Supplementary material (ESI) for Soft Matter

Supplementary Information

Reactive inkjet printing of calcium alginate hydrogel porogens – a new strategy to open-pore structured matrices with controlled geometry

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Characterization of sodium alginate and alginate solutions.

The general strategy for identifying an appropriate alginate for printing involved preparing solutions of different grades of sodium alginate as aqueous solutions, and then loading them into an inkjet printer to see if they were printable. Due to the challenges in characterizing sodium alginate, the short-term strategy was to find a grade of sodium alginate that works, and characterize it. Many different grades of sodium alginate were tried, but most were found to be unsuitable for printing in the intended concentrations (*ca*. 1 wt-%). In most cases, the resulting solutions were too viscous to print using a drop-on-demand inkjet printer. The grade of sodium alginate that was found to work the most reliably was a low viscosity grade of sodium alginate isolated from brown algae, purchased from Sigma-Aldrich (catalog number A0682, batch number 106K0113.) Being a biopolymer, the physical properties of sodium alginate such as molar mass and copolymer composition can vary from batch-to-batch; to wit, we felt that it would be useful to other researchers to have some additional characterization information for this material, so as to help make material selection easier for other researchers. Viscosity measurements were conducted using an AMVn viscometer (Anton Paar, Graz, Austria) with the capillary/ball combination of the measuring system. A solution of 1 wt-% sodium alginate in distilled water was prepared and filtered using a 1.2 µm pore glass fiber syringe filter, and measured. Measurements were taken six times each from three different angles, namely 30- , 50- , and 70 °, and the average of all of these measurements was taken. Using this approach, the dynamic viscosity was found to be 30.5 ±0.2 mPa·s at 20 °C, and 26.17 ±0.2 mPa·s at 25 °C.

Sedimentation velocity measurements were performed using an XL-1 ultracentrifuge (Beckman Instruments, Palo Alto, CA). Experiments were carried out in a conventional double-sector Epon centerpiece, with a 12 mm optical path length and a four-hole rotor. The alginate solution was used without further purification. Cells were filled with 450 μL of solution and solvent, respectively. The experiments were performed with a rotor speed 40,000 rpm. The rotor temperature was equilibrated for approximately 2 h at 20 °C in the chamber of an Optima XLI. Sedimentation profiles were obtained every 120 sec by interference optics. The sedimentation runs were evaluated by the program SEDFIT. The data were modeled using the c(s) analysis in SEDFIT, which is based on a numerical resolution of the Lamm equation, allowing for estimations of the weight–average frictional ratio.[1] The sample was investigated at three different concentrations, parameter c[η] characterized degree of dilution was in the range where $0.12 \leq c[\eta] \leq 0.53$. Translation diffusion coefficients were determined from dynamic light scattered (DLS) using DynaPro plate reader (Wyatt Technology), at 20 °C. The density measurements were carried out in the density meter DMA 02 (Anton Paar, Graz, Austria) according to the procedure of Kratky, *et al.*[2]

Combination of the velocity sedimentation (s_0) and translational diffusion coefficients (D_0) leads to the Svedberg equation used for the molar mass determination from the hydrodynamic data using:[3]

$$M_{sD} = \frac{R \cdot T \cdot s_0}{D_0} \cdot (1-\bar{v}\rho_0)$$

where R is the universal gas constant, $\bar{v}$ is the partial specific volume, ρ_0 is the density of the solvent.

Table 1. Sedimentation coeffcient at zero concentration s_0, diffusion coefficient at zero concentration D_0, intrinsic viscosity $[\eta]$ and the average molar mass M_{avg}.

Sample	s_0 [S]	D_0 ($cm^2 \cdot s^{-1}$)	[η] ($cm^3 \cdot g^{-1}$)	$M_{avg} \pm \Delta M$ ($g \cdot mol^{-1}$)
A2158 0.05M	2.28	6.05×10^{-8}	530	187,000 ± 3,000
A2158 0.2M	2.47	6.36×10^{-8}	450	

Setup of the instrumentation for inkjet printing of calcium alginate microbeads

For the drop-on-demand reactive inkjet printing of calcium alginate, a customized Autodrop inkjet printer was used from Microdrop Technologies (Norderstedt, Germany), fitted with an undampened 100 μm inner-diameter dispenser head (Fig. 1) A solution of 1 wt-% sodium alginate and 0.01% brilliant blue G was prepared in distilled water, and filtered using a 1.2 μm pore syringe filter immediately prior to use. The head was loaded with roughly 1 mL of filtered colored alginate solution, moved into position over the reservoir for printing.

