep Kumar, Raj Pal Singh *

Division of Polymer Science and Engineering, National Chemical Laboratory, Dr. Homi Bhabha Road, Pune, Maharashtra 411 008, India

Received 12 April 2008; received in revised form 17 July 2008; accepted 12 August 2008
Available online 29 August 2008

Abstract

The present paper reveals the potential uses of novel hybrids of chitosan-g-lactic acid and sodium montmorillonite (MMT) in controlled drug delivery and tissue engineering applications. The drug-loaded novel nanohybrid films and porous scaffolds have been prepared by solvent casting and freeze-drying of the grafted polymer solution, respectively. Sodium Ibuprofen was loaded into nanohybrids of chitosan-g-lactic acid/sodium montmorillonite (CS-g-LA/MMT). Grafting of lactic acid and the drug loading were characterized by Fourier transform infrared spectroscopy. Formation of intercalated nanocomposites was confirmed by X-ray diffraction. Mechanical properties measurements have shown improvement in modulus and strength with expense of elongation by MMT reinforcement. The nanohybrids were found to be stable regardless of pH of the medium. The cell proliferation profile also shows that prepared nanohybrids are biocompatible. MMT reinforcement was found to control the drug (Ibuprofen) release rate in phosphate buffer saline solution (pH 7.4). MMT clay is therefore a viable additive for formulating sustained drug delivery systems based on lactic acid grafted chitosan.

Keywords: Nanocomposites; Chitosan; Clay; Drug delivery systems; Controlled release

1. Introduction

The properties such as inherent biodegradability, biocompatibility and tunable mechanical properties are essential for the scaffolds in tissue engineering applications. The scaffolds should also be durable, stress-resistant, flexible, pliable and elastic with reasonable tensile properties, which could bear the stresses exerted by different parts of the body having varying contours. According to Peppas et al. [1], the increase in flexibility of the scaffolds could improve the contact between the scaffold material and the tissue, hence promoting penetration of the polymeric chains into the tissue to form strong adhesion. The surface morphology and structural integrity of the scaffolds should also allow the cells to attach and proliferate. In therapeutic applications, the efficiency of a drug lies in targeting specific body parts and maintaining a desired concentration level for longer period of time. For example, Ibuprofen (Ibu), which is a non-steroidal anti-inflammatory drug (NSAID), is used for relief of rheumatoid arthritis and osteoarthritis. However, its use is limited due to side-effects, which are often consequences of high plasma levels following the administration of conventional formulations [2]. By designing controlled delivery systems, the desired concentration of drug can be maintained without reaching a higher toxic level or dropping below the minimum effective level. For this purpose, the interaction between drug and lamellar host has been considered. The idea is to store the drug in the interlayer region of the lamellar host and allow the drug release as a consequence of diffusion and/or de-intercalation process. The montmorillonite (MMT) clay is composed of thin silicate layers, which are parallelly stacked by rather weak interactions such as van der Waals forces and Coulombic interactions. MMT exhibits enhanced gel strength, mucoadhesive capability to cross the gastrointestinal (GI) barrier and adsorb bacterial and metabolic toxins such as steroidal metabolites. Because of

* Corresponding author. Tel./fax: +91 20 25902615.
E-mail address: rp.singh@ncl.res.in (R.P. Singh).

1742-7061/$ - see front matter © 2008 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2008.08.007

these advantages in biomedical applications, it has taken the credit to be called medical clay [3]. In addition, of particular relevance, the drug (Ibu), which is cationic in nature, can also facilitate drug loading into interlayer regions of MMT and assist to achieve adequate sustained release property [4]. Chitosan (CS), a biopolymer, is the deacetylated product of chitin. Due to its biodegradability, biocompatibility and avirulence, CS has been used in many biomedical applications. The properties of high mechanical strength, hydrophilicity, good adhesion and non-toxicity of CS offer it as food additive, anticoagulant and wound healing accelerator. Though CS has the ability to form films, the tensile properties of pristine CS film are poor (due to its crystallinity). Thus, the modification (chemical modification, blending and graft copolymerization) of CS has gained its importance for tailoring the desired mechanical properties [5–7]. Since CS is alkaline in nature, by combining it (as graft copolymer or blend) with the biodegradable polymers like polylactic acid, which generate acidic by-products, the local toxicity at the implant site can be reduced [8–10]. Earlier, we have reported the preparation and characterization of nanohybrids based on chitosan-g-lactic acid and montmorillonite (CS-g-LA/MMT) [11]. Generally, the nanohybrid materials are derived from organic and inorganic solids interacting at the nanoscale level. These organic–inorganic hybrids show extraordinary and versatile properties, as they could be formed from a large variety of biopolymers and various nanoscale particles such as layered silicates (clay minerals) [12], carbon nanotubes [13], hydroxyapatite [14], and gold–silver nanoparticles [15]. In the present study, we investigate the effect of MMT on physical properties, such as the microstructures, swelling behavior, cell growth, and drug-release behavior of the prepared CS-g-LA/MMT hybrids.

