Supporting Information

Dopamine-functionalized sulphated hyaluronic acid as a titanium implant coating has enhanced biofilm prevention and promotes osseointegration

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1.0 NMR Characterization of sHA2-DA (17.1)

The degree of substitution (% mol/mol of DA *vs* sHA2 r.u.) of sHA2-DA (17.1) derivative was confirmed by ^{1}H-NMR spectroscopy in D_2O on a Bruker Advance spectrometer operating at 300 MHz. A derivatization degree of 17.7 ± 0.5 % mol/mol was determined. The signals used for the determination were: ^{1}H-NMR (D_2O) δ 1.9-1.7 (s, 3H, sHA2-NHCO·C*H*$_3$); 2.8-2.6 (m, 2H, sHA2-CONH(C*H*$_2$)$_2$·C$_6$H$_3$-(OH)$_2$); 6.9-6.6 (m, 3H, sHA2-CONH(CH$_2$)$_2$·C$_6$*H*$_3$-(OH)$_2$) (Fig. S1).

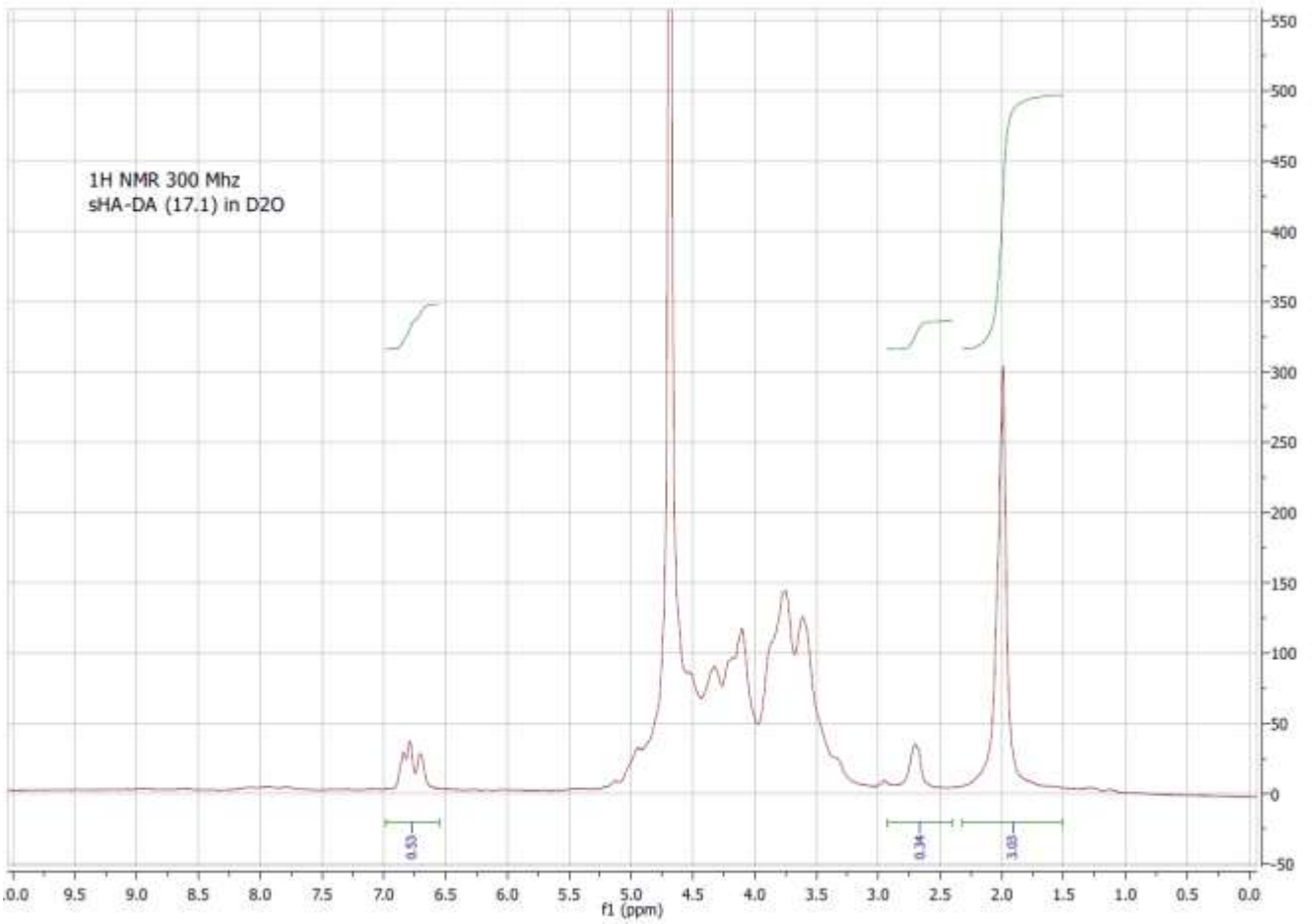

Figure S1. [1]H NMR spectrum of sHA-DA (17.1) derivative (D_2O, 300 MHz, 302 K)

2.0 GPC Characterization of sHA2-DA (17.1)

The degree of substitution (% mol/mol of DA vs sHA2 r.u.) of sHA2-DA (17.1) derivative was determined by GPC-UV analysis by means of a HPLC Agilent 1200 series, equipped with a UV detector set at 280 nm. Bio Gel TSK-30 column was eluted with a mobile phase (80%: MES 0.5M, pH 5 and 20%: ACN) at 20 °C; flow rate: 0.8 mL/min; injection loop: 20 µL. All the acquired chromatograms were processed with EZChrom Elite software, using dopamine hydrochloride as standard (Fig. S2; blue line, RT= 18.3 min; purity: 100.0 %), at 280 nm. sHA2-DA (17.1) polymer elutes at 9.5 min (Fig. S2; red line, purity: 99.6 %), with calculated derivatization degree of 17.1% mol (of DA vs sHA r.u.). The unreacted DA in the sHA2-DA (17.1) sample was quantified as 0.4 % of the total area.

