Use of Gelatin as Tannic Acid Carrier for Its Sustained Local Delivery

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Supporting Information

Tannic acid release

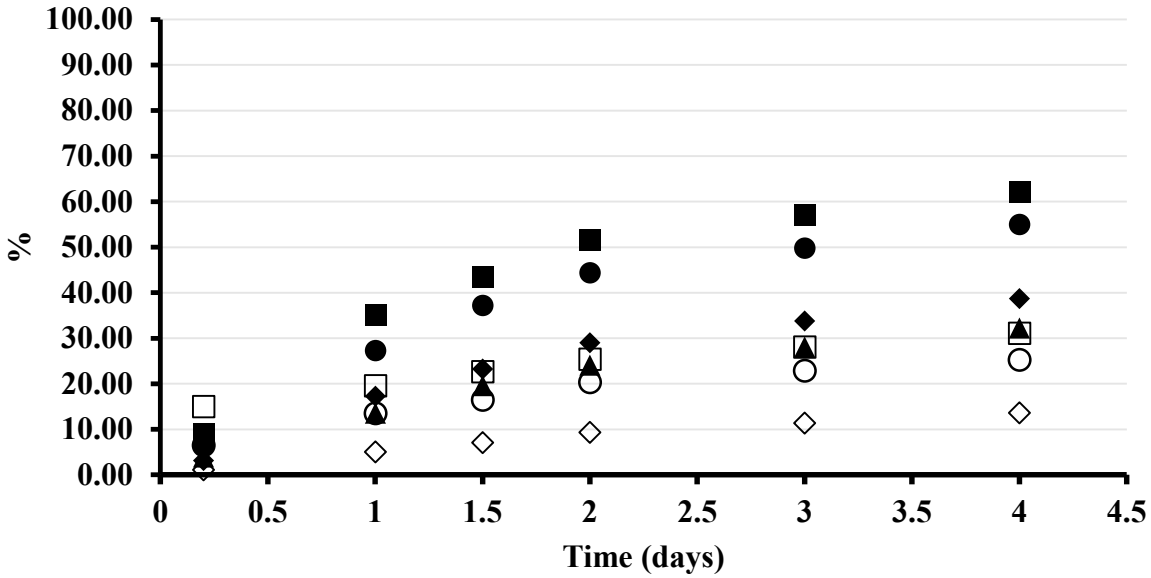

Figure S1. Release of tannic acid by the time from a G/TA$_x$ matrix made from 1mg gelatin and expressed as a percentage from the initial TA content. (◇) G/TA$_{15}$ (○) G/TA$_{30}$ (□) G/TA$_{50}$ (▲) G/TA$_{70}$ (◆) G/TA$_{100}$ (●) G/TA$_{150}$ (■□) G/TA$_{200}$.

Matrix Size Measurement

Matrix size was monitored by the time by following its area. G/TA$_x$ Matrixes were prepared in a 24 well plate from 1 mg of gelatin and various concentrations in TA. Once prepared the matrixes were incubated in a citrate/phosphate buffer at pH = 7.0 at room temperature. At various time points, the 24 well plate was scanned using an Aficio MPC3002 (RICOH) plate scanner. Images were transferred as TIFF files in the ImageJ software. Area measurement of each matrixes was performed using the circle selection tool.

