DATA SHEET

VitroINK® 3D

VitroINK® 3D is a ready-to-use tunable bioinkfor 3D bioprinting. The pure hydrogel matrix structure is good for cell spheroid formation and supports the growth of cells requiring low cell-matrix interactions.

VitroINK® is a family of the ready-to-use, xeno-free tunable bioink system that requires no UV, no temperature/pH curing, or chemical cross-linking. The system is ready-to-use at room temperature, neutral pH, transparent, and has excellent visibility after printing and cell culture. Due to the unique shear-thinning and rapid recovery mechanical property, VitroINK® can well maintain the printed structure without UV or other special curing methods. Simply adding the cell culture medium after printing can further stabilize the printed structure and support cell growth. Cells can pre-mix with VitroINK® by using our VitroINK® mixing kit to get different mixing ratios (3:1 or 10:1). Multiple biological functional ligands are incorporated for different versions of VitroINK®, which promote cell attachment, cell-matrix interactions, cell proliferation, motility/migration and differentiation for many different applications.

In this datasheet, we evaluate the rheological properties of VitroINK® 3D, including the time sweep, viscosity, temperature ramp, and repeated amplitude sweep (shear-thinning and recovery) tests. The printability properties of VitroINK® were also tested by using two bioprinters respectively (Cellink INKCREDIBLE and Allevi II). The line width and parameter optimization index (POI) value of the bioink after extrusion from different sizes of nozzles were measured at different printing pressures and speeds. The adjusted printing parameters after mixing VitroINK® with cell culture media at different ratios were also evaluated. This datasheet aims to provide a guideline to understand the biophysical properties of VitroINK® 3D and help customers to set up the optimal printing conditions for different applications.

Material and method

Rheological properties Test

Time sweep, viscosity, temperature ramp, and repeated amplitude sweep (shear-thinning and recovery) tests were performed by using a dynamic rheometer. The mixing samples were prepared by using VitroINK® mixing kit to mix VitroINK® and cell culture medium at 3:1 or 10:1 ratios. The time sweep testing was performed at 1 Hz, 0.1% shear strain for 30 min. The Viscosity was tested at shear strain from 0.1 to 100 %. The temperature ramp was tested from 20 to 40 °C with 2 °C /min temperature increase rate and 1Hz, 0.1% shear strain. The shear-thinning the recovery property of the hydrogel was tested on a repeated time sweep and amplitude sweep testing program: the sample was loaded and tested with time sweep for 10 min, then shearing by using the amplitude sweep with 0.1 to 500% shear strain for 4 min. After the first amplitude sweep, the sample was immediately tested with the second time sweep test for 10 min and follow with the second amplitude sweep test. For final hydrogel strength, samples were prepared in a 35 mm petri dish then covered with cell culture medium. The samples were incubated in a CO_2 incubator for 24 hours before the time sweep testing.

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Printability test

The printability of VitroINK® 3D was determined by the line test. This test was carried out by the two bioprinters: INKREDIBLE+ (Cellink, USA) and ALLEVI2 (Allevi, USA), in order to determine the best printing parameters (nozzle size, print pressure, and print speed) for VitroINK® 3D. On INKREDIBLE+, VitroINK® 3D was printed under different pressures (25kpa-60kpa, increment of 5kpa), different printing speed (1mm/s-20mm/s, increment of 2mm/s) and with different nozzle sizes (22G,25G,27G). On ALLEVI2, VitroINK® 3D was printed under the pressure range from 6psi (41.47kpa) to 13psi (89.63kpa), which was increased by 1psi (6.895kpa) each time. The range of print speed and nozzle sizes were the same as the tests done by INKREDIBLE+. The width of each printed line was measured under a microscopy, and each line width recorded was taken by the average of 3 to 9 lines. The POI values were calculated for each different printing conditions, by using the following formula: POI $=\frac{1}{W_{line}*N_G*P}$, where W_{line} stands for the width of each line, N_G is the nozzle size, and P is for print pressure. The optimized printing parameter for VitroINK® 3D was determined by the highest POI value in each printing condition.

The printability of VitroINK® 3D mixed with different cell culture media (DMEM, DMEM/F-12, and RPMI) was tested by INKREDIBLE+ using the method of line test above. The printing parameters of highest POI at unmixed sample were selected as a reference, and then printing parameters were adjusted to get the optimized printing parameters for VitroINK®/medium mixture.

