**Supplementary Materials**

Self-assembled CNF/rGO/Tannin composite: Study of the Physicochemical and Wound Healing Properties

**Katherina Fernández** 1,\*, **Ayleen Llanquileo 1, Montserrat Bustos 1, Valentina Aedo 1, Isleidy Ruiz 1, Sebastián Carrasco 1, Mauricio Tapia 1, Miguel Pereira  2, Manuel F. Meléndrez 3, Claudio Aguayo 4, Leonard I. Atanase  5,6**

1 Laboratorio de Biomateriales, Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad de

 Concepción, Box 160-C, Concepción, Chile

2 Laboratorio de Productos Forestales, Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad de Concepción. Box 160-C, Concepción, Chile

3 Grupo Interdisciplinario de Nanotecnología Aplicada (GINA). Laboratorio de Materiales Híbridos (HML). Departamento de Ingeniería de Materiales (DIMAT), Facultad de Ingeniería, Universidad de Concepción, Edmundo Larenas 270, Box 160-C, Concepción, Chile

4 Departmento de Inmunología y Bioquímica Clínica, Facultad de Farmacia, Universidad de Concepción, Concepción, Chile

5 Faculty of Medical Dentistry, Apollonia University of Iasi, 700511, Iasi, Romania

6 Academy of Romanian Scientists, 050045 Bucharest, Romania

**\*** Correspondence: kfernandeze@udec.cl;

**1. Materials and Methods**

*1.1 Materials*

Graphite powder (flakes; mesh 325) was purchased from Asbury Online (Asbury Carbons, New Jersey, USA). Dopamine hydrochloride (𝐷𝐴 − 𝐻𝐶𝑙, ≥ 98.0%) was purchased from Sigma-Aldrich Company (St. Louis, MO). The other chemicals and solvents, such as tris(hydroxymethyl)aminomethane (buffer Tris), sulfuric acid (H2SO4, 98%), phosphoric acid (H2PO3, 85%), oxygenated water (H2O2, 60 vol), silver nitrate (AgNO3, 0.1 N), potassium permanganate powder (KMnO4, 99.9%) and hydrochloric acid (HCl, 37% v/v), were purchased from Merck (Darmstadt, Germany). These chemicals were used as received, without further purification. Milli-Q® water and distilled water were used throughout the study. Cellulases Quimizime B was provided by CHT group (Santiago, Chile).

*1.2. Material characterization*

*X-ray diffraction (XRD).* The X-ray diffraction (XRD) was used to determine the oxidation degree of GO and the crystallinity of rGO/NCF and rGO/CNF/TA composites. The measurements were carried out on the X-ray diffractometer (Bruke Axs, D4 Endeavor, USA) with reference target: Cu Kα radiation (λ=1,541841 Å; 2,2 kW), voltage: 40 kV, and current: 20mA. The samples were measured from 2 to 50° during 141 s with steps of 0.02°.

*Fourier Transform Infrared Spectroscopy (FTIR).* The FTIR was used to investigate the chemical nature of interaction of rGO/NCF and rGO/CNF/TA composites. The spectra were recorded in the Perkin Elmer UATR Two FTIR Spectrometer. The wavenumber range analyzed was 4000-500 cm-1 and a total of 40 accumulated scans were acquired.

*Raman analysis.* The Raman was used to identify the spectra features and structural properties of GO and rGO/NCF and rGO/CNF/TA composites. The data were acquired by high-resolution confocal (LabRamHR Evolution Horiba Jobin Yvon microscope, Japan) at 633 nm of wavenumber in the excitation laser line, a power of 13.3 mW and 1.96 eV. The laser spot was focused on the samples using an optic Objective Olympus 100x VIS and a NUV camera (B/S UV 50/50 + Lens F125 D25). The measurements were carried out in quadruplicate at room temperature, and with a laser intensity constant to avoid damaging on the samples. In addition, for the calculation of either the intensity and the area of the D and G bands were applied a Lorentzian function in the spectral region 1000-1800 cm-1.

*X-ray photoelectron spectroscopy (XPS).* XPS technique was used to quantitatively identify the surface chemistry of rGO/NCF and rGO/CNF/TA composites, also the raw components NCF, TA and rGO were analyzed. The measurements were carried out in a Surface Analysis Station 1 (STAIB model RQ300/2, USA) at ultravacuum conditions (< 10-9 bar) equipped with a hemispherical electron analyzer (SPEC PHOIBOS 100, Germany). The photoelectrons were excited with non-monochromatic radiation Mg Kα (1486.6 eV) and analyzed with a constant energy step of 1 eV. The X-ray source was used with a strength of 300 W.

*Thermogravimetric analysis (TGA).* TGA technique was used to evaluate the thermal stability of gelatin, GO and rGO/NCF and rGO/CNF/TA composites. The measurements were carried out in a Cahn-Versatherm thermogravimetric analyzer with sensitivity of 0.1 μg, heating rate of 10°C/min under nitrogen atmosphere (100 mL/min) and a temperature range from 30°C to 800°C.

*Scanning electron microscopy (SEM).* The SEM analysis was used to investigate the micromorphology of rGO/NCF and rGO/CNF/TA composites. SEM images were recorded using a JEOL JSM-6380LV, Japan model microscope at 10 kV. The aerogels were coated using a gold sputter coater and their surfaces were observed at different resolutions. In addition, the SEM images were processed using ImageJ® software to determinate the average pore sizes.

*Surface charge measurements*. The surface charge was determined through ζ-potential measurements using the Dynamic Light Scattering principle (SZ-100 Nano particle analyzer, Horiba Scientific, Japan). The measurements were carried out for the GO and gelatin-GO aerogels. Samples 1.0 cm3 in volume were dissolved in Milli-Q® water pH 6.5, shaken and sonicated for 20 min to achieve homogeneity. Finally, the samples were measured in triplicate.



 f

20 μm

**Figure S1.** SEM images a) GO, b) rGO/CNF5, c) rGO/CNF15, d) rGO/CNF25, e) rGO/CNF50 f) rGO/CNF25/TA.



**Figure S2.** Swelling behavior of neat materials and composite samples on PBS fluid on time.

**Table S1.** Phenol composition and content of the *Pinus Radiata* bark extract

|  |  |
| --- | --- |
| Compounds  | Content (mg per gram of extract)  |
| (-)-Catechin  | 13.8 |
| Taxifolin  | 13.9 |
| p-Hydroxybenzoic acid  | 7.6 |
| Homovanillic acid a  | 6.7 |
| Quercetin  | 4.3 |
| Proanthocyanidin *B-2*  | 3.3 |
| (+)-Epicatechin  | 2.8 |
| Dihydroxybenzoic acid b  | 2.7 |
| Dihydroxybenzoic acid b  | 1.8 |
| Syringic acid a  | 1.1 |
| 3,4-dihydroxyphenyl acetic acid  | 0.9 |
| Dihydroxybenzoic acid b  | 0.2 |
| Epigallocatechin  | n.d. |

n.d.: not detected, a: tentatively identified compounds, b: not recognized isomers.

**Table S2.** Average molecular weight number (Mn) of pine extracts at different percentages of sample development, determinate by GPC.

|  |  |
| --- | --- |
| % sample development | Mn |
| 10 | 438 |
| 25 | 749 |
| 50 | 1355 |
| 100 | 63277 |
|  |  |