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Preparation of Chitosan Based Scaffolds Using Supercritical Carbon Dioxide

Kumari Rinki,[1] Pradip K. Dutta,[1,2] Andy J. Hunt,[2] James H. Clark,[2] Duncan J. Macquarrie[2]*

Summary: A novel chitosan-formaldehyde porous derivative (scaffolds) was prepared by reaction of 85% deacetylated chitosan with 37% aq. formaldehyde using supercritical carbon dioxide (sc. CO_2). Prior to reaction, the chitosan hydrogel was prepared in 1% aq. acetic acid (AcOH) and formaldehyde. The prepared hydrogel was subjected to solvent exchange. The identity of the Schiff base was confirmed by Fourier transform infrared spectroscopy (FTIR). The chitosan-derivative was evaluated by thermal analysis, scanning electron microscopy, and porosimetry analysis. Overall, the sc. CO_2 assisted chitosan derivative opens new perspectives as biomedical material.

Keywords: chitosan; formaldehyde; FTIR; hydrogel; sc. CO_2

Introduction

One of the most critical medical problems which man is facing today is the tissue loss or organ failure. Tissue engineering has emerged as a field to solve these critical medical problems.[1] Tissue engineering involves the regeneration of damaged tissue. This task demands a combination of molecular biology and materials engineering, since in many applications a scaffold is required, that provides a temporary artificial matrix for cell seeding.[2] A scaffold is a support, either natural or artificial, that maintains tissue contour.

Hydrogels are highly swollen, hydrophilic polymer networks that can absorb large amounts of water and drastically increase in volume.[3] They are widely used in the biomedical field as drug-delivery devices, wound dressings and scaffolds for tissue engineering.[4] Chitosan, an undisputed biomaterial has many distinctive properties like antimicrobial activity, nontoxicity, biocompatibility, biodegradability, remarkable affinity to proteins and cell adhesion. Such kind of properties of chitosan draws attention of researchers in biomedical applications. Various chitosan derivatives have been designed to use it in various ways.[5–8] Scaffolds must meet certain fundamental characteristics such as high porosity, appropriate pore size, biocompatibility, biodegradability and proper degradation rate.[9] Scaffolds can be fabricated by different methodologies, including solvent casting, phase separation and 3D- printing. These fabrication methodologies use solvents like chloroform, methylene chloride and dioxane to achieve the final porous structures. It has been reported that residual solvents may remain at high level for some polymer formulations. Since these solvents are toxic to cells, the residual solvents must be removed to comply with FDA regulations.[10] Supercritical carbon dioxide has been used as a solvent or a foaming agent to prepare microcellular structures for both biodegradable and nonbiodegradable polymers and thus is a promising process medium for fabrication of tissue engineering scaffolds.[10–15] The use of supercritical carbon

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[1] Department of Chemistry, M. N. National Institute of Technology, Deemed University, Allahabad 211004, India
E-mail: pkd_437@yahoo.com
[2] Green Chemistry Centre of Excellence, Department of Chemistry, University of York, Heslington, York, YO10 5DD

dioxide offers many advantages over conventional solvents: CO_2 is non-toxic, nonflammable, inexpensive and readily available in high purity from a variety of sources.[16] The present work describes the development of chitosan based scaffolds, with potential biomedial applications *vis-à-vis* it is also important to ensure that chemical processes used in converting biomolecules to useful materials through green chemistry approach. From biomedical application point of views, it is a benign technique. We herein report the preparation of chitosan-formaldehyde based scaffolds using sc. CO_2.

Experimental Part

Materials

Chitosan (degree of deacetylation: 85%, Sigma-Aldrich) and formaldehyde (37% aq., Sigma-Aldrich) were used as such.

Measurements

The characterization of the chitosan derivative was carried out by using FTIR technique (Bruker ATR), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) with STA 600 thermal analyzer, scanning electron microscopy (SEM) with Jeol electron microscopy. The porosity measurement was done by using the Micromeritics Porosimeter: ASAP2010 model.

Sample Preparation

1% of chitosan solution was prepared by using 1% aq. acetic acid. 37% (w/v) aq. formaldehyde was added slowly to the chitosan solution and stirred until the chitosan solution turned into a more viscous gel and the magnet bar was stopped. The prepared hydrogel was then subjected to solvent-exchange into acetone.

