Supporting Information

Biomimetic HyperbranchedPoly(amino ester)-based Nanocompositeas Smart Bone Adhesive for Sternal Closure

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Experimental Section

Materials: Dopamine hydrochloride, trimethylolpropanetriacrylate, triethylamine, 4-methoxy-benzaldehyde, iron(III) chloride ($FeCl_3$) (reagent grade, 97%), potassium permanganate ($KMnO_4$), dimethyl sulfoxide (DMSO) and hydroxyapatite (reagent grade) were purchased from Sigma. Tetrahydrofuran (contains 0.025% BHT as stabiliser), hexane 95%, ethanol (99%), acetone 99.8+% were purchased from Fisher Scientific. KRYPTONITE™, a commercially available bone adhesive, was obtained as a gift from Dr. Mark Da Costa. PMMA sheets (60mm x15mm x3mm) were purchased from Goldstarplastics. Sternal bones were obtained from a meat factory and kept at -20ºC before use. For cell culture, Dulbecco's Modified Eagle's Medium (DMEM) was obtained from Sigma, fetal bovine serum (FBS), trypsin/ethylenediaminetetraaceticacid, penicillin–streptomycin(pen/strep) and phosphate-buffered saline (PBS, pH 7.4) were obtained from Gibco BRL. AlamarBlue® assays were purchased from Invitrogen Corporation and used per manufacturer's protocol.

Poly (dopamine-co-acrylate) (PDA) synthesis: Controlled poly-condensation polymerizations were conducted by a one-pot and one-step procedure, in which trimethylolpropanetriacrylate monomer (2.96 g, 10 mmol), dopamine hydrochloride monomer (1.90g, 10 mmol) and DMSO (10 g) were simultaneously added. 4- methoxy-benzaldehyde as inhibitor (60 µL) was added into the mixture to avoid the possibility of free radical polymerization. The mixtures were stirred until a clear solution formed. Oxygen was removed by bubbling argon through the solution for 10 mins at room temperature. TEA (1.42 g, 14 mmol) which was used to modify the pH value to around 8 in the synthesis reaction was added at last when bubbling through argon. The polymerizations were carried out for 2 hours, 2.5 hours and 3 h respectively at 60ºC in order to get a PDA polymer with low, medium and high molecular weights. Then excessive trimethylolpropanetriacrylate monomer (2.96 g, 10 mmol) was added to react for another 2 hours for endcapping the secondary amine groups left in the polymer. After polymerization, the product was diluted with THF(15 mL) and the clear polymer solution was separated from TEA saline precipitate by centrifugation (20mins, 4000 rpm). The clear polymer solution was added dropwise into precipitation agent (500 mL, hexane:ethanol = 1:1) with 4- methoxy-benzaldehyde (100 µL) under stirring conditions in order to precipitate the pure PDA polymer. The impurities and the monomer residuals were poured out with the precipitation agent after being kept still for 20 mins. Finally, the polymer at the bottom of the beaker was dried at room temperature in a vacuum oven to remove the residual solvent. The product yields were all above 80%.

Crosslinking of PDA by different curing agents and the help of additives: For all

mechanical tests, PDA was first dissolved in acetone to a concentration of 30 wt% by vortexing for 1 min. The curing conditions were optimized by shear lap tests of30 wt% PDA polymer (60 µl) with 20 mmol/L $FeCl_3/H_2O$(10 µl) and $KMnO_4/H_2O$(10 µl) as crosslinker and ethanol(10µl) as additive on PMMA sheets. The fully mixed adhesive fluid was applied at room temperature to both surfaces of PMMA substrates within a gluing area of 20 mm *15 mm. When doing *ex-vivo* (compression and tension) tests on lamb sternal bone, PDA adhesives were prepared by ultrasonic mixing PDA solution (50 wt%, 600µl) with crosslinker (20 mmol/L $FeCl_3$, 100 µl), additives (ethanol, 100µl) and hydroxyapatite(80 mg,10%w/v) as a re-enforcer and then the fully mixed putty adhesive fluid was applied at room temperature to both surfaces of lamb sternal bones which were put in PBS buffer solutions before tests. The two bone pieces were then bonded. Gauze saturated with PBS was wrapped around the samples to maintain hydration throughout the duration of the curing time. For comparison purposes, PMMA sheets and lamb sternal bone were adhered together using a commercially available bone cement (KRYPTONITE™) which was prepared according to manufacturer's instructions. The samples were left curing at 37^{o}C incubator for a period of 4 hours, 12 hours, 1day, 4 days and 7 days before strength testing.