In order to ensure that the droplets of alginate did not agglomerate during the dispensing process, the inkjet printer stage was customized with a VP 710SM profile Alligator microplate vertical magnetic tumble stirrer (V & P Scientific, San Diego, California). This was found to be critical; without a source of constant, gentle agitation of the solution, the droplets would impinge onto the same spot on the liquid surface; instead of individual microbeads, the resulting product is an undefined, amorphous mass of calcium alginate.

The alginate was printed into a stirred reservoir containing a 5 mL aliquot of filtered solution of 15 wt% calcium chloride in water in a 6 well tissue culture plate. After optimizing the printing conditions for stable droplet formation, the system was allowed to dispense 1 mL of alginate into the calcium chloride bath over the course of approximately 4 hours (voltage: 71 V, pulse width: 46 μs, frequency: 500 Hz). The product of this exercise was a slurry composed of deep blue microspheres in a colorless, transparent liquid, which began to precipitate as soon as stirring ceased.

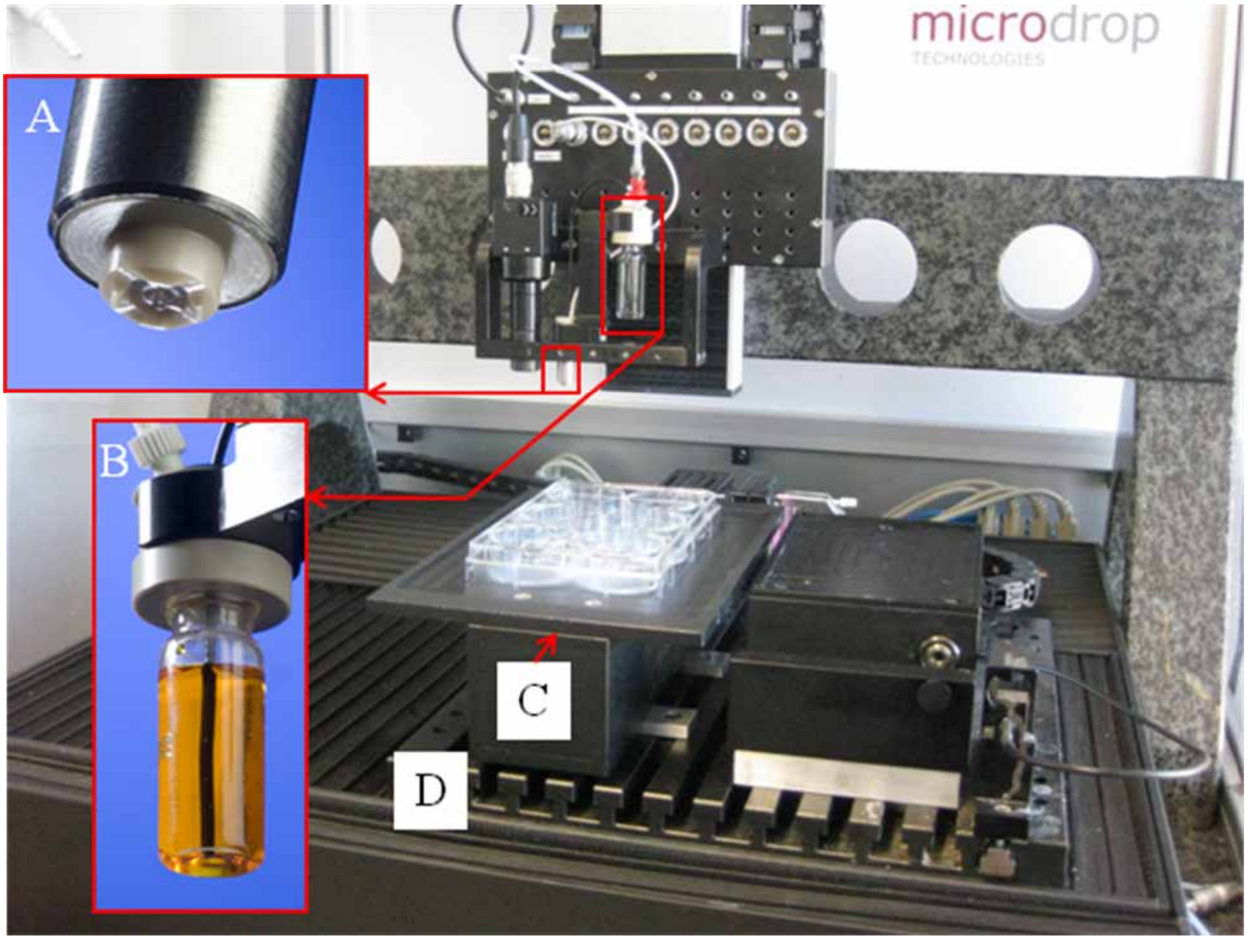

Figure 1. Customized drop-on-demand printer with tumble stirrer, including: A, dispensing head; B, fluid ink reservoir; C, magnetic stirrer; D, positioning platform.