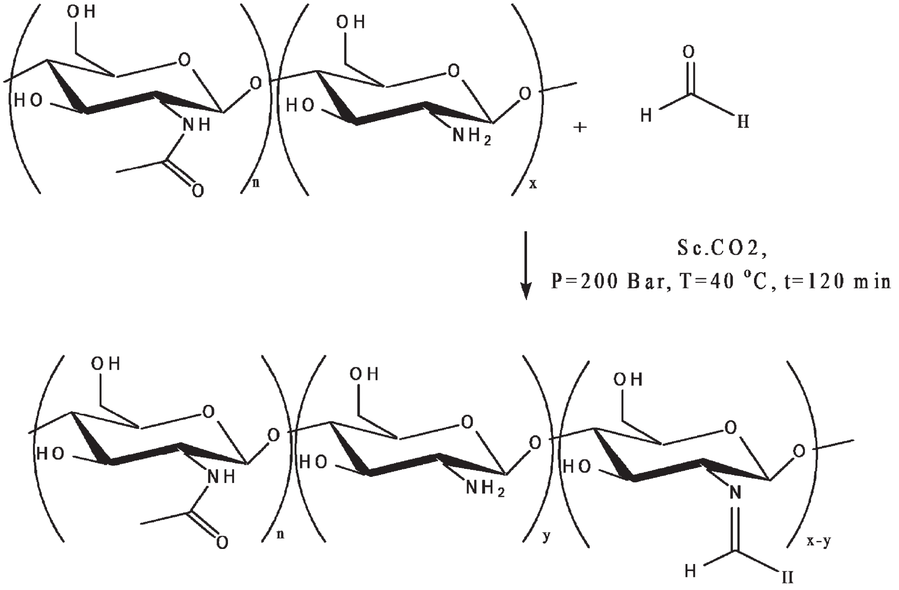
Supercritical Fluid (SCF) Drying

The chitosan-formaldehyde hydrogels were placed inside a sealed chamber of the SCF reactor (Thorr Co, USA). The temperature and pressure were raised to 40 °C and 200 bar respectively. The reaction was left for 2 h and a flow of CO_2 was then applied through the sample in order to replace all the organic solvent with CO_2. The pressure was then released slowly to the atmosphere and the temperature was reduced to 20 °C.

Results and Discussion

The current research in tissue engineering has emerged the need for new classes of biodegradable polymers with the potential for specific or controllable bioactivity. In this paper we have described procedures for fabricating tissue scaffolds from chitosan, an enzymatically degradable polysaccharide with broad potential by a greener technique. The polymer's hydroxyl and amino groups provide several possibilities for derivatization or grafting of desirable bioactive groups, and chitosan's pH-dependent solubility allows use of relatively mild processing methods. This feature is particularly important if incorporation of bioactive species is desired prior to forming a three dimensional microstructure. Furthermore, the removal of excessive chemicals from the scaffolds can be additionally controlled. Formation of dense chitosan membranes and extruded fibers has been extensively described and characterized elsewhere,[17] and provide alternate approaches for biomedical implant fabrication.

Chitin is the precursor of chitosan, which is the major structural molecule in arthropod cuticles. Interactions with matrix proteins and connective tissue components are numerous and undoubtedly intimate in that environment. Chitosan is formed by the partial deacetylation of chitin which creates the potential for a variety of interactions in the mammalian implant environment. Interactions with mammalian proteins have not been extensively characterized; however, collagen is the exception. However, many interesting interactions with mammalian tissues have been reported. The cells involved ranged from osteoblasts and fibroblasts to macrophages and keratinocytes.[18–20] The cellular interactions have been positive in most cases



Scheme 1.
Preparation of chitosan-formaldehyde derivative (Schiff base).

from the tissue repair and regeneration standpoint.[21]

The preparation of chitosan-formaldehyde derivative (Schiff base) is shown in Scheme 1. The hydrogel was subjected to solvent exchange to remove water and thus formation of aerogel by the use of sc. CO_2 with the retaining of microstructures is desig-

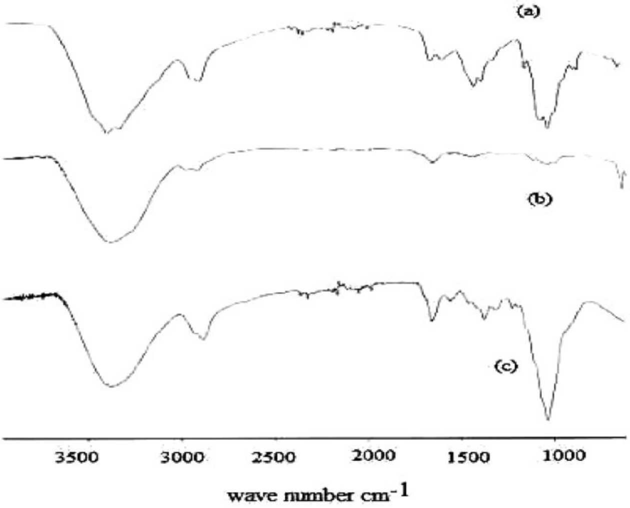


Figure 1.
FTIR spectra of (a) chitosan; (b) formaldehyde and (c) chitosan-formaldehyde derivative.

