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Evaluation of in vitro bioactivity of Chitosan/*Mimosa tenuiflora* composites

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ABSTRACT

[Porous biocomposite scaffolds of Chitosan/*Mimosa tenuiflora* (Ch/Mim) were fabricated for tissue engineering applications by thermally induced phase separation and lyophilization techniques. The in vitro bioactivity evaluation of the scaffolds was carried out by analyzing the apatite layers produced on them using SBF as the incubation medium. The apatite formation was analyzed using FTIR spectroscopy and Field Emission Scanning Electron microscopy coupled to Energy-Dispersive Electron X-ray Spectroscopy. The cumulative results obtained from IR spectra and SEM-EDS suggest that the developed composites might have potential applications in tissue engineering.

1. Introduction

Mimosa tenuiflora (Willd.) Poiret is a species of the Mimosoideae, a subfamily of the Fabaceae botanical family that is distributed from Mexico to Venezuela and Brazil [1]. It is a plant with a high content in tannins and large amount of starch [2]. *M. tenuiflora* is traditionally used as an aqueous extract [3] for the treatment in wounds and venous leg ulcers due to its healing and antiseptic properties [4]. It has been used in the treatment of burns and infections in both humans and animals because of its antimicrobial and cicatrizing effects [4,5].

The chemical composition of the *M. tenuiflora* cortex has been identified as a group of alkaloids, mainly N-N-dimethyltryptamine, serotonin, triperpenoid glycosides, and steroidal saponins: 3-O-β-D-glucopiranosil campesterol, 3-O-β-D-glucopiranosil estigmasterol, and 3-O-β-D-glucopiranosil β-sitosterol [4].

On the other hand, Chitosan is a semi-crystalline polysaccharide derived from chitin and possesses antimicrobial activity [6]. Due to its properties, Chitosan is a good candidate for preparation of biomaterials that could substitute missing or damaged tissue [7].

One of the most important characteristics of a bioactive material is the possibility that a biologically active carbonate hydroxyapatite layer can form on their surface [8]. Calcium phosphate is the main constituent of bone tissue [9]. This substance could be formed on the material surface by an inorganic chemical reaction similar to that occurring in bones. This ability has been related to osteoconduction and bone bonding [10], because the properties of the apatite layer can affect cell viability and proliferation [8]. In order to predict bioactivity, a simulated body fluid (SBF) has been used [11].

With these characteristics, it could be reasonable to believe in the bioactivity of a Chitosan/*M. tenuiflora* composite. So, in this study, a biocomposite of Chitosan/*M. tenuiflora* composite was manufactured through a thermally induced phase separation method. The composite was used to investigate the in vitro bioactivity of scaffolds by analyzing the apatite layers produced in them using SBF as an incubation medium.

2. Materials and methods

Materials: Chitosan was purchased from Carbomer, Inc (United States). The bark of *M. tenuiflora* was obtained directly in the region of Jiquipilas Chiapas. Ethanol was provided by CTR Scientific (Mexico). Glacial acetic acid (Mallinckrodt, United States) was used as a solvent. SBF was prepared in our laboratory according to a previously published method [7].

Preparation of composites: Chitosan/*M. tenuiflora* was prepared using the thermally induced phase separation technique. Briefly,

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Chitosan and *M. tenuiflora* were dissolved in 1% (%v/v) aqueous acetic acid solution. The solutions were mixed together at 80/20 ratio. Finally, the composite was frozen and freeze dried for 2 days.

FTIR spectroscopy: To evaluate the Chitosan/*M. tenuiflora* composites and mineralized scaffold, FTIR spectra were recorded using a transmission mode in an IR spectrometer (Nicolet 6700, Thermo Scientific, USA). For each spectrum, 100 scans at16 cm^{-1} resolution were averaged.

Characterization of morphology and in vitro bioactivity: A previously studied method was used for the in vitro bioactivity study [7]. Briefly, porous samples of approximately 250 mm^3 were soaked in 5 mL of tetraethoxyl orthosilicate for 2 h in a vacuum oven at 60 °C. The samples were rinsed with ethanol and were dried in air at room temperature for 1 day. Then, the samples were soaked in 5 mL of 1.5 × SBF, pH 7.4, in an incubator at 37 °C. After the incubation period, the specimens were washed carefully with deionized water and finally dried.

Field Emission Scanning Electron Microscopy (FE-SEM, JEOL JSM-7000 F) was applied to investigate the morphology of the composites and Field Emission Gun Scanning Electron Microscopy (Jeol JSM-7000 F) coupled with Energy Dispersive X-ray Spectroscopy (15 kV) to analyze the dispersion and distribution of apatite particles and to detect the concentration of calcium and phosphorous on the surface after the treatment for the different experimental conditions. The Ca/P ratio was estimated for the various conditions. Also, the pore size was measured using the Scandium Universal SEM Imaging Platform software.

