

Supporting Information

Polysaccharide films built by simultaneous or alternated spray: a rapid way to engineer biomaterial surfaces

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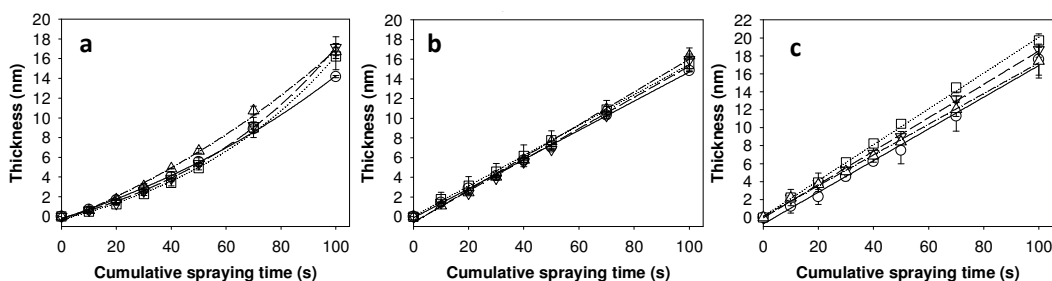


Figure S-1: Thickness of polysaccharide films obtained by alternated spray as a function of the cumulative spraying time of (a) CHI and HA, at 150 mM NaCl and pH 4, with a constant CHI spraying rate of $(4.1 \pm 0.5) \times 10^{-4}$ mol/s and for different sprayed [HA]/[CHI] charge ratio: 0.22 (\circ), 0.54 (∇), 0.58 (\square) and 1.93 (\triangle) (b) CHI and ALG, at 150 mM NaCl and pH 4, with a constant CHI spraying rate of $(3.9 \pm 0.2) \times 10^{-4}$ mol/s and for different sprayed [ALG]/[CHI] charge ratio: 0.39 (\circ), 0.76 (∇), 0.96 (\square) and 3.96 (\triangle) (c) CHI and CS, at 150 mM NaCl and pH 4, with a constant CHI spraying rate of $(4.1 \pm 0.2) \times 10^{-4}$ mol/s and for different sprayed [CS]/[CHI] charge ratio: 0.32 (\circ), 0.97 (∇), 1.08 (\square) and 3.08 (\triangle). The data represents the mean of two film buildups and the error bars the standard deviation on those two values.