The Dropjet continuous printer was fitted with a 100 μm inner diameter nozzle, which was loaded with 10 mL of sodium alginate solution, as illustrated in Figure 2. The solution was processed using a frequency of 90 kHz and a back pressure of 380 kPa, where it was dispensed downwards into a reservoir containing 50 mL of 15 wt-% calcium chloride, which was sitting 100 cm below the nozzle. This distance between the nozzle and the calcium chloride bath is necessary; it was observed that if the nozzle is maintained at too close of a distance (*e.g.* 1 to 10 cm from the surface of the liquid), the resulting stream is too forceful, and there is a great deal of turbulence where the stream impinges on the bath surface, resulting in highly irregular beads.

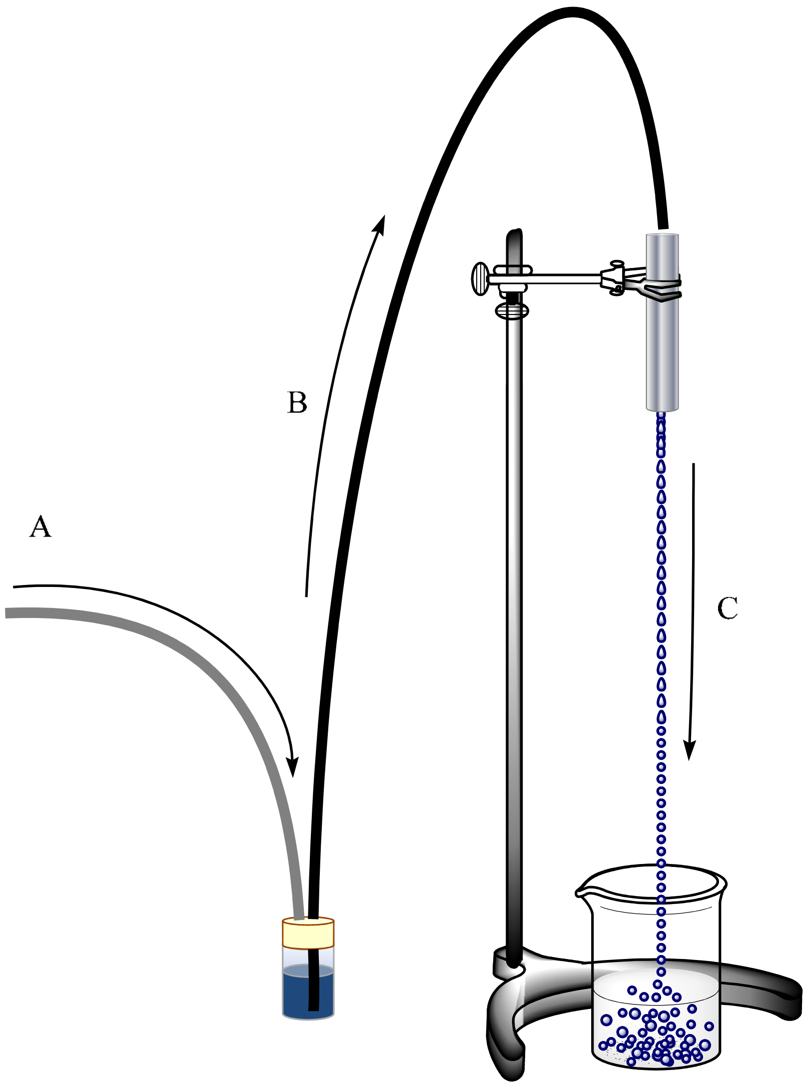

Figure 2. Schematic diagram for the Dropjet continuous inkjet system. Pressurized air is applied to the alginate reservoir through tube *A*, which drive the alginate solution through the transfer line *B*, into the vibrating dispenser head. As the vibrating jet is ejected from the nozzle, it breaks up into a stream of droplets *C*, which are collected in a calcium chloride hardening bath.

1. P. Schuck, *Biophys. J.*, 2000, **78**, 1606-1619.
2. O. Kratky, H. Leopold and H. Stabinger, *Methods Enzymol.*, 1973, **27**, 98-110.
3. T. Svedberg and K. O. Pedersen, *The Ultracentifuge*, Oxford University Press, New York, NY, 1940.