3. Results and discussion

FTIR of the samples: Fig. 1 shows that the N–H band of Chitosan in the composite was affected and shifted from 1543 cm^{-1} to 1553 cm^{-1}. On the other hand, the peak that corresponds to the primary alcohol groups of Chitosan (–C–O stretching) was shifted from 1418 cm^{-1} to 1459 cm^{-1} for the composite. Furthermore, it is possible to observe a reduced intensity on the band at 1021 cm^{-1} that could imply an interaction of the ether bond of Chitosan with the *M. tenuiflora*. All these events suggest that there is an interaction between the amino group of the Chitosan and carboxyl groups in *M. tenuiflora* or hydroxyl groups in Chitosan and carboxyl groups of the *M. tenuiflora* in the blend.

Characterization of morphology and in vitro bioactivity: Pores with sizes around 50–100 μm are essential for bone ingrowth [12]. As shown in Table 1, the composite Chitosan/*M. tenuiflora* has more homogenous porosity than that of Chitosan, with an average pore size of 211.61±13.74 μm. The composite also showed an open-pore structure with high porosity (Fig. 2). The pore size and porosity obtained is shown in Table 1.

According to SEM images (Fig. 2e and f), the particles formed in vitro have a spherical shape. Some large particles with a diameter of about 10 μm and smaller crystals around 3 μm were observed. With an increase in immersion period a more compact mineral layer was formed on the surface. The images revealed that apatite crystals grow in a layer-by-layer manner. The Ca/P peak intensity ratio for Chitosan at 28 days was 1.13, which might

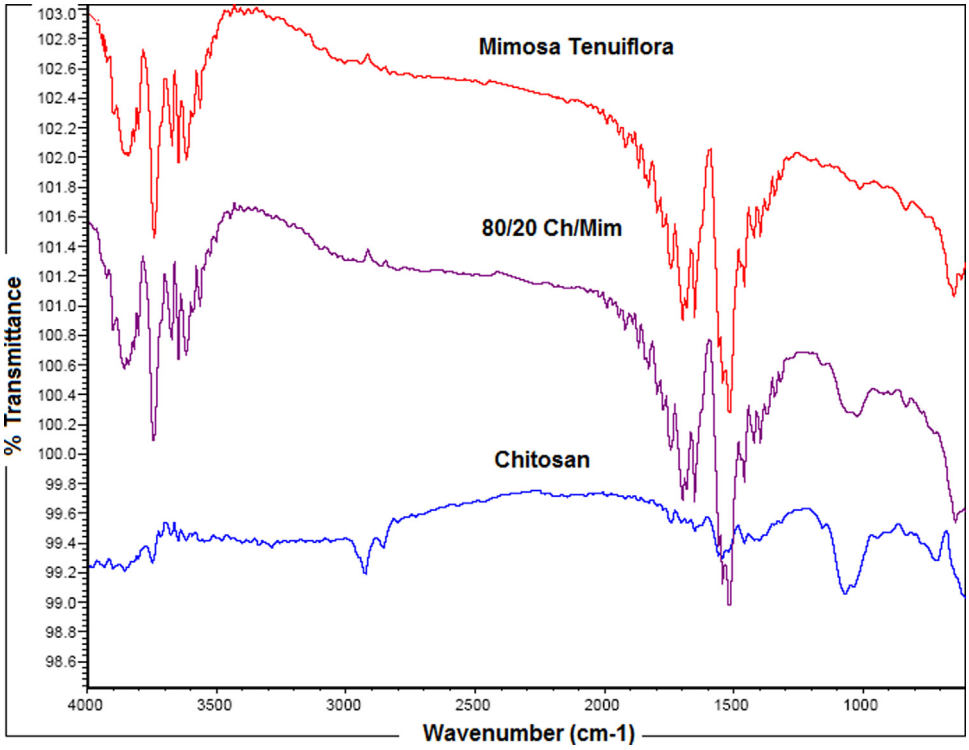

Fig. 1. FTIR spectra of *Mimosa tenuiflora*, Chitosan and 80/20 Ch/*Mimosa tenuiflora* composite before incubation.

Table 1
Pore size distribution. It shows the average pore size (μm) and the distribution of porosity in Chitosan and 80/20 Chitosan/*Mimosa tenuiflora*.

Composite	Average pore size (μm)	< 50 μm (%)	50–100 μm (%)	100–200 μm (%)	200–300 μm (%)	300–400 μm (%)	400–500 μm (%)	> 500 μm (%)
Chitosan	150.56 ± 14.65	15	30	35	10	4	1	5
80/20 Ch/Mim	211.61 ± 13.74	12	16	24	22	12	12	2