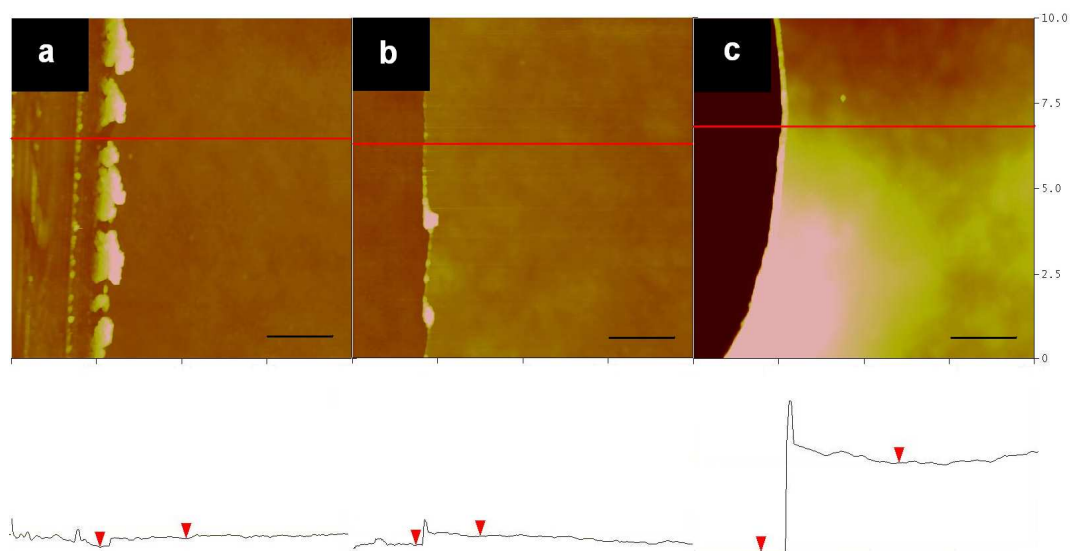


Figure S-2: AFM topography (**top**) and line profiles (**bottom**) showing the growth of CHI/HA films built by simultaneous spraying (**a**) 25 s, (**b**) 50 s and (**c**) 200 s of cumulative spraying time with a CHI spraying rate of 3.18×10^{-4} mol/s and HA/CHI molar charge ratio of 0.6. The solutions were prepared at pH 4 in 150 mM NaCl. The scale bar represents 2 μ m. The films were scratched in to check the full coverage of the substrate. The z scale is of 100 nm. The vertical distance between the two red arrows is of (**a**) 2 nm, (**b**) 15 nm and (**c**) 67 nm.

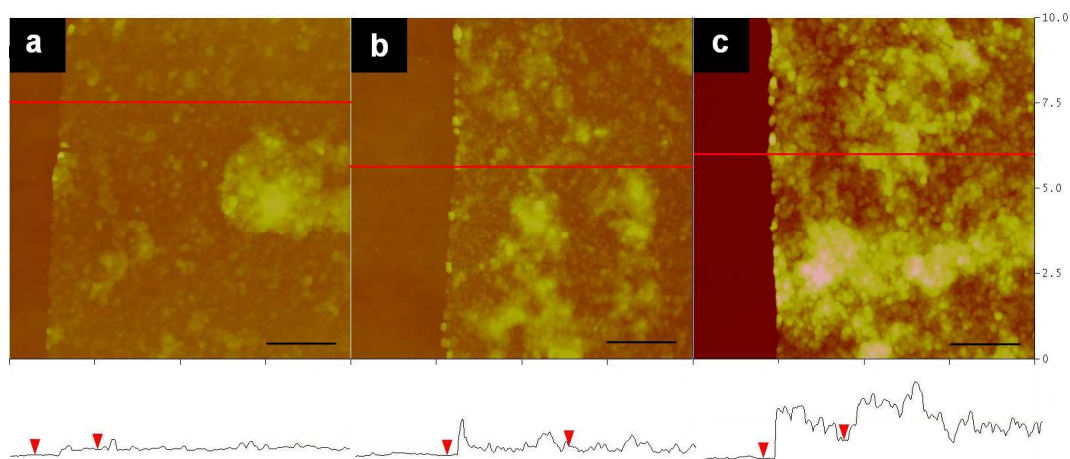


Figure S-3: AFM topography (**top**) and line profiles (**bottom**) showing the growth of CHI/ALG films built by simultaneous spraying with (**a**) 25 s (**b**) 50 s and (**c**) 200 s of cumulative spraying time with a CHI spraying rate of 3.8×10^{-4} mol/s and ALG/CHI molar ratio of 0.9. The solutions were prepared at pH 4 and 150 mM NaCl. The (x, y) scale bar represents 2 μ m. The films were scratched in to check the full coverage of the substrate. The z scale is of 150 nm. The vertical distance between the two red arrows is of (**a**) 4 nm (**b**) 17 nm and (**c**) 30 nm.

