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Research Paper

Effect of mechanical and electrical behavior of gelatin hydrogels on drug release and cell proliferation

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ABSTRACT

The present study was aimed to explore the effect of the mechanical and the electrical properties of the gelatin hydrogels on the mammalian cell proliferation and drug release properties. FTIR analysis of the hydrogels suggested that gelatin retained its secondary protein structure. A decrease in the diffusion constant of the water molecules was observed with the increase in the gelatin concentration in the hydrogels. The mechanical and the electrical stabilities of the hydrogels were enhanced with the increase in the gelatin content. Stress relaxation and creep studies were modeled using Weichert and Burger's models, respectively. The relaxation time (stress relaxation study) did not follow a concentration-dependent relationship and was found to affect the MG-63 cell (human osteoblast) proliferation. The impedance profile of the hydrogels was modeled using a (RQ) Q model. Release of ciprofloxacin from the hydrogels was inversely dependent on the rate of swelling. The release of the drug was not only dependent on the Fickian diffusion but also on the relaxation process of the gelatin chains. The inhomogeneous constant of the constant phase element representing the hydrogel-electrode interface indicated improved cell proliferation rate with a decrease in the inhomogeneous constant. In gist, the rate of cell proliferation could be related to the relaxation time (stress relaxation) and the inhomogeneous constant of the sample-electrode constant phase element (electrical study) properties, whereas, the drug release properties can be related to the bulk resistance of the formulations.

1. Introduction

Gelatin is a natural polymer obtained from the animal sources. Three-dimensional gelatin matrix has long been used for mammalian cell culture, tissue engineering and controlled delivery applications (Hoffman, 2012). Gelatin dissolves in warm water. Gelatin solution forms a reversible hydrogel having sol-to-gel transition temperature at ~35 °C.

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This change is governed by the physical interactions amongst the gelatin molecules. An increase in the temperature above ~35 °C results in the disruption of these interactions and conversion of the hydrogel into solution. The stability (thermal, mechanical and chemical) of the gelatin hydrogels may be improved by crosslinking with various multi-functional chemical reagents (e.g. glutaraldehyde, genipin, carbodiimide etc.) (Nguyen et al., 2015). Amongst the crosslinking agents, glutaraldehyde is commonly used. The effect of glutaraldehyde crosslinking on the bioadhesive properties of the gelatin hydrogels has been explored by Sung et al. (1999). Gelatin mediated crosslinking takes place *via* imine and acetal group formations (Zhang et al., 2015). An alteration in the gelatin content and the glutaraldehyde concentration helps tailoring the physicochemical properties (swelling, mechanical and thermal) of the gelatin matrices (Amadori et al., 2015).

None of the above-mentioned literature reports observations related to the effect of the relaxation (stress relaxation and creep-recovery studies) and electrical properties of the hydrogels and correlate with the cell proliferation and drug release properties. We hypothesize that the complex mechanical and the electrical properties might play an important role in the cell proliferation and growth. The basis of the hypothesis was due to the following aspects. Scientists around the world are developing artificial matrices of different physical and mechanical properties for modulating the cell growth (Haugh et al., 2011). Though the scientists have only studied the effect of matrix stiffness on the cell proliferation (Mason et al., 2013), the complex mechanical properties like stress relaxation and creep-recovery parameters might also influence the cell proliferation. This is because the cells try to analyze the mechanical properties of the matrix in its immediate environment during migration (an important step for cell proliferation). If the relaxation of the matrices occurs very quickly then the cell might not be able to judge the matrix property accurately and hence this may lead to altered cell growth profiles. Similar to the alteration in the mechanical properties, a group of scientists are working on the effect of cell proliferation over the conducting polymers. Changes in the electrical properties have been found to alter the cell proliferation rate (Li et al., 2013). Many of the scientists have studied the electrical properties of the polymer and have tried to correlate their results with the cell growth. No reports were found which investigated the prediction of equivalent electrical circuits and the effect of its components on the cell growth. This is important because, even though the materials are modeled using capacitive components, in practicality, the capacitive elements are not idealistic in nature. The inhomogeneous nature of the non-ideal capacitive elements might tailor the interactions amongst the matrix and the cells. Hence, understanding of the complex mechanical and electrical parameters is important with respect to the cell proliferation. The electrical conductivity is dependent on the reactance of the capacitive elements of the materials and might alter the diffusional characteristic of the drugs within the delivery vehicles. Due to this reason, it seems justified, to understand the effect of the properties of the circuit elements on the release properties of the drugs from delivery matrices.

In this study, glutaraldehyde-crosslinked gelatin hydrogel, of varying gelatin:crosslinker ratios were subjected to conventional physicochemical studies like swelling, mechanical and electrical studies. The hydrogels were prepared using Type-B gelatin, extracted from bovine collagen. Type-B gelatin is commonly used for tissue engineering and drug delivery applications (Abruzzo et al., 2012; Graulus et al., 2015; Huber et al., 2015; Siqueira et al., 2015). All the data obtained were analyzed using number of relevant mathematical models. The mechanical results were interpreted in terms of time-dependent parameters (e.g. delayed elasticity and relaxation time). Finally, cell proliferation on the hydrogels and drug release from the hydrogel were studied. The results were analyzed in the context of the time-dependent parameters (mechanical studies) and electrical parameters to find out an appropriate correlation between the physical properties of gelatin and its functionality.

2. Materials and methods

2.1. Materials

Gelatin (Type-B; 225 bloom; IP: 5.05; Extra pure), extracted from bovine skin, was procured from Himedia (Mumbai, India) (Varghese et al., 2014). Hydrochloric acid and glutaraldehyde (25% solution, for synthesis) were purchased from Himedia (Mumbai, India) and Merck Specialities Pvt. Limited (Mumbai, India), respectively. Ciprofloxacin was received as a gift from Aristo Pharmaceuticals (P) Ltd., India. The chemicals were used as received. Double distilled water was used throughout the study.

2.2. Preparation of the hydrogels

Gelatin solutions were prepared by dissolving accurately weighed gelatin (10 g, 15 g and 20 g) in 60 g of water (60 °C). The final weights of the solutions were adjusted to 100 g using water (60 °C). 0.5 ml of glutaraldehyde solution was put into 20 g of the gelatin solution, mixed well and poured into the cylindrical molds (height: 23.23 mm; diameter: 14.30 mm). The molds were kept at room temperature (25 °C) for 5 min. 0.01 ml of 0.1 N hydrochloric acid was used as a catalysis reagent. The hydrogels containing 10%, 15% and 20% gelatin were regarded as G1, G2 and G3, respectively. Ciprofloxacin was used as a model drug. It was loaded in the hydrogels at a concentration of 1% (w/w). The drug was added to the gelatin solution before adding glutaraldehyde solution. The rest of the process remained same. The drug loaded hydrogels were regarded as G1C, G2C and G3C, respectively.

2.3. FTIR analysis

The IR spectra of the hydrogels were acquired using a Bruker spectrometer (Alpha), attached to a ZnSe reflection attenuated total reflectance (FTIR-ATR) module. The scanning was done in the wavenumber range of 4000–450 cm^{-1}. The spectral resolution was set at 4 cm^{-1}.

2.4. Swelling study

The prepared hydrogels (weight=W_0) were immersed in water (37 °C). The weight of the hydrogels was measured at regular intervals (weight=W_t). The study was conducted for