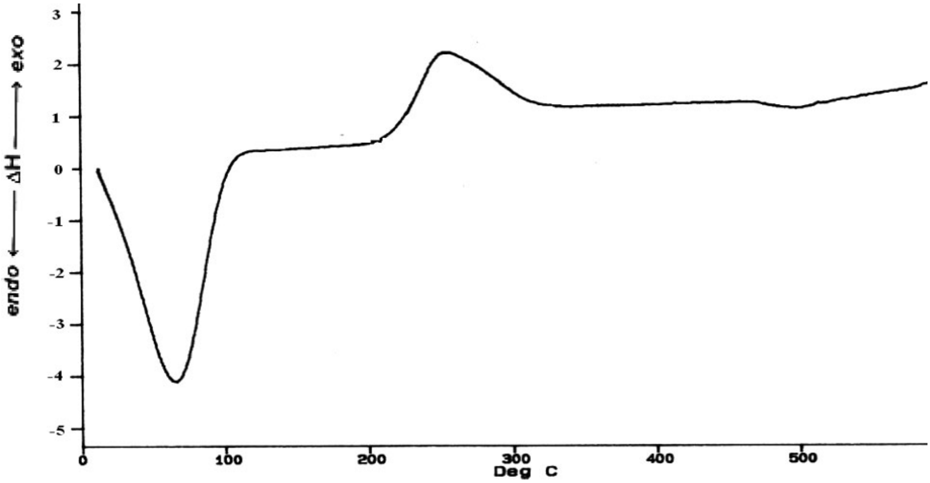


Figure 2.
DSC curves of chitosan derivative.

nated as scaffolds. The scaffolds shows light pink color due to the imine linkage (CH_2=NH–) called Schiff base.

FTIR Spectroscopy

The FTIR spectra of chitosan, formaldehyde and chitosan-formaldehyde derivative are shown in Figure 1. The native chitosan shows a prominent peak at 3450 cm^{-1} for the hydroxyl group and 1592 cm^{-1} for the amino group. Figure 1 (b) shows the spectra of formaldehyde. The characteristic peaks of formaldehyde are located at 1750 cm^{-1} for the carbonyl group and one near 2820 cm^{-1} for C–H stretching asymmetric and the other near 2720 cm^{-1} for C–H stretching symmetric. Figure 1(c) shows a significant peak at 1560 cm^{-1} due to the imine bond (C=N) formed by crosslinking reaction between the amino groups in the aldehyde group in formaldehyde. This characteristic peak confirms the formation of Schiff base after the reaction of chitosan with formaldehyde.

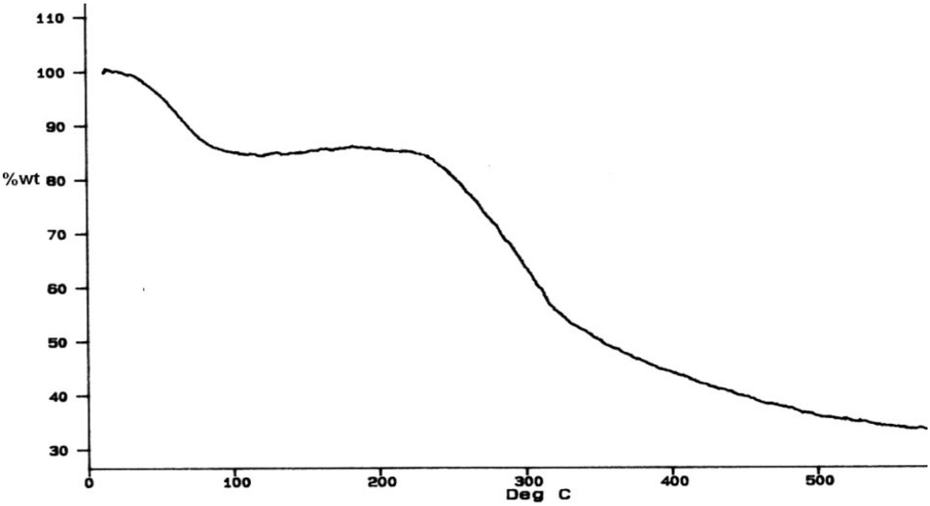


Figure 3.
TGA curves of chitosan derivative.

Differential Scanning Calorimetry & Thermogravimetric Analysis

The DSC thermograms of the chitosan derivative is represented in Figure 2. The exothermic peak at 255 °C corresponds to thermal degradation of chitosan derivative. The endothermic peak at 80 °C is associated with loss of water from the chitosan derivative.