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Rheological Properties

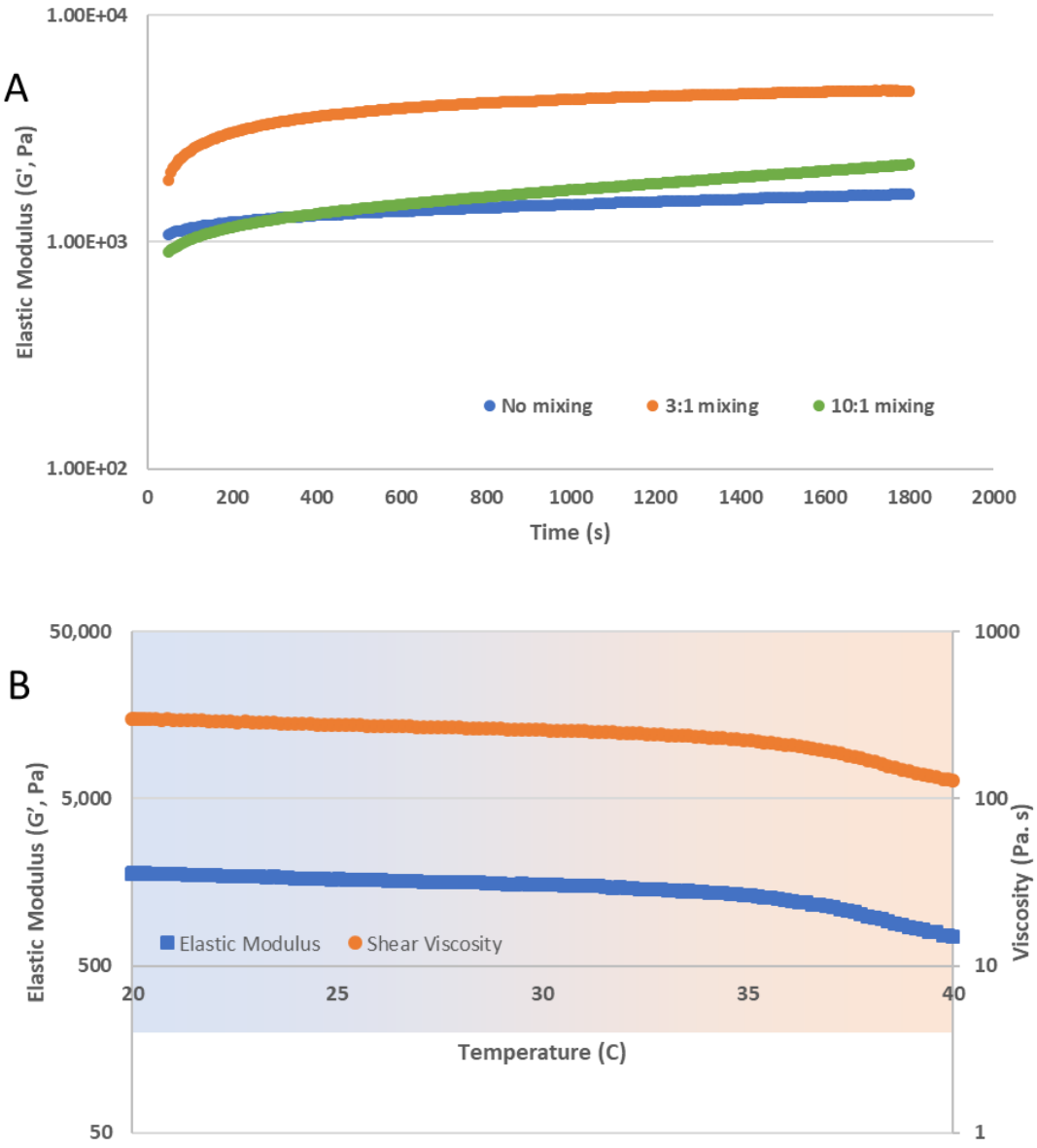

Figure 1. Rheological properties of VitroINK® 3D under the time sweep and temperature sweep tests. A) The time sweep test of VitroINK® 3D without or with 3:1 and 10:1 (v/v) mixing with DMEM medium. The elastic modulus was tested at room temperature and showed a feasible range for extrusion printing before incubation with cell culture medium. **B)** the elastic modulus and viscosity properties of VitroINK® 3D under the temperature sweep testing (20 to 40°C). The elastic modulus and viscosity are relatively stable at 20 to 36°C. When temperature higher than 36 °C, the elastic modulus and viscosity decrease. The data indicate the VitroINK® has a wide range of working temperature and can be easily used at room temperature or physiological temperature.