In-vitro adhesion strength measurements: Shear-Lap stress measurements were performed on PMMA sheets to get the longitudinal shear modulus following the procedures described in ASTM standard F2255-05. Slight modifications to the protocol were used where appropriate. Samples of PMMA measuring 60 mm x15 mm x 3 mm were used as bone substitute for shear lap tests. A Zwick tensile tester with a 1000 N load cell was used, which comprised of a fixed member that carried one substrate holder and a movable member that carried the loading cell, two compatible holders which fixed the test specimens between two members and a driving mechanism that allowed the moving member to move at a constant rate as required. Nylon was selected as the material for use as the substrate holders. Nylon provided sufficient strength to withstand the shear stresses imposed on the piece. G-clamps were used to fix the samples onto the holders. The bonded fixtures were loaded until failure at a cross-head speed of 5 mm/min. A minimum of five samples per group were tested using this approach.

Ex-vivo mechanical strength measurements: The Instron 4467 materials testing systems were used both in compression and lateral distraction testing. The adhesion strength at failure by loading compression and lateral distraction strength were measured with lamb sternal bone specimens bonded while wet. Each sternum was cut vertically in half as in a median sternotomy using a band-saw and then was cut horizontally into 10 samples. After cutting the samples, 5 pairs of samples were tied together with elastic bands and placed in PBS buffer for 24 hours.

Compression measurements were performed on lamb sternal bone following the procedures described in ASTM standard D905–08e1 with modifications. The machine had interchangeable clamps to allow for compression tests. A compressor was chosen specifically for this test to focus the applied force on the adhesion site. This would ensure the most accurate results from the experiment. Before the experiment the bonding samples were mounted on two blocks to prevent the compressor from just crushing the bone and to ensure that the results obtained were for the strength of the adhesion at the site of the cut. A third block was used to keep the space between the mounting blocks consistent for all experiments. The bonding site was carefully aligned with the centre of the compressor. The rate of the crosshead movement was 5 mm/min. After testing, the bonding area of the bone at the site of adhesion was measured.

The lateral distraction tests were performed on lamb sternal bones following the procedures described in ASTM standard D2095-96 with modifications. The bones were drilled through using a 3 mm drill bit and hand drill. The hole was drilled a little back from the adhesion site

to prevent damaging the surface area. After drilling, the samples were glued and left curing for certain periods, then the bonding samples were clamped sideways in the machine by a pair of vices which move at a constant rate of 5 mm/min in the opposite direction. A metal strip with holes was used to fix the bones to the vice. The metal strip was held to the bone using a 3 mm threaded bar and nuts. The ends of the metal strip that were not connected to the bone were clamped in the vices. For some of the irregular samples it was necessary to cut off the excess meat to ensure the samples were straight and could be placed in the machine without placing an initial pressure on the adhesion. After testing each sample the metal strip and bar was removed and replaced each time and a new sample was tested. After each test the bonding area was measured and the lateral distraction strength was calculated from this using the standard formula.

Degradation study: Degradation was assessed by percentage of weight loss every five days over a period of one month. The adhesive was prepared in a glass vial (5 mL) and immersed in PBS buffer solution (PH = 7.4) in an incubator set at 37°C. A minimum of three samples per group were tested using this approach. Each glass vial was weighed before preparing the adhesive. At every time point, after PBS buffer solution was removed, the adhesive sample was washed with distilled water, dried and weighed again. The overall mass loss was calculated from the recorded initial and final mass values.[1]

Cellular metabolic activity study: 3T3 fibroblasts were cultured in DMEM supplemented with 10% FBS and 1% Pen/Step under standard sterile cell culture conditions at 37^{o}C and 5% CO_2. The cells were seeded in 48 well-plates at a concentration of 10, 000 cells per well. PDA adhesives, with $FeCl_3$ and $KMnO_4$ as crosslinker and ethanol as additive, were added to the cells one day after seeding to result in a final concentration of 2 mg/ml, 500µg/ml and 150µg/ml. To assess the cell viability one, four and seven days post the addition of the adhesive, the alamarBlue® cell metabolic activity method was used as described previously.[2] Cell viability was obtained by normalizing metabolic activity to cells receiving no treatment.