2. Materials and methods

2.1. Materials

CS of low molecular weight ($M_v = 1.5 \times 10^5$, degree of deacetylation = 85%) was obtained from Aldrich. L-Lactic acid (purity 92%) was purchased from M/s Spectrochem and used as such for graft copolymerization. Ibuprofen was purchased from Aldrich. Sodium MMT, with cation exchange capacity (CEC) of 76.4 meqv/100 g, was received from M/s. Southern Clay Inc., USA.

2.2. Preparation of nanohybrids and drug loading

The nanohybrid films were prepared by following the procedure from our earlier report [11]. It involves aqueous dispersion of clay, suspension of CS in L-lactic acid, mixing them, drying and dehydration. First, the suspension of CS was added into aqueous dispersion of clay, heated up to 60 °C with continuous degassing for 40–45 min. This solution was casted and dried for 8 h at 60 °C under air and subsequently at 60 °C in vacuum oven for 8 h to obtain films. For obtaining porous scaffolds, the solution of CS-g-LA/MMT was freeze-dried at −56 °C using 9 well tissue culture plates. The dimensions of obtained cylindrical scaffolds are about 20 × 20 mm. The drug-loaded CS and CS-g-LA/MMT nanohybrid films and porous scaffolds were prepared by following the above-mentioned procedure. The dispersion of Ibuprofen (0.1 g), in the reaction mixture was done just after degassing and cooling. After 24 h of stirring, the Ibu-loaded solution was casted to obtain films and freeze-dried to obtain porous scaffolds. The formulations are given in Table 1.

2.3. Characterization

The wide-angle X-ray diffraction (XRD) patterns of the samples were obtained using a Rigaku (Japan) X-ray diffractometer with CuK_α radiation at 50 kV in the scan range of 2θ from 2 to 30° at a scan rate of 2° min^{-1}. The *d*-spacing was calculated by Bragg's formula where $\lambda = 0.154$ nm. The Fourier transform infrared (FTIR) spectra were obtained from the sample under attenuated total reflectance mode on a Perkin–Elmer Spectrum GX. The efficiency of LA grafting was evaluated by analyzing the yield (%) after drying and by ^{1}H-NMR spectroscopy of resultant copolymer. Surface morphology was investigated by scanning electron microscopy (SEM) (JEOL Stereoscan 440, Cambridge).

2.4. Swelling behavior

The water absorption behavior of the film samples were followed according to ASTM D570 as presented in our earlier study [11]. The swelling behavior of the porous scaffolds was investigated by exposing them to media of different pH: 1 N HCl, 1 N NaOH, and simulated body fluid (SBF) (pH 7.4) solutions. The shape retention was quantified by measuring the changes in diameter as a function of immersion time in the media. Three specimens were

Table 1
Formulations of Ibuprofen-loaded nanohybrids of chitosan-g-lactic acid and sodium montmorillonite

S. No.	Chitosan (g)	Lactic acid (ml)	MMT(g)	Ibu (%)	Drying process	Sample code
1	1	1	–	–	Vacuum	NTCS*
2	1	1	–	–	Vacuum	CL
3	1	1	0.05	–	Vacuum	CLM
4	1	1	–	10	Vacuum	CLI
5	1	1	0.05	10	Vacuum	CLMI-1
6	1	1	0.10	10	Vacuum	CLMI-2
7	1	1	0.05	–	Freeze	FCLM
8	1	1	–	10	Freeze	FCLI
9	1	1	0.05	10	Freeze	FCLMI-1
10	1	1	0.10	10	Freeze	FCLMI-2

* NTCS is neat chitosan without grafting.

ID	Title	Pages
2349	Cell proliferation and controlled drug release studies of nanohybrids based on chitosan-g-lactic acid and montmorillonite	8