Supplementary Information:

Facile immobilization of enzymes on electrospun poly(styrene-alt-maleic anhydride) nano fibres.

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Synthesis of Poly(St-alt-MA)

An alternating copolymer of styrene (extracted with *KOH*, dried over $MgSO_4$, distilled in vacuo) and maleic anhydride (Merck 99%) was synthesized by conventional free radical chemistry in a 1:1 molar ratio styrene : maleic anhydride. *15 g Styrene* monomer was dissolved in *200 mL MEK* (SASOL Chemicals, 99.75%) along with *14.12 g maleic anhydride* and *0.1182 g AIBN* (Riedel-DeHaën ; SIGMA-ALDRICH; Recrystallized from CH_3OH). The reaction mixture was degassed with N_2 (gas) for *30 min* and reacted over night under reflux at *60 ^oC.* The polymer was precipitated in *500 mL* of *iso-propanol* (SASOL Chemicals, *85%*). The polymer was dried in a vacuum oven at room temp, overnight to remove non reacted monomer and solvent. The total amount of polymer recovered was *27.03 g (%Yield = 92.80%)*. ^{1}H-NMR (Varian ^{Unity} Inova, 400MHz) in *d6-DMSO* was used to verify if the co-polymer was in fact synthesized and size exclusion chromatography (*SEC*) was done to establish the molar mass.

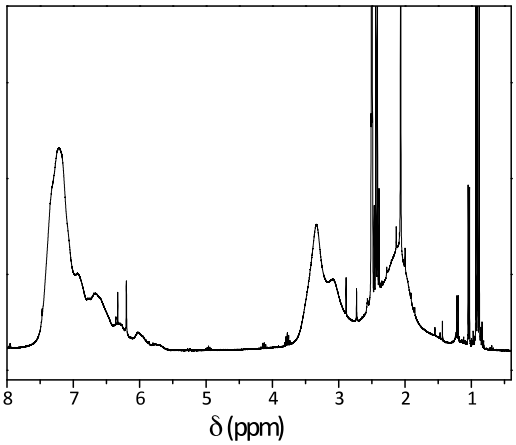

Fig 1: ^{1}H-NMR of poly(Sty-alt-MAnh) δ(d6-DMSO) [ppm]: J=6.5-7.5 Hz, benzyl unit; J=3-3.5,2H, anhydride unit; J=2.1, (C**H**$_2$-CH(C$_6$H5))

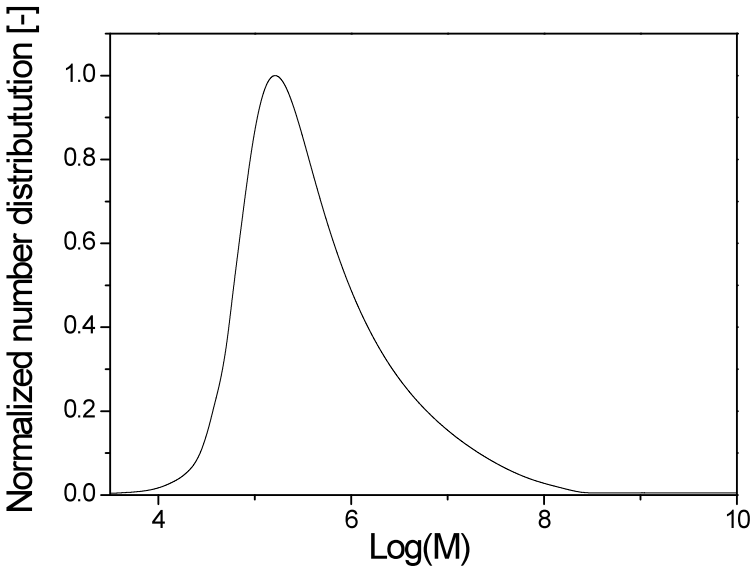

Fig 2: *SEC* analysis done in *THF* (M_w=*252 887Da, PDI= 4.6*)