Temperature raise evaluation: 50 wt% PDA polymer(6 ml) was mixed with hydroxyapatite (0.8g)first in a 20 ml glass vial on a digital hotplate stirrer and then 20 mmol/L $FeCl_3/H_2O$(1 ml)and ethanol(1ml)were added successively with the temperature monitored by a temperature probe.

Molecular weight analysis: The molecular weight of the sample was determined by gel permeation chromatography(GPC) (Varian 920-LC) apparatus equipped with an RI detector. The columns (30 cm PLgel Mixed-C, two in series) were eluted using dimethylformamide (DMF) and calibrated using a series of 12 near-monodisperse PMMA standards (Mp from 690 to 1,944,000 g mol^{-1}). The polymers were analysed in DMF at a concentration of 5.0 mg/mL. All calibrations and analysis were performed at 60°C with a flow rate of 1 mL/min.

Statistical analysis: All data were presented as means ± standard deviations, with sample number of at least 5. One way ANOVA was performed and a value of $p < 0.05$ (*) was considered to be statistically significant.

Data Analysis and Calculations

The max load at failure was recorded and used with the bonding area to calculate the max shear stress using the standard shear stress formula:

$\tau max = \frac{Fmax}{area} (Eq.1)$

τmax = max shear stress at failure

Fmax = max tensile load at failure

area = bonding area of adhesive

Our calculation for human sternal application is based on a mathematical model (Eq.2) shows that a distracting force of 58Kg would be imposed on the sternum during a ~13.3 KPa pressure generating by a normal cough, whereas the distending pressure of a maximal cough reaches 40KPa; imposing a force of 174Kg (1695N) which is consistent with the studies by Trumble and Dasika showing that forces from 160N to 400N and 550N to 1650N are imposed on the sternal midline during breathing and coughing respectively. [3,4,5] Casha AR. et al. reported that, for any closure technique to provide suitable stability, it must be able to resist twice the maximum potential stresses applied on the sternum. [6]

$T = RLP = 0.17 * 0.25 * 13.3 = 565$ N (~58Kg) (Eq.2)
T is the tension (N)
R is the cadaver's sternal radius (m)
L is the height of the chest (m)
P is the distending pressure (KPa)
T (1 day) $= RLP = 0.17 * 0.25 * 171.7 = 7297$ N (~745Kg)
T (4 day) $= RLP = 0.17 * 0.25 * 539.3 = 22920$ N (~2339Kg)
T (7 day) $= RLP = 0.17 * 0.25 * 633.2 = 26911$ N (~2746Kg)

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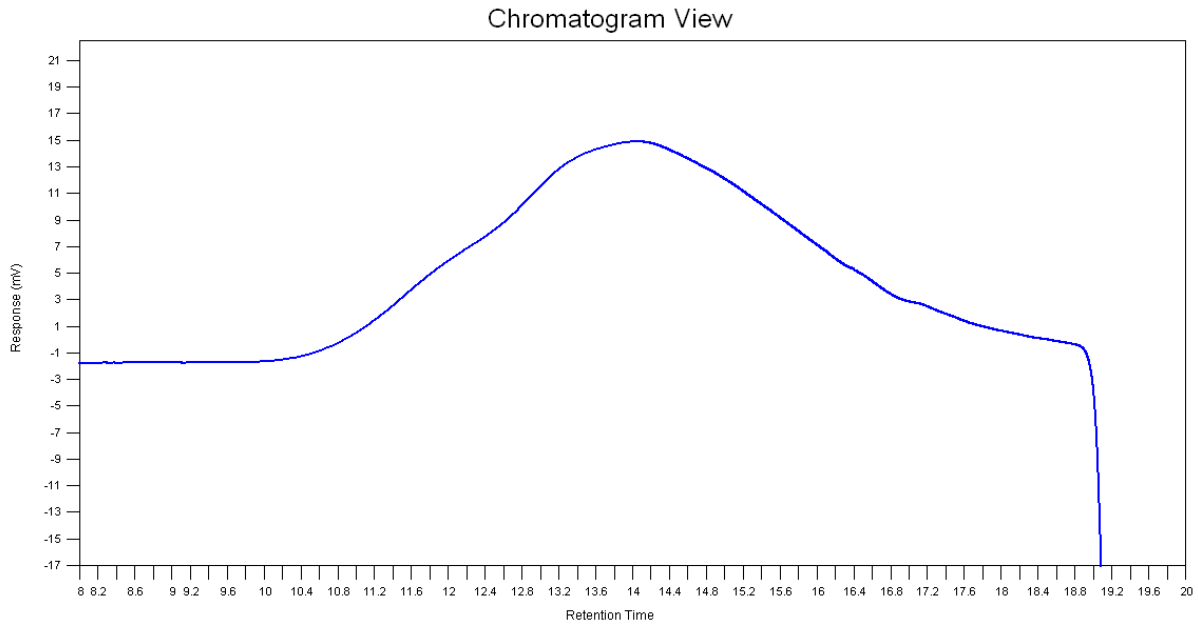