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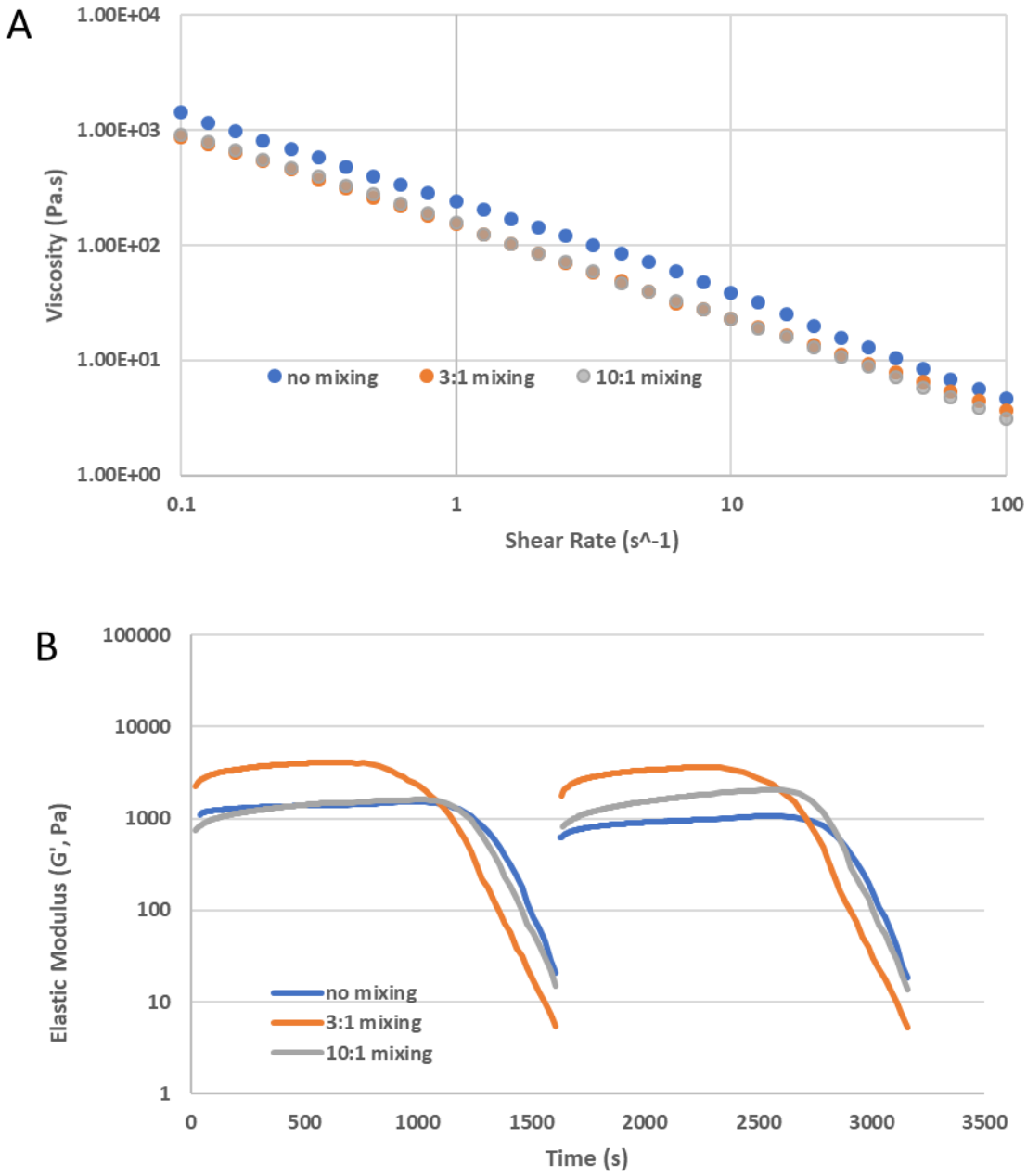

Figure 2. Shear-thinning and rapid recovery property of VitroINK® 3D. A) The shear-thinning property of VitroINK® under the shear rate from 0.1 to 100 HZ. **B)** the amplitude sweep test show the shear thinning and rapid recovery property of VitroINK® under the shear strain change from 0.1 to 500% in two circles. With this unique shear-thinning and rapid recovery mechanical property, VitroINK® can well maintain the printed structure after extrusion.

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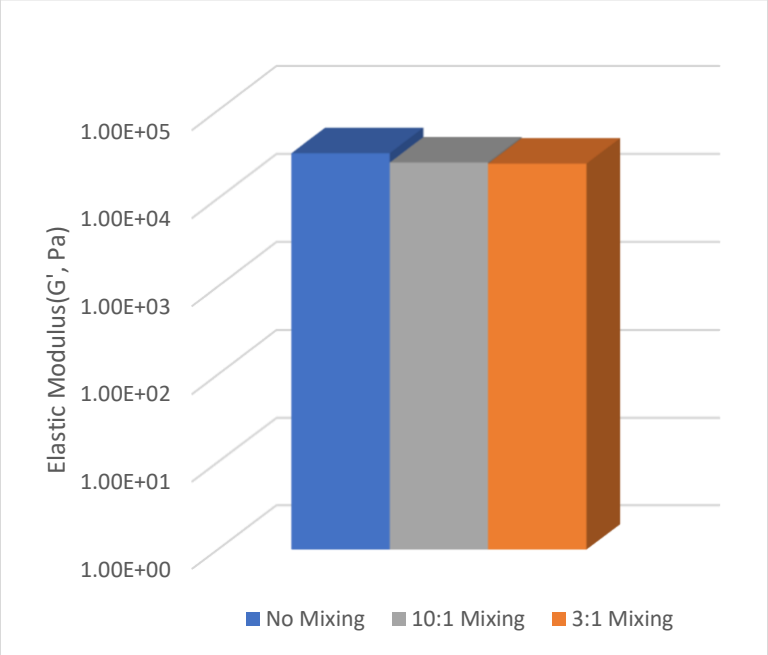

Figure 3. Gel strength of VitroINK® 3D after adding cell culture medium and overnight incubation. The VitroINK® 3D was mixed with the cell culture medium at different ratio and submerged to the medium for overnight incubation, which can further stabilize the printed structure and support cell growth.

