Supporting information

Micro-stratified architectures based on successive stacking of alginate gel layers and poly(L-lysine)/hyaluronic acid multilayer films aimed to tissue engineering

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Fig. S1 Vertical section image obtained by CLSM of PLL^{FITC}-AGL-PLL^{FITC} with the deposition time of PLL^{FITC} on the top of AGL of (a) 5 min (b) 25 min. AGL was obtained by spraying the alginate solution (Δt = 10 s) followed by the spraying of a 0.05 M $CaCl_2$ solution during 5 s. PLL^{FITC} was prepared in 0.05 M $CaCl_2$ solution. The scale bar represents 20 µm.

Fig. S2 Evolution of the thickness of AGL versus the spraying time (Δt) of the alginate solution on a PLL^{FITC} monolayer. The thickness was determined on CLSM vertical section images of the PLL^{FITC}-AGL-PLL^{FITC} architecture. After the spraying of the alginate solution, AGL was obtained by dipping the architecture into a 0.05 M $CaCl_2$ solution for 2 h. A minimum of 5 different areas were imaged on three different samples to determine the standard error shown as error bars.

Fig. S3 Opposite of the QCM-D resonance frequency shift ($-\Delta f/\nu$) at 15 MHz after deposition of PLL and HA layers on a quartz crystal coated with SiO_2. Multilayer films were built in a 0.15 M NaCl solution (○) and in a 0.05 M $CaCl_2$ solution (■). The ratio 3:1 between the molar concentrations of NaCl and $CaCl_2$ was used to have the same ionic strength in both salt solutions.

Materials and methods

Confocal Laser Scanning Microscopy (CLSM)

Confocal laser scanning microscopy investigations of the films were performed in rinsing solution ($CaCl_2$ or NaCl solution). CLSM observations were carried out with a Zeiss LSM microscope using a 40×/1.4 oil immersion objective and a 0.43-µm z-section interval. For all CLSM observations unless otherwise stated, the adsorption of poly(L-lysine)-fluorescein (PLL^{FITC}) was performed by dipping the architecture for 5 min in a PLL^{FITC} solution (prepared at 1.5×10^{-3} M in a $CaCl_2$ or a

NaCl solution) and rinsed with a $CaCl_2$ or a NaCl solution before its observation. FITC fluorescence was detected upon excitation at 488 nm, through a cut-off dichroic mirror and an emission band-pass filter of 505-530 nm (green). Virtual vertical sections can be visualized, allowing the film thickness to be determined (Figs 2-7 and Figs S1, S2). The architectures were never dried.

Quartz Crystal Microbalance – Dissipation (QCM-D) Measurements

The construction of multilayer films was monitored *in situ* by quartz crystal microbalance using the axial flow chamber QAFC 302 (QCM-D, D300, Q-Sense, Götenborg, Sweden). The QCM technique consists in measuring the resonance frequency changes (Δf) of a quartz crystal induced by polyelectrolyte adsorption on the crystal, when compared to the crystal in contact with a rinsing solution (aqueous NaCl or $CaCl_2$ solution). The quartz crystal is excited at its fundamental frequency (5 MHz), and the measurements are performed at the first, third, fifth and seventh overtones (denoted by ν = 1, 3, 5 and 7, respectively) corresponding to 5, 15, 25, and 35 MHz, respectively. Changes in the resonance frequencies, Δf, and in the relaxation of the vibration once the excitation is switched off are measured at these four frequencies. The relaxation measurement gives access to the change in the dissipation factor, D, of the vibration energy stored in the resonator. The crystal used is coated with a ~50-nm SiO_2 film. The measurement methodology has been addressed in details elsewhere.[1] A 0.15 M NaCl solution was injected into the measurement cell by gravity. After stabilization of the signals, 500 µL of the poly(L-lysine) (PLL) solution containing either NaCl or $CaCl_2$ was injected, left in the cell for 10 min, and rinsed for 10 min with the 0.15 M NaCl solution. During the whole process, the frequency shifts were continuously recorded as a function of time. The same procedure was used for the deposition of hyaluronic acid (HA) by adding 500 µL of its solution also containing either NaCl or $CaCl_2$. The construction was pursued by alternate depositions of PLL and of HA. A positive shift in the opposite of the normalized frequency shift, $-\Delta f/\nu$, can be associated, in first approximation, with an increase of the mass adsorbed on the crystal (Fig. S3).

Reference :

1. J. Zhang, B. Senger, D. Vautier, C. Picart, P. Schaaf, J.-C. Voegel and P. Lavalle, *Biomaterials*, 2005, **26**, 3353-3361.