FigureS1. Gel Permeation Chromatography trace for the hyperbranched PDA polymer

Table S1. Hyperbranched PDA molecular weight after purification

Polymer	**M_n (KDa)**	**M_w (KDa)**	**PDI**
PDA	6.3	96.1	15.3

TableS2. Intensity values for the hydrogen atom groups assigned to the poly(dopamine-co-acrylate), obtained from Figure S2.

a: 6.80	d: 2.66	g: 23.49	j: 10.97
b: 3.15	e: 2.70	h: 98.83	k: 6.73
c: 2.74	f: 21.68	i: 22.36	l: 11.50

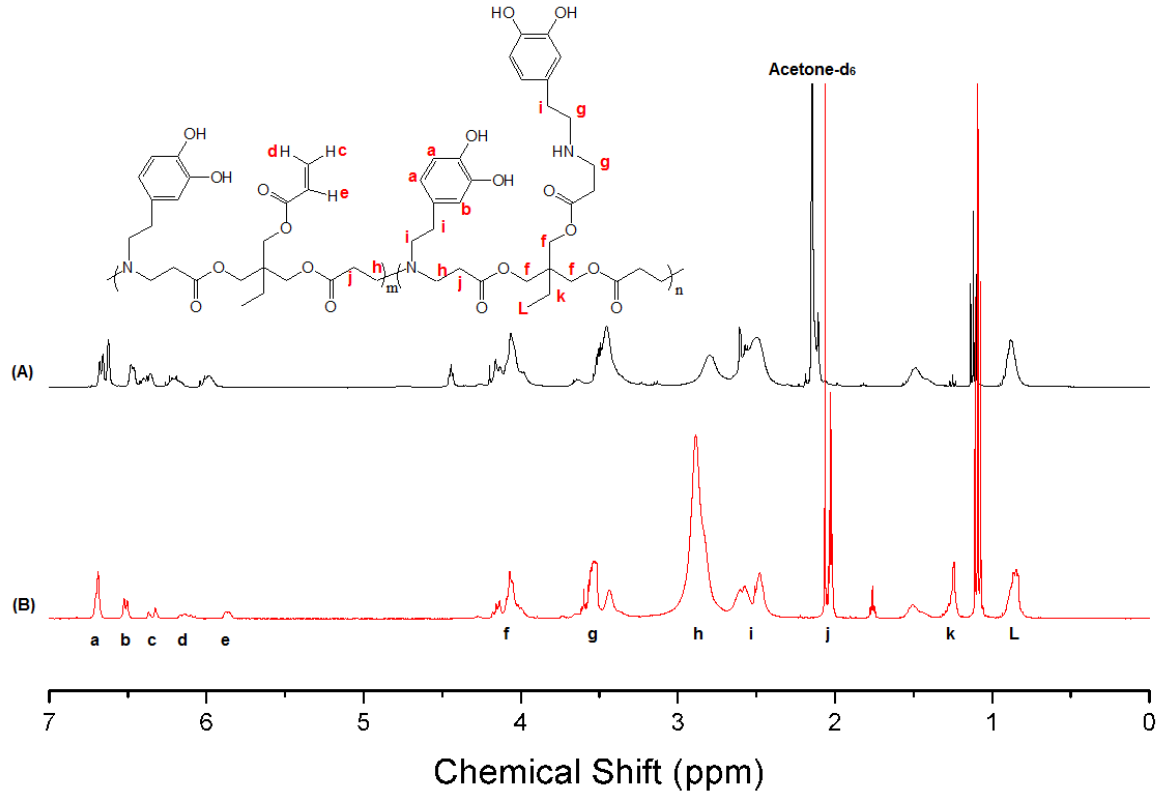

Figure S2.[1]H NMR spectrum (400 MHz, acetone-D_6) of purified hyperbranched PDA (A) without endcapping (B) with endcapping by triacrylate as intensity (vertical axis) versus chemical shift (in ppm on the horizontal axis). Signal from spectrum have been assigned hydrogen atom groups (atoL)from the structure on the left. With endcapping, the integral proton value of peak h ascribed to tertiary amine is bigger than the integral proton value of peak g ascribed to secondary amine and it is conversely contrary without endcapping.

TableS3. Feeding ratio, final composition ratio, vinyl group, DOPA and branching ratio of PDA polymer.