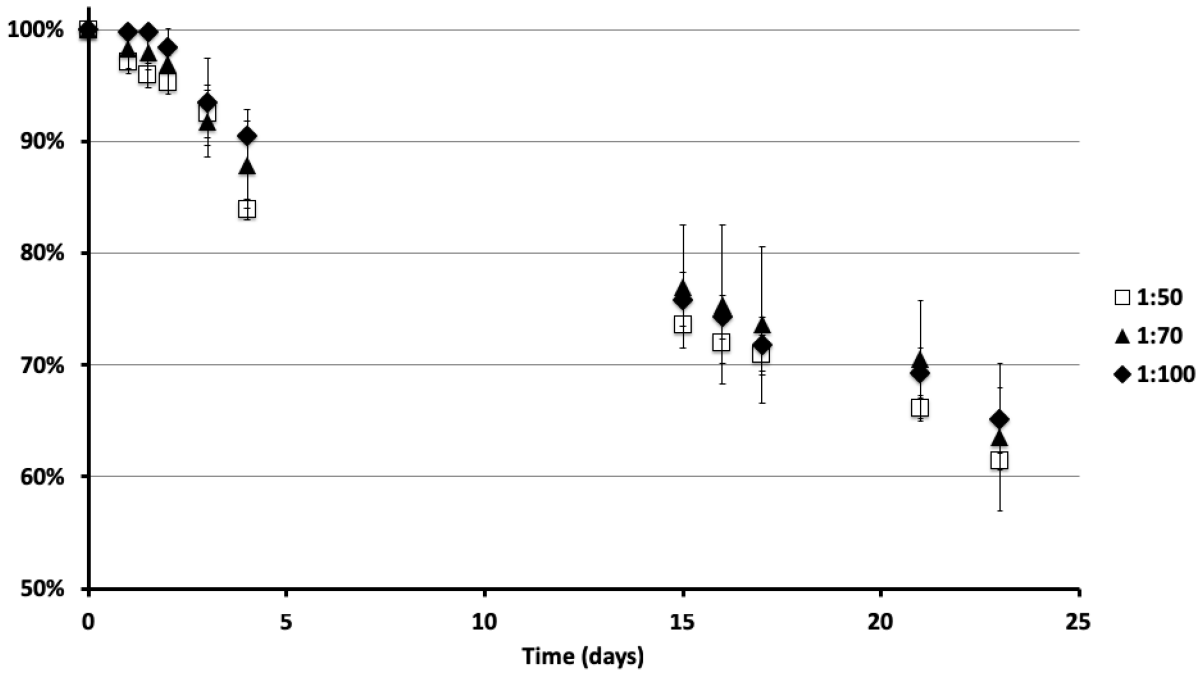

Figure S2. Time evolution of the area of G/TA$_x$ matrixes incubated in citrate/phosphate buffer pH = 7 at room temperature. One could note the shrinking over the time period. There is no difference in the three different G/TA molecular ratios tested.

Tannic Acid Cytotoxicity

The cytotoxicity of TA was evaluated using pNPP test, representing the activity of the cellular acid phosphatase. The experiment was performed on NIH 3T3, a mouse fibroblast cell line (ATCC® CRL-1658). Cells were cultivated in DMEM + 1 g/L glucose (EMEM, LGC Standards) + 10% FBS + 100 □g/mL penicillin + 100 g/mL streptomycin.

24h prior the experiment, cells were seeded in a 96 well plate at a concentration of 1.10^4 cell/well to allow cell adhesion. Then 200 L of complete medium with various concentration of tannic acid were added to each well (7 samples per TA concentration). After 24 h incubation at 37 °C at 5% CO_2, wells were washed three times with 200 L of PBS, and 200 L of pNPP solution (sodium acetate 0,1 M + 0.1% triton X-100 + 10 mM pNPP, pH = 5.5) were added. After 2 h of incubation at 37 °C the reaction is stopped with 10 L of 1 N NaOH solution and 100 L of each well transferred to a new 96 well plate. Absorbance at 405 nm was then read on a microplate reader (Xenius, Safas, Monaco). Cell viability was express as percentage $\pm SE$ compared to the positive control. The experiment was repeated three times. Only one is represented as representative.

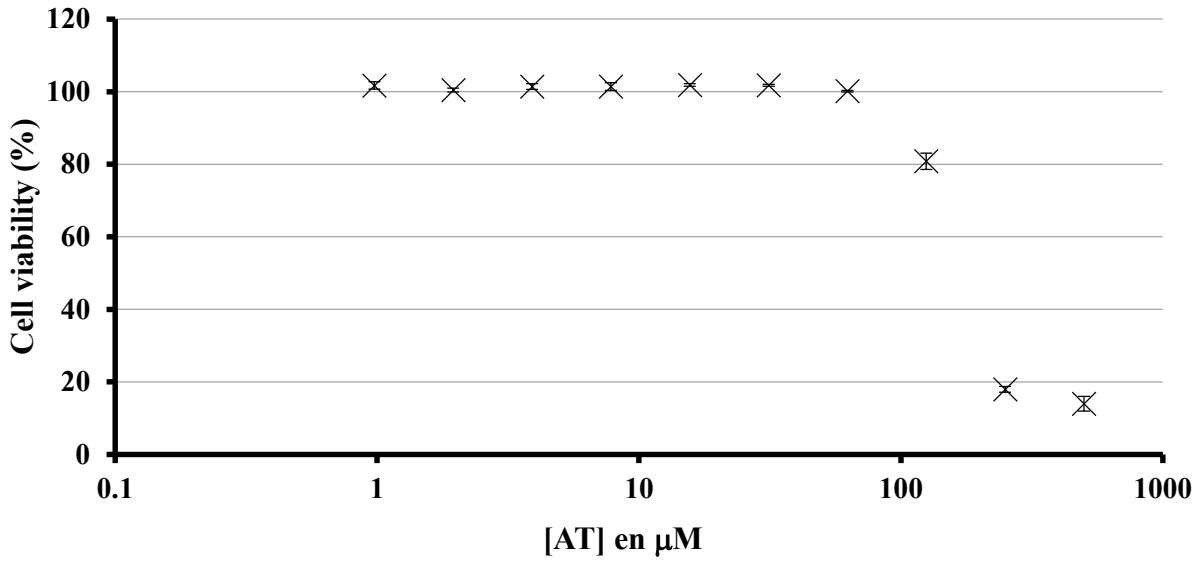

Figure S3. TA cytotoxicity on NIH3T3 cells after 24 h incubation.