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Pintability test

VitroINK® 3D with INKREDIBLE+

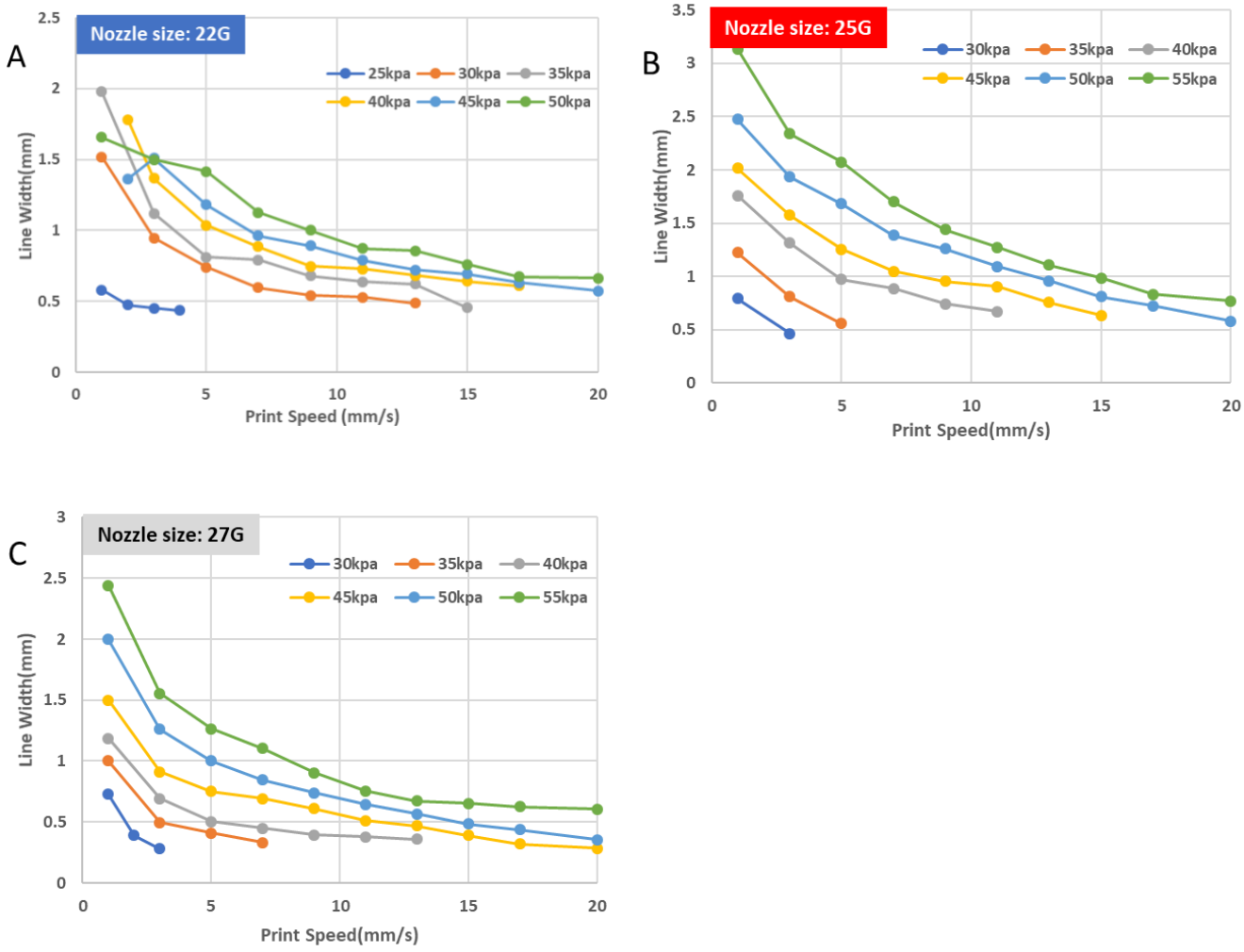

Figure 4. Printed line width of VitroINK® 3D with 22G, 25G, 27G nozzle at different pressures and print speeds. The line width decreases as the print speed increases. At lower printing pressures, lines can only be printed continuously under low print speed.