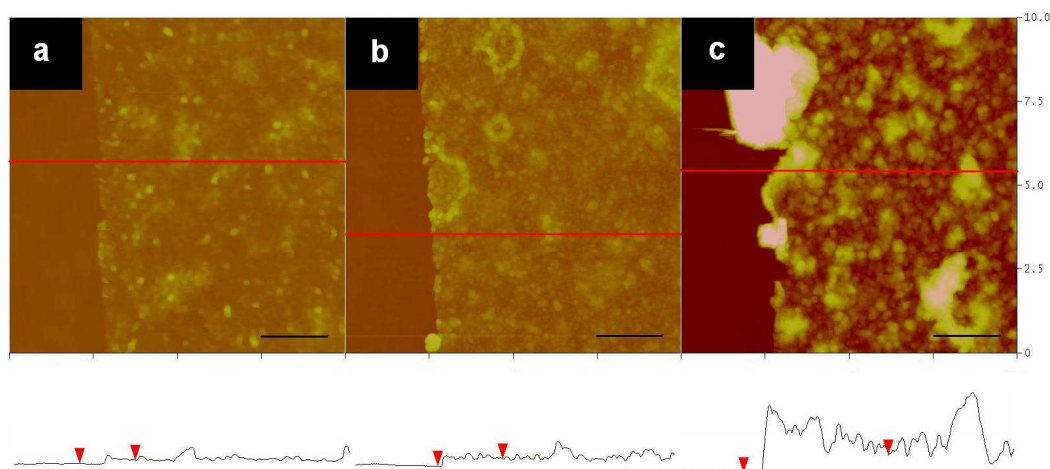


Figure S-4: AFM topography (**top**) and line profiles (**bottom**) showing the growth of CHI/CS films built by simultaneous spraying on silicon wafer with (a) 25 s, (b) 50 s and (c) 200 s of cumulative spraying time with a CHI spraying rate of 3.68×10^{-4} mol/s and CS/CHI molar ratio of 1.05. The solutions were at pH 4 in 150 mM NaCl. The scale bar represents 2 μ m. The films were scratched in to check the full coverage of the substrate. The z scale is of 250 nm. The vertical distance between the two red arrows is of (a) 6 nm (b) 13 nm and (c) 30 nm.

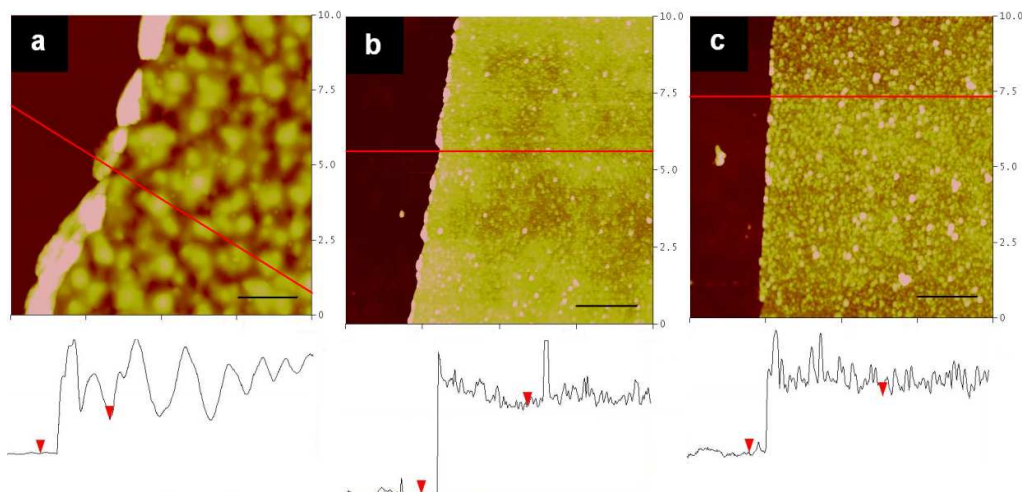


Figure S-5: AFM topography (**top**) and line profiles (**bottom**) of (a) CHI/HA film of 23 bilayers (b) CHI/ALG film of 9 bilayers and (c) CHI/CS film of 7 bilayers built by alternated spraying process at a polyanion/CHI molar charge ratio of 0.6, 0.9 and 1.05 respectively. The solutions were prepared at pH 4 and 150 mM NaCl. The scale bar represents 2 μ m. The films were scratched in to check the full coverage of the substrate. The z scale is of (a) 180 nm, (b) 20 nm and (c) 30 nm. The vertical distance between the two red arrows is of (a) 36 nm and (b, c) 12 nm.