The TGA curve, represented in Figure 3 shows that the chitosan derivative has two main decomposition stages with the first occurs in the range of 50°–100 °C, attributed to the evaporation of water. The second decompositon stage occurs in the range of 200°–300 °C, denotes the thermal degradation of chitosan derivative.

Morphology

Chitosan scaffolds before sc. CO_2 treatment are shown in Figure 4.The scanning electron micrographs (SEMs) of the native chitosan are shown in Figure 5(a) & 5(b). It exhibited a nonporous, smooth membranous phases consisting of dome shaped orifices, microfibrils and crystallites (Figure 5(b)). It also exhibited flat lamellar phases on which a large number of protruding microfibrils are evident.

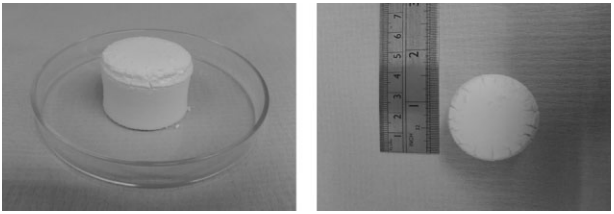


Figure 4.
Chitosan scaffolds before sc. CO_2 treatment.

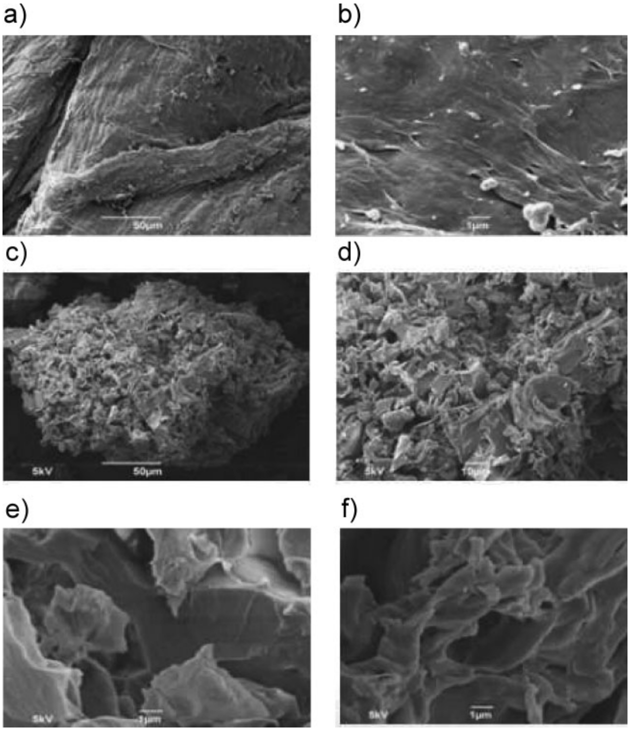


Figure 5.
Scanning electron micrographs of native chitosan (a) and (b); chitosan derivative (c)–(f).

The electron micrographs of chitosan derivative are shown in Figure 5(c), 5(d), 5(e) & 5(f). The SEMs of chitosan derivative exhibited a polyphasic microporous structure. The pore dimensions are non-uniform with thin walls.

Porosimetry Measurement

Control of scaffold pore morphology is critical for controlling cellular colonization rates and organization within an engineered tissue. Furthermore, angiogenesis is a requirement for some scaffold application scenarios and can be grossly affected by material porosity. Pore morphology can also be expected to significantly affect scaffold degradation kinetics and the mechanical properties of the developing tissue. While controlled rate freezing is limited by the magnitude and directionality of thermal gradients, it does provide a simple, straightforward and reproducible way of introducing directional pores into a polymer structure. The methods detailed here allow optimization of pore morphology over a physiologically relevant range and serve as an additional method of tailoring scaffold properties for particular tissue requirements. The *Brunauer-Emmett-Teller* (BET) surface area was calculated using adsorption data in the BET region ($P/P_0 = 0.30040457$). The pore size distribution of the chitosan derivative was determined from desorption branches of the isotherms by the *Barret-Joiner-Halenda* (BJH) method. The BJH pore size is defined as the maximum of the BJH pore size distribution.[22] The chitosan derivative demonstrated a BET surface area of 19 m^2/g, with a BJH total pore volume of 0.076096 cm^3/g and an average pore diameter (4V/A) of 13.3347 nm and the median pore diameter of 35.0967 nm.

Conclusion

In conclusion, the studies described in this paper and elsewhere indicate that chitosan has excellent potential as a structural base material for a variety of engineered tissue systems. The chitosan scaffolds prepared by supercritical carbon dioxide (sc. CO_2) is porous and the moderate surface area is expected for better proliferation for biological study. Moreover, the use of sc. CO_2 as a reaction medium offers many advantages over conventional solvents. From biomedical application point of views (especially, hygienic) the green chemistry approach is a benign technique.

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