Electrospinning

The polymer was dissolved in a *1:2 DMF:acetone* solution (*15 wt%*). The collector (aluminium foil) was set up *15 cm* below needle tip with an offset of *4 cm* to the centre of the collector. The polymer solution was fed through a Teflon tube (Hamilton, P/N 8651/00) from a glass syringe mounted in a syringe pump (Harvard, Model 33 Twin Syringe Pump) to the needle (Hamilton, 26 Gauge). The needle was positioned at an angle of *ca*. 30°. A flow rate of *0.01 mL/min* and the high voltage supply (Voltage supply: 25 kV, 400 micro Amps, 10 Watt, high output voltage supply) set at *15 kV* appeared to be the optimum parameters for spinning. The following table consist of all the parameters used in the different electrospinning experiments.

Experiment:	**WC013**				
Sample:		**#1**	**#2**	**#3**	**#4**
Parameters:	**Volts**	**15kV**	**15kV**	**15kV**	**15kV**
	Distance	**15cm**	**15cm**	**15cm**	**15cm**
	Flow Rate	**0.01mL/min**	**0.02mL/min**	**0.03mL/min**	**0.04mL/min**
	Offset	**4cm**	**4cm**	**4cm**	**4cm**
	Concentration	**15wt% SMA**	**15wt% SMA**	**15wt% SMA**	**15wt% SMA**

Table 1: Same experimental setup but different flow rates for electrospinning

Experiment:	WC014			
Sample:		#1	#2	#3
Parameters:	Volts	10kV	15kV	20kV
	Distance	15cm	15cm	15cm
	Flow Rate	0.01mL/min	0.01mL/min	0.01mL/min
	Offset	4cm	4cm	4cm
	Concentration	15wt% SMA	15wt %SMA	15wt% SMA

Table 2: Same experimental setup but electrospinning done at different voltages

SEM analysis was done after vacuum gold coating of some of the resulting fibres at *500X* and *3000X* magnification respectively.

The following images were obtained from the SEM analysis:

WC013_Sample1

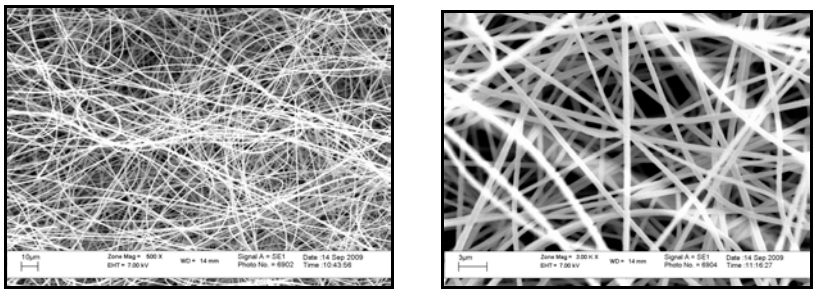

Fig 3: *Poly(Sty-alt-MAh)* fibres Zone Magnification *X500* and *X3000*

WC013_Sample2

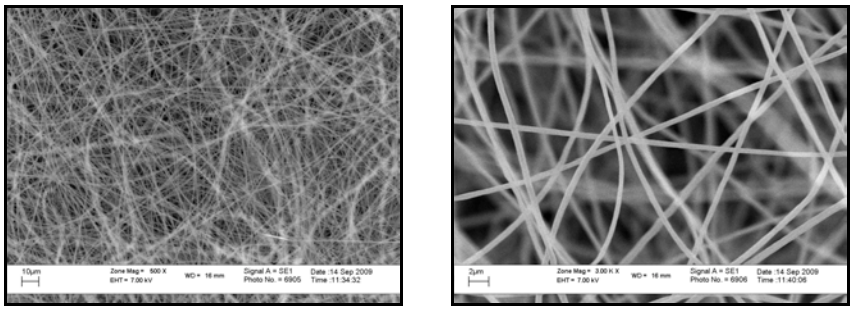

Fig 4: *Poly(Sty-alt-MAh)* fibres Zone Magnification *X500* and *X3000*

WC014_Sample3

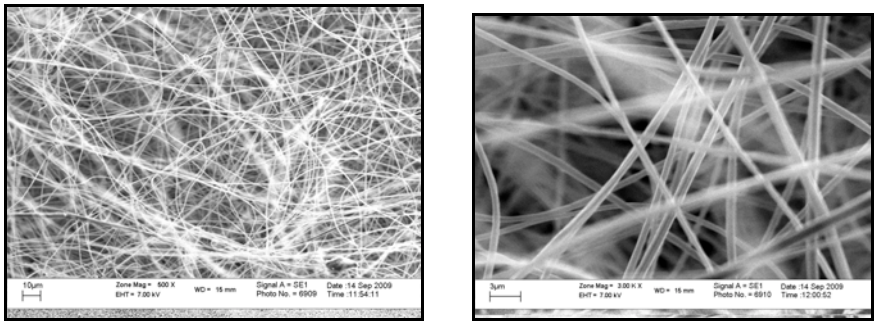

Fig 5: *Poly(Sty-alt-MAh)* fibres Zone Magnification *X500* and *X3000*

WC013_Sample4

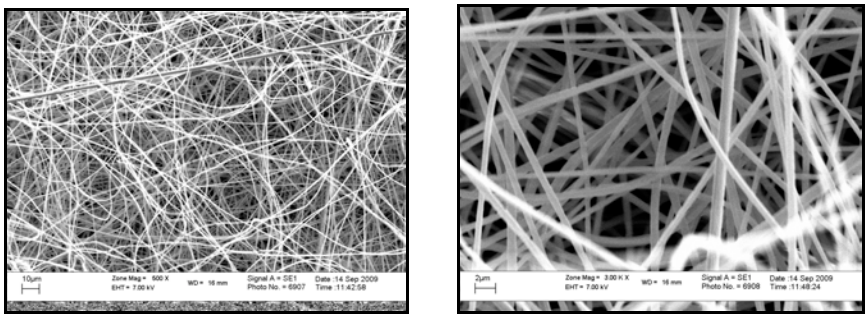

Fig 6: *Poly(Sty-alt-MAh)* fibres Zone Magnification *X500* and *X3000*

From the images it is clear that fairly uniform fibres were obtained and that different spinning conditions, all resulted in fibre diameters *ca*. 300 nm.

Immobilization of *Horseradish Peroxidase (HRP)* on *poly(Sty-alt-MAnh)*

A 4 mg/mL solution was made up by dissolving *HRP* in *PBS* buffer (8 g NaCl; 0.2 g KCl; 11.5 g Na_2HPO_4; 0.2 g KH_2PO_4; pH 7.4) and placed along with the electro spun fibres in a petri dish and incubated for one hour on a Belly Dancer laboratory shaker. The fibres were subsequently washed three times for 10min at a time with PBS-Tween buffer solution (*PBS, 0.01% Tween 20*). The substrate solution *ABTS/H_2O_2* (*6 mg ABTS (2, 2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)*) and *37% H_2O_2, 6 µL* in *12 mL* citrate buffer *0.1 M*, *pH 5*) was then introduced to the fibres and a colour change was observed. The colour change was noted relative to what happens when the *ABTS/H_2O_2* is introduced to fibres where no *HRP* was immobilized and incubated for the same time period in the *PBS* buffer.

Fig 7: Colour change (dark blue) observed relative to a control with no immobilized enzyme and the two substrate solutions used in the left picture after half an hour.

Immobilization of Horseradish peroxidise (*HRP)* and Glucose oxidase (*GOX)* on *poly(Sty-alt-MAnh)*

The same approach was taken as above for the immobilization of *HRP*. The *GOX* was immobilized by incubating the fibres in a petri dish with a *10 mg/mL* solution of *GOX-PBS* (*PBS pH 7.55*). The membranes was washed 3 times for *10 min* at a time with *PBS-Tween* (*PBS, 0.01% Tween 20*) The substrate solution of final concentration *100 µL/mL Ampliflu Red/PBS* was made up of two solutions, *1 mg/mL Dextrose (D-glucose)/PBS* and *10 mmol/L Ampliflu Red/PBS*. The substrate solution was introduced to the membranes and the colour change observed relative to a control. Two experiments were done, one where both *HRP* and *GOX* were immobilized on one membrane and another where *HRP* and *GOX* were immobilized on two separate membranes. In the experiment where the enzymes were immobilized on separate membranes the substrate solution (*Ampliflu Red/PBS/Dextrose*) was introduced to the *GOX* membrane first; the solution was then transferred from there to the membrane with *HRP* and the subsequent colour change was observed relative to a control.