Triacrylate: Dopamine feeding ratio	**Triacrylate: Dopamine composition ratio[a)]**	**Vinyl groups ratio in triacrylatecomposition[b)]**	**DOPA ratio[c)]**	**Free vinyl groups ratio[d)]**
1:1	1:0.86	23.5%	55%	45%

(The calculations to assess the amount of functional groups in the polymer were done according to the values on Table S.2.[a)]Dopamine : triacrylate composition ratio: (a+b)/l = 0.86 : 1; [b)]Vinyl groups ratio in triacrylate composition: (c+d+e) / (l×3) = 23.5 %; [d)]Free vinyl groups ratio = 1×3×23.5% / (0.86 + 1×3×23.5%) = 45 %; [c)]DOPA ratio = 0.86 / (0.86 + 1×3×23.5%) = 55%)

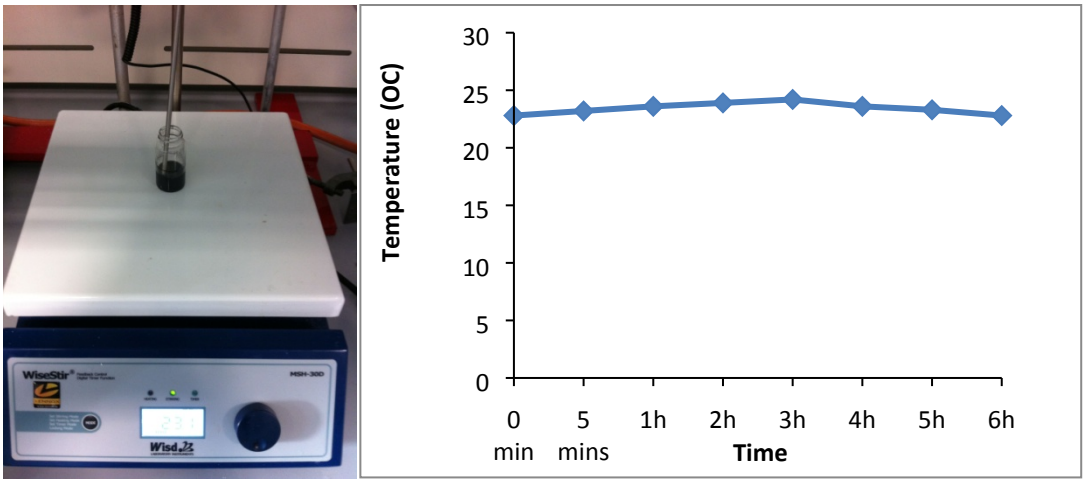

Figure S3.Hotplate stirrer digital MSH-30D with temperature probe was used to monitor the temperature after mixing the crosslinker and additives with PDA polymer. Very minimal heat production occurs, however the temperature increases to 23.2^{o}C from 22.8 ^{o}C in 5 mins and arrives at peak temperature(24.2 ^{O}C) after 3h curing and return to 22.8^{o}C after 6h curing.