Electronic Supplementary Material (ESI) for RSC Advances.

On-column enzymatic synthesis of melanin nanoparticles using cryogenic poly(AAM-co-AGE) monolith and its free radical scavenging and electro-catalytic properties

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Supplementary data

S1. Experimentals:

S1.1. Synthesis of poly(acrylamide-co-allylglycidyl ether) monolith column

The monolith column was synthesized by mixing the monomer units of AAm (400 mg), MBAAm (100 mg) and AGE (100 µL) in 10 mL of degassed ultrapure water. The total concentration of monomers was 6% in the reaction volume. Further, APS (11 mg) and TEMED (9.5 µL) was mixed sequentially into monomer solution. This solution was added into a plastic syringe (Becton Dickinson, India) columns (20 mm height × 8 mm diameter) and was incubated at -12 °C for 16 h in a liquid cryobath (Grant, Germany) to carry out free-radical polymerization. Monolithic column of poly(AAm-co-AGE) was obtained after thawing of these frozen gels at room temperature (25 ± 2 °C) followed by washing with ultrapure water. Finally, these monolith columns were dried and stored at room temperature for further study.

S1.2. Physical properties of poly(AAm-co-AGE) monolith column

A. Morphology analysis

Poly(AAm-co-AGE) monolith matrix was morphologically examined using sample size of 8 mm diameter and 3 mm thickness. The size and distribution of pores in the matrix was examined by scanning electron microscopy (SEM). For SEM analysis, sample was dried for overnight in vacuum

desiccators and was further surface coated with gold using ion sputter coater (Hoyeon Tech., Model-HC 21, South Korea) at 20 mA for 3 min. The gold coated samples were mounted on the sample holder of SEM (FEI Quanta 200) and the analysis was performed at high vacuum (5 kV) with spot size 3.5 mm.

B. Porosity analysis

According to the Archimede's principle, total porosity of the poly(AAm-co-AGE) matrix was calculated theoretically using the value of sample mass at their different time point during the experiment. For that mass of the sample was recorded at initial stage (W_d), then mass of the sample by placing it in the specific gravity bottle that was filled with water. The weight difference of bottle filled with water and bottle filled with water and test sample was calculated, which was referred as submerged mass of sample (W_{sub}). Finally, the water saturated sample mass (W_s) was recorded. These values were used to calculate the porosity of sample using equation (1):

$$\text{Porosity (\%)} = [(W_s - W_d)/ (W_s - W_{sub})] \times 100 \quad (1)$$

C. Swelling kinetic and swelling ratio of poly(AAm-co-AGE) matrix

Swelling kinetic of poly(AAm-co-AGE) matrix was determined by gravimetric weight change during sample immersion in the water and retention capacity was calculated. In this method, initial dry weight (W_d) of sample was taken and then sample was incubated in water and their increase in weight was continuously recorded at pre-defined time intervals (W_t). The maximum weight was considered as water equilibrated sample weight (W_e). These values were used in the equation (2) to calculate the percentage swelling kinetics of poly(AAm-co-AGE) matrix.

$$S_K = [(W_t - W_d)/ W_e] \times 100 \quad (2)$$

The initial dry weight (W_d) and swollen weight at equilibrium (W_e) of the sample was used in the equation (3) to calculate the swelling ratio (S_R) of poly(AAm-co-AGE) matrix:

$$S_R\ (\%) = [(W_e - W_d) / W_d] \times 100 \quad (3)$$

D. Hydraulic permeability and flow rate analysis

According to Darcy law, a relationship between the flow of liquid and pressure was analyzed for determining the hydraulic permeability (κ) of poly(AAm-co-AGE) matrix as per the method described elsewhere.[S1] Poly(AAm-co-AGE) matrix (5 mm height and 8 mm diameter) was placed in the laboratory made permeability measurement setup. To this, a constant water flow to maintain the water pressure was applied in the sample from top for the period of 1 min. The amount of water come out from the outlet of the system was weighed. However, in the control experiments, no sample was used. The obtained values were applied in the equation (4) for calculating the hydraulic permeability.

$$\kappa = \frac{\Delta X}{A \times M_{B2}} \times \frac{2\pi^2 r^4}{\left(M_{B1}/M_{B2}\right)^2 - 1} \quad (4)$$

Where, κ is the hydraulic permeability, A is the flushing area of the sample, ΔX is the thickness of the sample, M_{B1} and M_{B2} are the mass of water flowed from the outlet of control setup and test setup, respectively.