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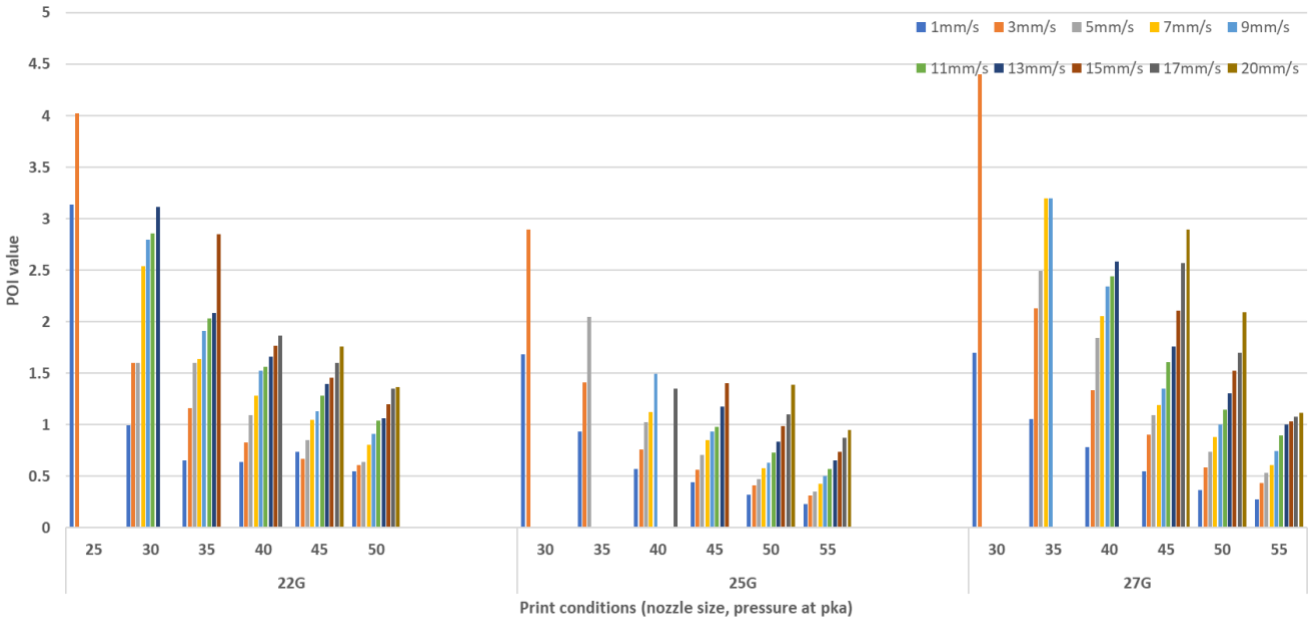

Figure 5. POI value of VitroINK® 3D with 22G, 25G, 27G nozzle at different pressures and print speeds. For all three nozzle sides, print speed at 3 mm/s give the highest POI value at low pressure. Under a fixed pressure, the faster print speed gives a higher POI value.

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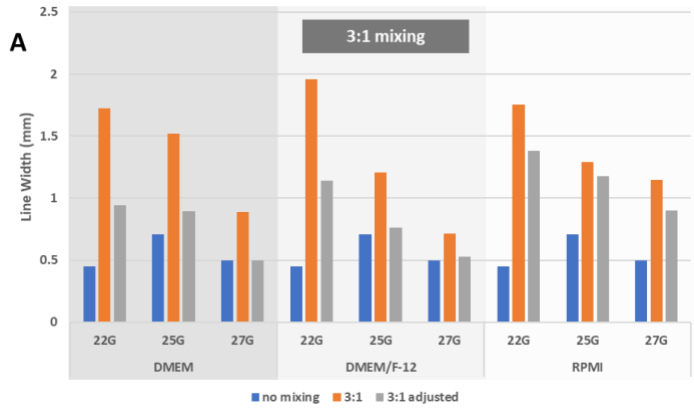
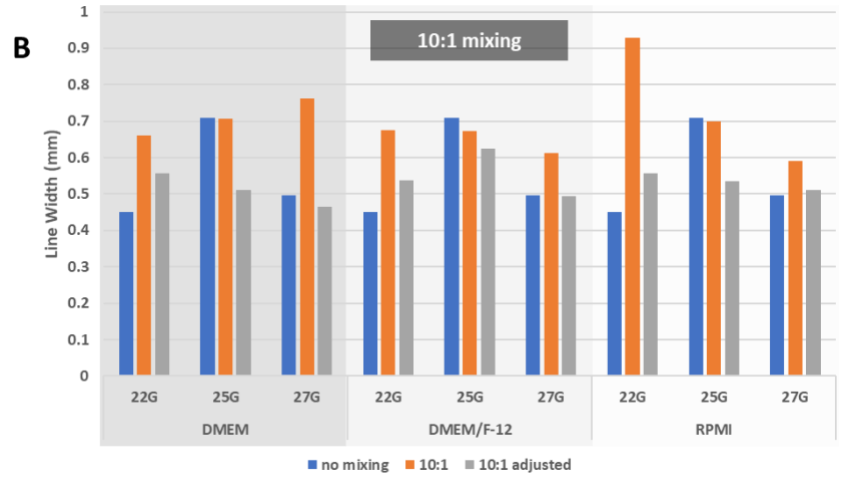
Adjusted printing conditions after mixing VitroINK® with Cell culture medium



		22G	25G	27G
No mixing	Pressure (kpa)	25	35	35
	Speed (mm/s)	3	3	3
3:1	Pressure (kpa)	25	35	35
	Speed (mm/s)	3	3	3
3:1 adjusted	Pressure (kpa)	25	35	35
	Speed (mm/s)	7	7	7



		22G	25G	27G
No mixing	Pressure (kpa)	25	35	35
	Speed (mm/s)	3	3	3
10:1	Pressure (kpa)	25	35	35
	Speed (mm/s)	3	3	3
10:1 adjusted	Pressure (kpa)	25	35	35
	Speed (mm/s)	5	5	7

Figure 6. Adjusted printing conditions after mixing VitroINK® with Cell culture medium. Mixing at 3:1 **(A)** or 10:1 **(B)** ratios by using VitroINK® mixing kit. The line width increase after mixing. Adjust the printing speed from 3 to 7 or 5 mm/s can bring the line width down.