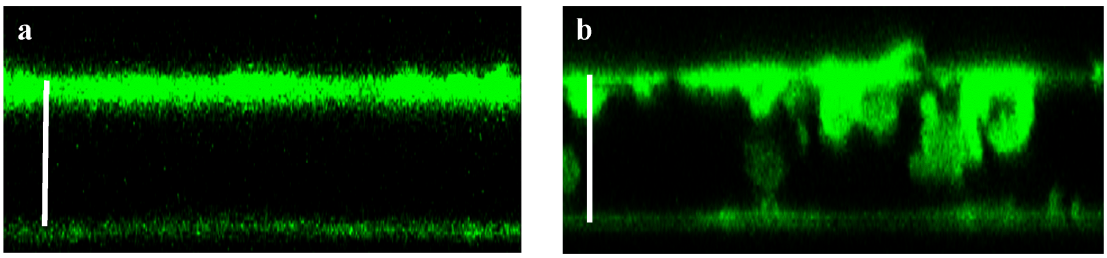
a
b

Fig. S1

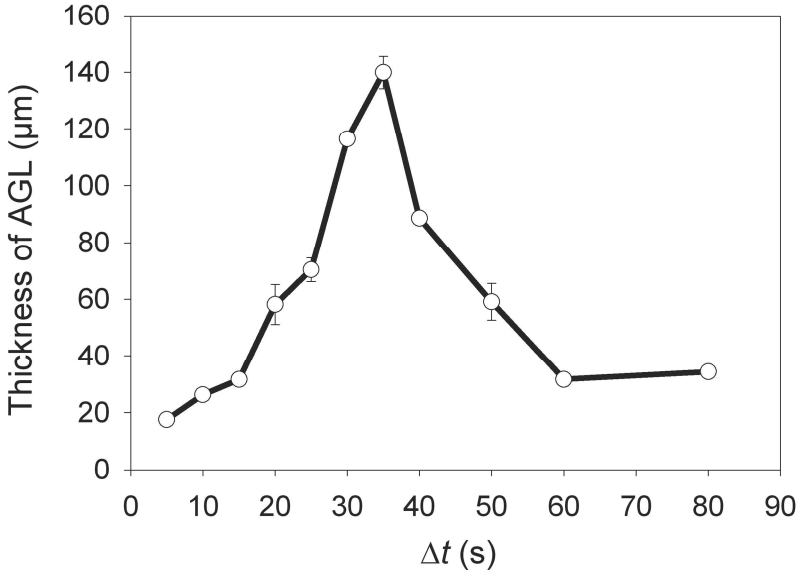
Thickness of AGL (μm)
160
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
Δt (s)

Fig. S2

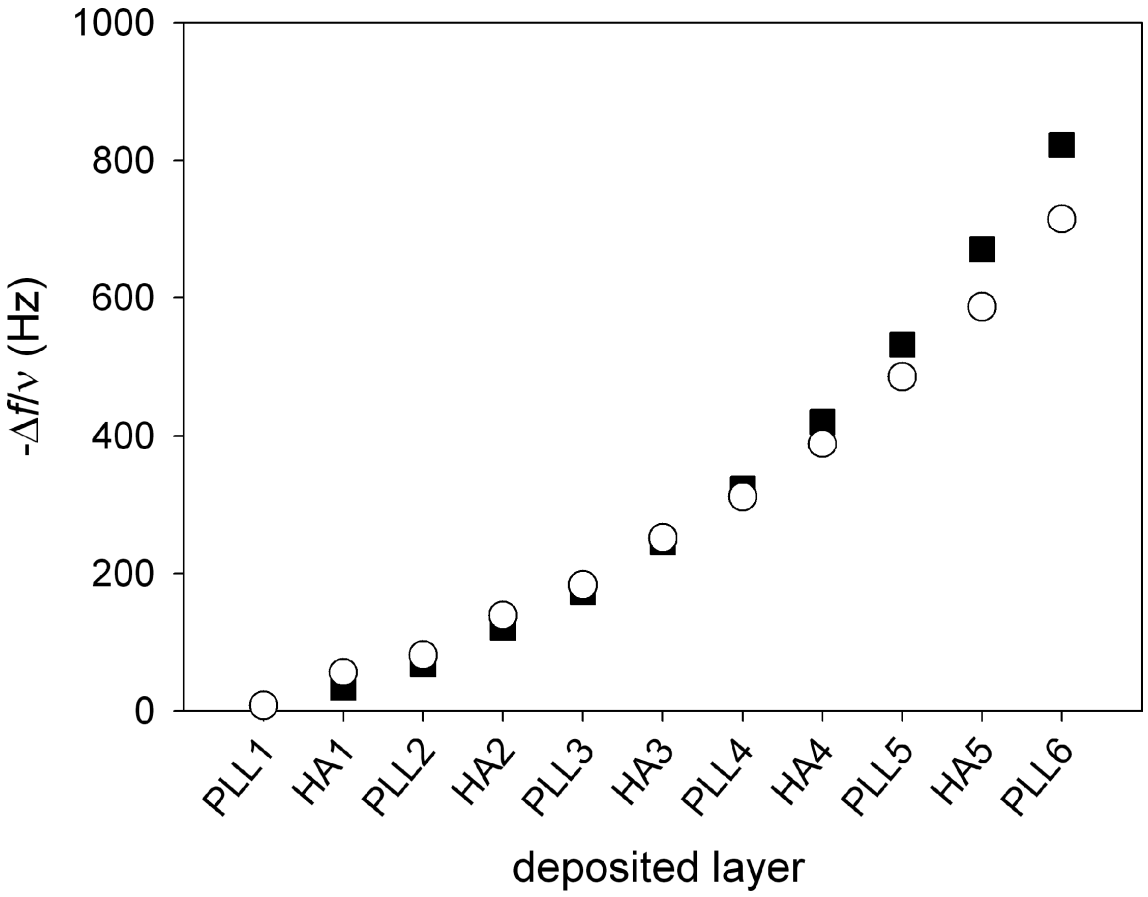
1000
800
600
400
200
0
-Δf/ν (Hz)
PLL1
HA1
PLL2
HA2
PLL3
HA3
PLL4
HA4
PLL5
HA5
PLL6
deposited layer

Fig. S3