The flow of aqueous medium through the poly(AAm-co-AGE) matrix was determined using a peristaltic pump as described by Adrados et al.[S2] The flow of liquid through the pump was set as a control flow rates of the system. For obtaining the maximum flow rate of the poly(AAm-co-AGE) matrix, the cylindrical monolith (20 mm height and 8 mm diameter) was placed in a plastic syringe mould and then connected in between the flow path of a pre-set pump. The maximum water flow (mL min^{-1}) limit was registered till the monolith was not showing any back pressure, which was defined as a flow rate capacity of the column.

E. Rheological analysis of poly(AAm-co-AGE) matrix

The rheological properties of poly(AAm-co-AGE) matrix was examined under the dry and wet conditions on Modular compact rheometer (MCR 302, Anton Paar, Germany). The monolith was manually cross-sectioned in to small sections (height; 2 mm and diameter; 8 mm) using surgical blade. The test sample was then placed between the plates of rheometer and applied a force at the rate of 1 N s^{-1} with constant frequency (1Hz) and amplitude (0.1%) up to 50 N. The data points were recorded up to the end of test using associated software Rheoplus. The change in value of complex modulus with respect to increasing force was further used to draw a plot. The obtained relationship between complex modulus and force of dry and wet poly(AAm-co-AGE) matrix were used to define the rheological stability of this monolith for bioprocess application.

S3. Results and discussion

S 3.1 Rheological characterization of poly(AAm-co-AGE) monolith

Rheological study of the monolith suggested its high visco-elastic property in dry and wet conditions (Fig. S1). In the dry condition, up to 20 N force, poly(AAm-co-AGE) matix showed stable complex modulus (~ 1×10^7 Pa), however, after that continuous increase in the complex modulus (from 1×10^7 Pa to 2.65×10^7 Pa) was observed up to 50 N. This result suggests that the matrix can maintain its mechanical integrity up to 20N without increasing resistance against the applied force. However, the matrix showed increase in complex modulus as the force was increased above 20 N.

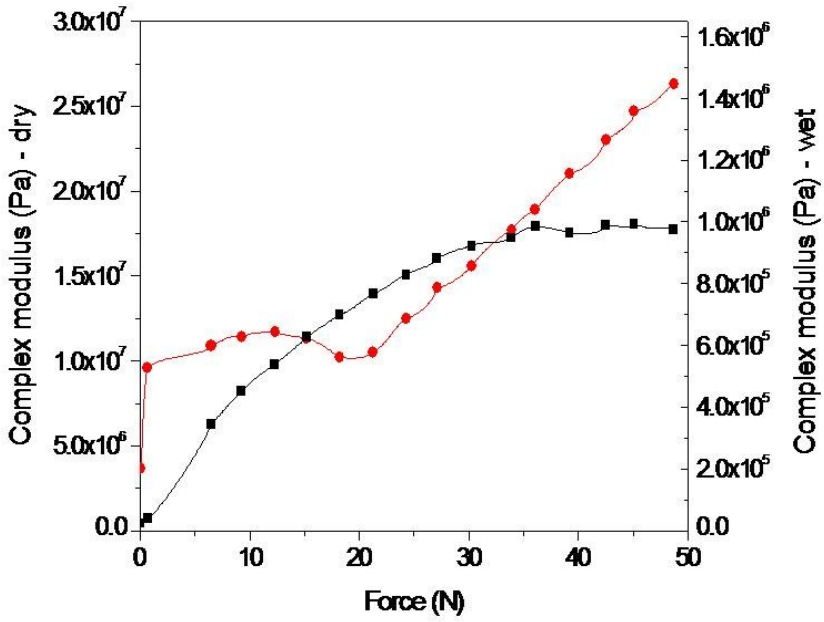

Fig. S1 The rhelogical behavior of scaffolds in its dry (red circle) and wet (black square) state suggests its pliable mechanical property (B).

Unlike dry matrix, the water saturated poly(AAm-co-AGE) matrix showed increasing complex modulus with respect to applied force. The complex modulus was 2.5×10^5, 6×10^5 and 9.8×10^5 Pa at 5, 20 and 50 N, respectively. Additionally, there was no fracture deformation was observed up to 50 N at a constant frequency (1Hz). However, upon hydration of matrix, a significant decrease in complex modulus was observed (i.e. from 10^7 to 10^5 Pa), which suggest that water saturated monolith are more pliable in nature compared to dry monolith and also maintain its structural integrity. These results are suggesting amiable mechanical stability of matrix for high flow bioprocessing application. Consequently, poly(AAm-co-AGE) monolith with amiable structural integrity and macroporous geometry was further utilized for on-column biosynthesis of Mel-NPs.

Supplementary Reference:

S1. J. Li and A. F. T. Mak, *J. Biomater. Appl.* 2005, **19**, 253-266.

S2. B. Adrados, I. Galaev, K. Nilsson and B. Mattiasson, *J. Chromatogr. A.* 2001, **930**, 73-78.