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Table of Printed line width of VitroINK 3D under different pressure, print speed and nozzles

Print Speed	1 mm/s	3 mm/s	5 mm/s	7 mm/s	9 mm/s	11 mm/s	13 mm/s	15 mm/s	17 mm/s	20 mm/s
	25kpa									
22G	0.5799±0.021	0.4516±0.015	NA	NA	NA	NA	NA	NA	NA	NA
25G	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
27G	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	30kpa									
22G	1.520±0.026	0.9465±0.084	0.7416±0.032	0.5968±0.006	0.5419±0.029	0.5313±0.037	0.4870±0.033	NA	NA	NA
25G	0.7912±0.021	0.4611±0.002	NA	NA	NA	NA	NA	NA	NA	NA
27G	0.7268±0.014	0.2805±0.006	NA	NA	NA	NN	NA	NA	NA	NA
	35kpa									
22G	1.978±0.051	1.121±0.033	0.8129±0.026	0.7923±0.044	0.6798±0.070	0.6386±0.025	0.6222±0.024	0.4558±0.013	NA	NA
25G	1.225±0.025	0.8086±0.021	0.5583±0.020	NA	NA	NA	NA	NA	NA	NA
27G	1.001±0.012	0.4960±0.019	0.4078±0.006	0.3312±0.008	NA	NA	NA	NA	NA	NA
	40kpa									
22G	1.979±0.021	1.369±0.017	1.037±0.016	0.8868±0.019	0.7463±0.019	0.7236±0.023	0.6840±0.031	0.6417±0.004	0.6100±0.041	NA
25G	1.757±0.057	1.314±0.038	0.9719±0.011	0.8873±0.023	0.7456±0.024	0.6703±0.013	NA	NA	NA	NA
27G	1.188±0.026	0.6919±0.063	0.5034±0.028	0.4500±0.036	0.3951±0.063	0.3798±0.054	0.3586±0.006	NA	NA	NA
	45kpa									
22G	1.756±0.067	1.510±0.054	1.182±0.018	0.9623±0.022	0.8921±0.012	0.7880±0.016	0.7236±0.036	0.6919±0.028	0.6306±0.023	0.5731±0.063
25G	2.017±0.062	1.576±0.036	1.254±0.021	1.046±0.010	0.9517±0.020	0.9048±0.035	0.7569±0.044	0.6343±0.009	NA	NA
27G	1.500±0.031	0.9095±0.025	0.7505±0.063	0.6914±0.022	0.6106±0.055	0.5118±0.034	0.4674±0.026	0.3903±028	0.3206±0.039	NA
	50kpa									
22G	1.657±0.025	1.498±0.020	1.415±0.065	1.126±0.020	1.000±0.022	0.8720±0.019	0.8578±0.019	0.7590±0.033	0.6729±0.013	0.6639±0.015
25G	2.472±0.031	1.933±0.027	1.683±0.021	1.385±0.055	1.260±0.023	1.091±0.007	0.9562±0.026	0.8094±0.14	0.7251±0.066	0.5775±0.110
27G	2.000±0.069	1.2591±0.078	1.001±0.063	0.8429±0.022	0.7379±0.044	0.6465±0.051	0.5662±0.036	0.4854±0.050	0.4368±0.036	0.3539±0.049
	55kpa									
22G	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
25G	3.134±0.055	2.339±0.010	2.075±0.062	1.702±0.049	1.438±0.033	1.273±0.008	1.108±0.009	0.9826±0.034	0.8326±0.033	0.76846±0.042
27G	2.439±0.022	1.555±0.038	1.2630.047	1.104±0.015	0.9024±0.026	0.7532±0.037	0.6722±0.051	0.6513±0.052	0.6249±0.039	0.6040±0.033