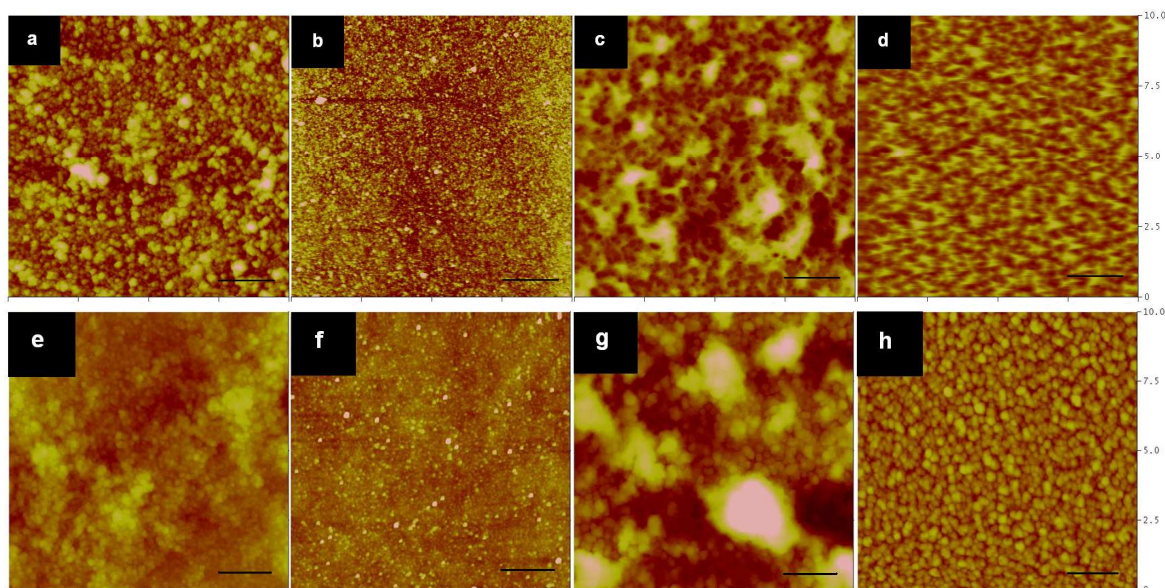


Figure S-6: AFM morphology in wet state of (a) CHI/ALG films obtained by simultaneous spraying with 125 s of cumulative spraying time, (b) CHI/ALG films obtained by alternated spraying with 25 bilayers, (c) CHI/CS films obtained by simultaneous spraying with 125 s of cumulative spraying time, (d) CHI/CS films obtained by alternated spraying with 25 bilayers and in dry state of (e) CHI/ALG films obtained by simultaneous spraying with 125 s of cumulative spraying time, (f) CHI/ALG films obtained by alternated spraying with 25 bilayers, (g) CHI/CS films obtained by simultaneous spraying with 125 s of cumulative spraying time, (h) CHI/CS films obtained by alternated spraying with 25 bilayers. The solutions were prepared at pH 4 and 150 mM NaCl. The films were built with CHI spraying rate of 4.27×10^{-4} mol/s and respectively with ALG/CHI molar charge ratio of 0.9 and CS/CHI molar charge ratio of 1.05. The (x, y) scale bar represents 2 μ m and the z scale is of (a, c, d, e, g and h) 300 nm and (b and f) 20 nm.

Table S-1: Film thickness, measured in the dry state by ellipsometry and in the wet state by AFM, swelling degree, defined as a ratio of wet and dry thickness, and roughness (RMS), determined from AFM images, of polysaccharide films, obtained by simultaneous and alternated spraying and used for cellular tests.

| Polysaccharides system | Type of spray | Thickness in dry state (nm) | Thickness in wet state (nm) | Swelling degree | Roughness in wet state (nm) |
|------------------------|---------------|-----------------------------|-----------------------------|-----------------|-----------------------------|
| CHI/HA | simultaneous | 51 ± 6 | N.D. | N.D. | N.D. |
| | alternated | 49 ± 6 | N.D. | N.D. | N.D. |
| CHI/ALG | simultaneous | 55 ± 7 | 128 | 2.3 | 38 |
| | alternated | 54 ± 1 | 104 | 1.9 | 3 |
| CHI/CS | simultaneous | 52 ± 6 | 82 | 1.6 | 40 |
| | alternated | 74 ± 8 | 98 | 1.3 | 33 |

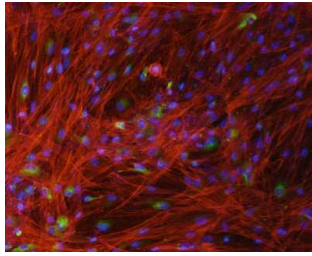


Figure S-7: Cytoskeleton visualization by actin filament staining with phalloidin® (red labeling) and detection of COL1 (green labeling) by immunochemistry and blue counterstaining of HGFs after 12 days of culture on bare glass.