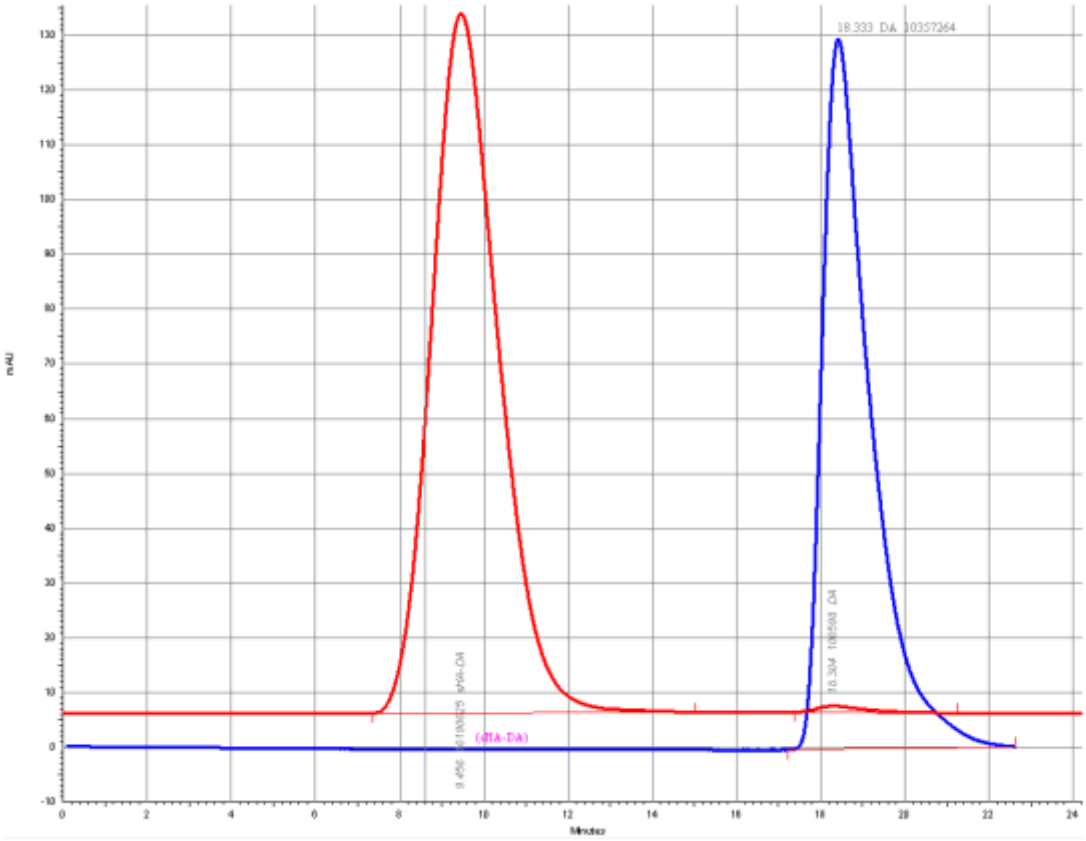

Figure S2: GPC chromatograms of dopamine hydrochloride (blue line) and sHA-DA (17.1) sample (red line).

3.0 Antibacterial activity

Titanium alloy rods were sprayed with a sHA2-DA or HA-DA solution, dried and then sprayed again with a gentamicin solution. Each titanium alloy rod was washed several times in pure water to remove the unbound gentamicin and finally incubated in 5 mL of a *S. aureus* solution ($6x10^6$ CFU/mL). After 6 h, 12 h, 1 day, 2 days, 6 days and 3 weeks, an aliquot of these solutions was collected, diluted and plated on agar for a CFU count. The row data are shown in table S1.

	Total Bacterial Count, S. aureous (CFU/mL)		
Time (h)	CTRL	HA-DA (18.2)	sHA2-DA (17.1)
6	240000000 ± 28800000	6900000 ± 897000	200000 ± 46000
12	310000000 ± 24800000	3700000 ± 518000	66000 ± 13200
24	400000000 ± 36000000	110000 ± 22000	13000 ± 2210
48	210000000 ± 16800000	910000 ± 200200	8000 ± 1760
144	50000000 ± 6000000	17000000 ± 3910000	4200 ± 1134
501	11000000 ± 1430000	19000000 ± 1520000	3100 ± 589

Table S1: Antibacterial activity at different time points due to antibiotic release from a titanium alloy rod, after the washing step, uncoated (CTRL) or previously coated with sHA2-DA (17.1) and gentamicin or HA-DA (18.2) and gentamicin.