Fig 8: Colour change (pink) observed relative to a control with no immobilized enzyme and the two substrate solutions after half and hour.

Binding efficiency of *HRP*

The binding efficacy of *HRP*(*BBI Enzymes, Sample for Stellenbosch University*) to the *Poly(Sty-alt-MAh)* fibres was determined with the use of antibodies. Primary antibodies (*Anti-HRP*) were raised in rabbits with an immunization schedule of *56 days*. The *HRP* was first incubated as previously discussed with subsequent wash steps. Then the fibres were incubated in casein buffer (*10 mM TRIS, pH 7.6, 0.15 M NaCl, 0.5 % Casein, 0.02 % Thiomersal*) for *20 minutes* in order to block all non specific sites where the antibodies may bind. For the controls only *PBS* was added. After *60 minutes* the *HRP* solution was discarded/decanted and the fibres were washed four times, four minutes each, with PBS buffer containing *0.1% Tween-20* (*PBS-Tween*). *Anti-HRP* antibodies (*1:10 000 in casein buffer*) was incubated with these fibres at *37 ° C* for *2 hours* followed by wash steps as mentioned previously.

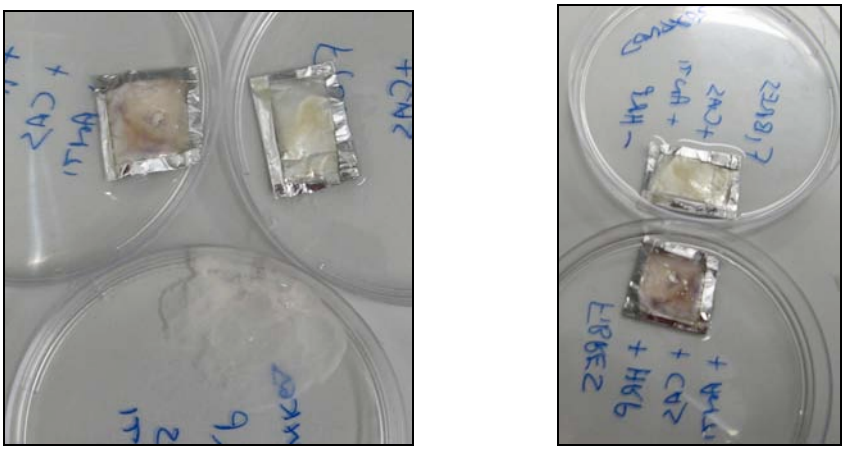

Fig 9: Colour change observed after incubation of primary and secondary *Anti-HRP* antibodies and the introduction of the *BCIP-T/NBT* substrate solution.

A secondary antibody (*Sigma, anti-rabbit IgG, catalogue number: A3687*), conjugated to alkaline phosphotase (1: 20 000), was then added to the fibres and incubated at 37 ° C for 2 hours, also followed by four washes with *PBS-Tween* as before, where after a *5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt /nitro blue tetrazolium (BCIP-T/NBT)* substrate, *33 ml* of *BCIP-T* (*50 mg/ml in DMF*) and *44 ml* of *NBT* (*75 mg/ml in 70% DMF*) in *10 ml* of alkaline phosphotase buffer (*100 mM TRIS, pH 9.5, 100 mM NaCl, 10 mM* $MgCl_2$), was added to the *poly(Sty-alt-Mah)* fibres and incubated at room temperature. The colour change was observed relative to the controls.

BCIP-T (Fermentas Life Sciences [Fermentas], Catalogue number R0821)

NBT (Fermentas Life Sciences [Fermentas], Catalogue number R0841)