Mean line width (mm) ± standard deviation

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VitroINK® 3D with ALLEVI2

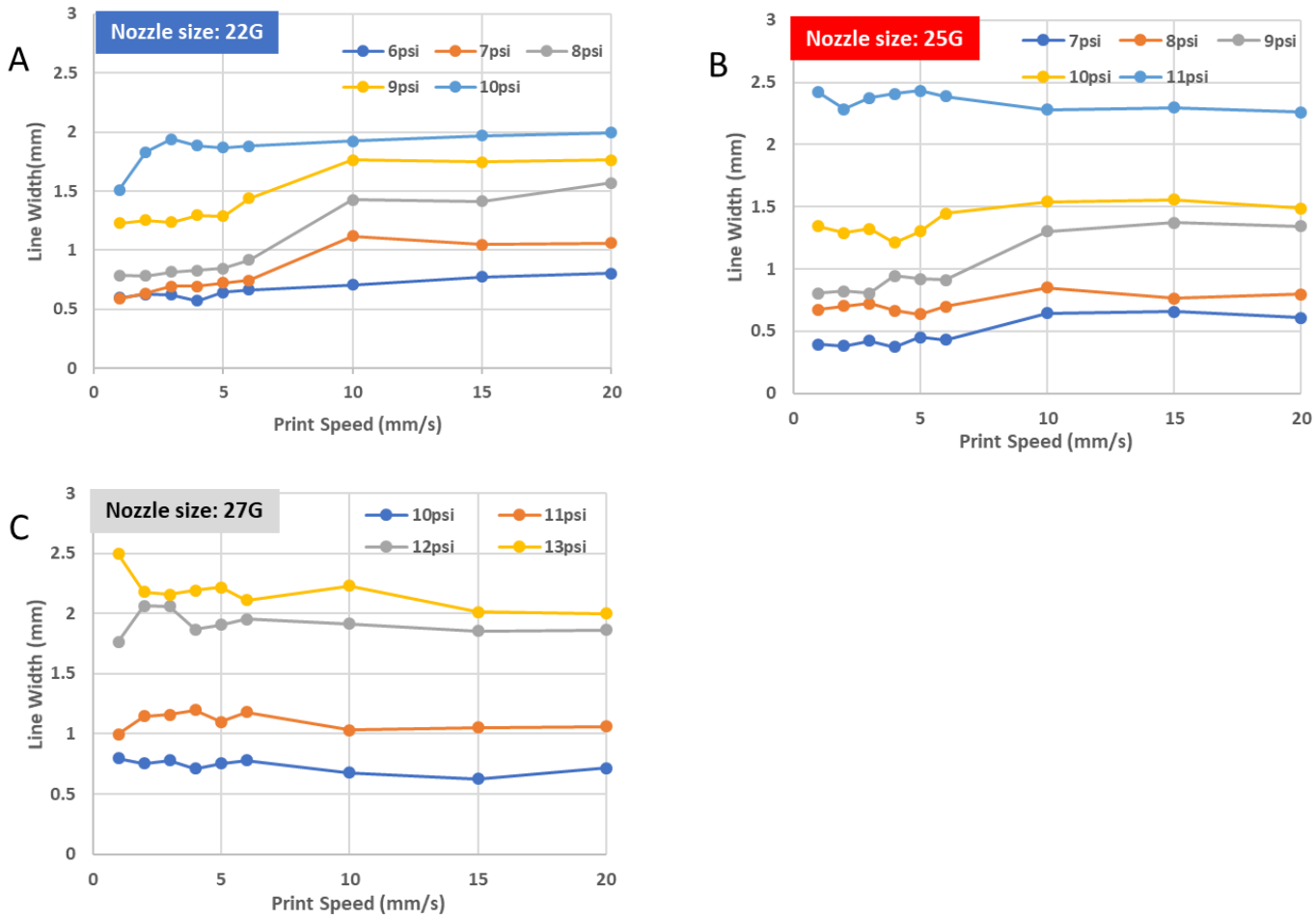

Figure 7. Printed line width of VitroINK® 3D with 22G, 25G, 27G nozzle at different pressures and print speeds. The line width remains relatively stable as the print speed increases.

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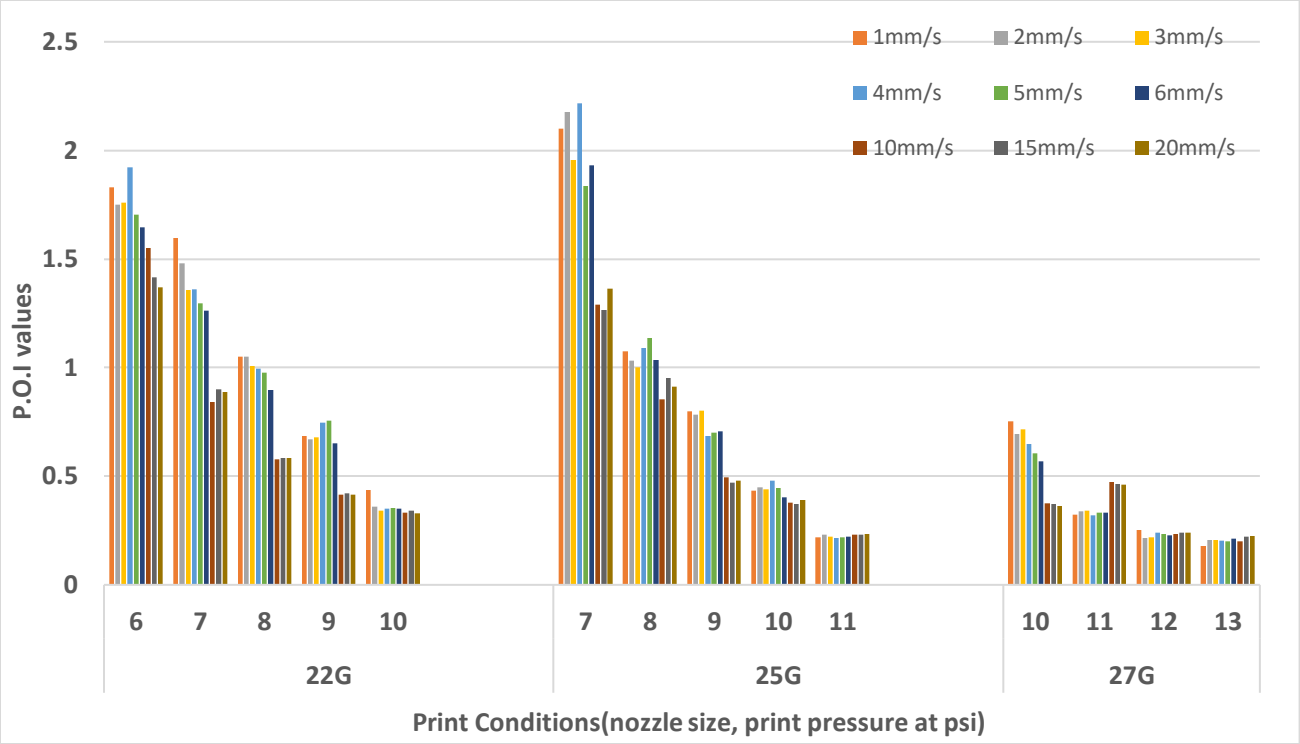

