Supplementary: Experimental Section

Title: **Design Considerations and Biomaterials Selection in Embedded Extrusion 3D Bioprinting**

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Preparation of gelatin support matrix: To create the gelatin support matrix, 5%, 7% and 10% (w/v) Porcine gelatin (No. G2500-1KG; Type A, Sigma-Aldrich) was mixed in 150 ml PBS to form a solution, and then gelled for 12 hours at 4°C, in a 500-ml Ball Mason jar. Next, 350 ml of PBS at 4°C was added to the jar, and its contents blended ("pulse" speed at 20,000 rpm) using a mechanical blade stirrer for a period of 20s, 50s, 80s, and 120 s in a consumer-grade blender (**Fig. S1-A**). Blend times longer than 120 s cannot be used because the gelatin particles will begin to dissolve and form a solution; we considered 120 seconds as the reference blend time in the derivation of master curves in the following steps. The blended gelatin was loaded into 50-ml conical tubes and centrifuged at 4200 rpm for 2 min, causing particles to settle out of suspension. The supernatant was removed and replaced with PBS at 4°C, to double the remaining volume. The volume was vortexed (Thermo Fisher) into suspension and centrifuged again. This process was repeated if needed until no bubbles were observed at the top of the supernatant, which indicated that most of the soluble gelatin was removed. The matrix was poured into a petri dish, a mold, or a container large enough to hold the object to be extruded (see **Fig. S1-B-ii**). Any excess fluid was removed from the gelatin support using Kimwipes (Kimberly-Clark), producing a material resembling Bingham plastic, demonstrated in a simple experiment discussed in **Fig. S1-B.** For long-term use, the matrix can be stored at 4°C for up to 10 days before it gets denatured and becomes a solution.

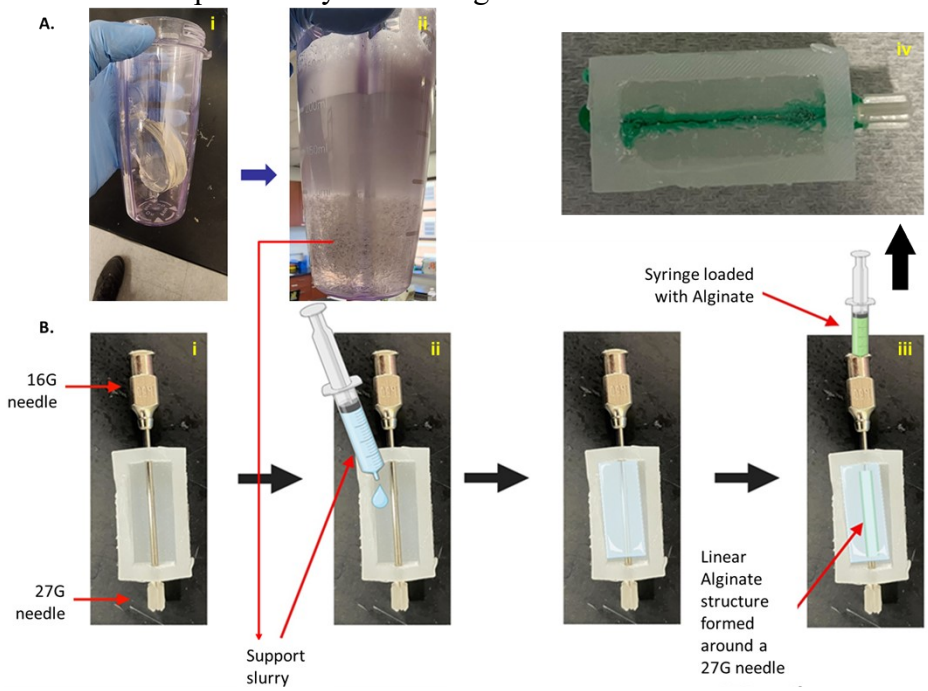

Figure S1. A) Matrix preparation composed of (i) preparation of a solidified gel, and (ii) blending the gel for 50s leading to form the matrix; B) Demonstrating the concept of Bingham plastic response in a prepared matrix: (i) a rectangular PDMS mold is equipped with a 27G needle placed coaxial inside a 16G needle, (ii) PDMS mold is filled with the blend/support matrix as prepared in the previous step, (iii) alginate bioink is extruded while retracting 16G needle, iv) at the end, a uniform and stable tubular alginate structure is formed around the 27G needle owing to the matrix's Bingham plastic response.

Preparation of agarose support matrix: To create the agarose support matrix, 2%, 3% and 4% (w/v) UltraPure™ agarose (No. 16500-100; Invitrogen) was mixed in 150 ml PBS. Agarose doesn't dissolve well in room temperature buffer, hence, to form a homogenous solution, the agarose powder is added to PBS in a glass bottle with the lid loosely attached and autoclaved for 20 mins at 121 °C (1 atm). Once the agarose is completely dissolved, it is

gelled for 12 hours at room temperature (22 ℃), in a 500-ml Ball Mason jar. Next, 350 ml of PBS at 22°C was added to the jar, and its contents blended for a period of 20, 50, 80 and 120s, then processed further like the gelatin matrix to prepare a bubble-free matrix for each concentration.

Rheology measurements: To measure the effect of blend time on gelatin and agarose matrix rheology (viscosity vs. shear rate), the gelatin and agarose matrix for varying blend times of 20 s, 50 s, 80 s, and 120 s were collected using spatula. The matrix was then loaded onto a Waters HR10 Rheometer and analyzed in plate-plate mode by running a frequency sweep (0.01 to 100 Hz) at 150 μm separation and 25°C. The viscosity-shear rate data was then recorded and used to illustrate a universal master curve. The raw data is accessible in Excel format in the Supplementary Information

The master-curve interpretation: two shift factors are used to unify the viscosity-shear rate data illustration for support matrices. The horizontal shift factor (α) is dependent on the blending time, t, and calculated by

$$\alpha = t_{ref}/t \quad \text{(Eq.1)}$$

where t_{ref} denotes to the reference blend time which is 120s. The vertical shift factor (β) is dependent on the gel concentration, C, and calculated by

$$\beta = C_{ref}/C \quad \text{(Eq.2)}$$

The reference concentration, C_{ref}, is assumed to be the lowest concentration (5% for gelatin and 2% for agarose matrices). The calculated shift factors are applied to each data point in all viscosity(η) - shear rate ($\dot{\gamma}$) curves using the following equations:

$$\dot{\gamma}_{shifted} = \alpha \cdot \dot{\gamma}$$
$$\eta_{shifted} = \beta \cdot \eta \quad \text{(Eq.3)}$$

where, $\dot{\gamma}_{shifted}$ denotes to time-shifted shear rate and $\eta_{shifted}$ denotes to concentration-shifted viscosity data. Finally, all η - γ curves are plotted in a single $\eta_{shifted} - \dot{\gamma}_{shifted}$ master plot (**Fig. 2 E,F**). This plot can be used as a guide to select a matrix. For any tailored matrix with a known viscosity at a given shear rate, α and β shift factors, one can calculate the relevant data point on this plot. If the data point fits into the same highlighted region as agarose/gelatin, the matrix's rheological properties would match a Bingham plastic and should be appropriate to be used in FRESH bioprinting.