Figure 8. POI value of VitroINK® 3D with 22G, 25G, 27G nozzle at different pressures and print speeds. For all three nozzle sides, print speed at 4 mm/s give the highest POI value at low pressure.

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Table of Printed line width of VitroINK 3D under different pressure, print speed and nozzles

Print Speed	1 mm/s	2 mm/s	3 mm/s	4 mm/s	5 mm/s	6 mm/s	10 mm/s	15 mm/s	20 mm/s
	6 Psi								
22G	0.6000±0.094	0.6282±0.072	0.6250±0.068	0.5720±0.056	0.6447±0.059	0.6673±0.054	0.7089±0.008	0.7764±0.014	0.8021±0.032
25G	NA	NA	NA	NA	NA	NA	NA	NA	NA
27G	NA	NA	NA	NA	N	NA	NA	NA	NA
	7 Psi								
22G	0.5898±0.063	0.6363±0.066	0.6944±0.076	0.6926±0.040	0.7259±0.029	0.7470±0.046	1.119±0.032	1.047±0.032	1.062±0.033
25G	0.3949±0.020	0.3809±0.029	0.4236±0.029	0.3740±0.018	0.4511±0.015	0.4294±0.024	0.6428±0.034	0.6555±0.047	0.6083±0.060
27G	NA	NA	NA	NA	NN	NA	NA	NA	NA
	8 Psi								
22G	0.7854±0.053	0.7840±0.034	0.8187±0.046	0.8291±0.037	0.8446±0.052	0.9171±0.132	1.119±0.032	1.047±0.032	1.062±0.033
25G	0.6740±0.030	0.7025±0.025	0.7241±0.020	0.6655±0.023	0.6380±0.029	0.6998±0.061	0.8502±0.059	0.7618±0.045	0.7954±0.047
27G	NA	NA	NA	NA	NA	NA	NA	NA	NA
	9 Psi								
22G	1.070±0.046	1.094±0.071	1.078±0.030	0.9803±0.051	0.9704±0.034	1.125±0.076	1.425±0.048	1.413±0.047	1.570±0.075
25G	0.8065±0.059	0.8224±0.054	0.8049±0.050	0.9428±0.146	0.9206±0.047	0.9111±0.048	1.302±0.069	1.317±0.033	1.343±0.038
27G	NA	NA	NA	NA	NA	NA	NA	NA	NA
	10 Psi								
22G	1.510±0.042	1.832±0.073	1.938±0.048	1.883±0.157	1.867±0.089	1.880±0.067	1.924±0.019	1.970±0.033	1.996±0.018
25G	1.342±0.087	1.291±0.074	1.323±0.030	1.212±0.138	1.304±0.052	1.445±0.035	1.539±0.023	1.556±0.044	1.489±0.022
27G	0.797±0.016	0.7558±0.091	0.7780±0.057	0.7130±0.081	0.7532±0.090	0.7807±0.166	0.6792±0.105	0.6264±0.072	0.7178±0.096
	11 Psi								
22G	NA	NA	NA	NA	NA	NA	NA	NA	NA
25G	1.252±0.054	1.375±0.023	1.426±0.045	1.535±0.069	1.425±0.075	1.447±0.120	2.28±0.045	2.220±0.108	2.223±0.023
27G	0.9988±0.058	1.146±0.052	1.164±0.071	1.198±0.092	1.102±0.054	1.179±0.088	1.032±0.030	1.051±0.024	1.062±0.014
	12 Psi								
22G	NA	NA	NA	NA	NA	NA	NA	NA	NA
25G	NA	NA	NA	NA	NA	NA	NA	NA	NA
27G	1.765±0.291	2.063±0.074	2.058±0.120	1.867±0.046	1.905±0.101	1.954±0.109	1.915±0.057	1.853±0.032	1.863±0.057

Mean line width (mm) ± standard deviation

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