

VitroINK® RGD Datasheet

VitroINK® RGD ready-to-use bioink system modified with RGD cell adhesive peptide, promoting the cell attachment and cell-matrix interactions. This RGD modified bioink can enhance cell adhesion, proliferation, motility/migration and differentiation in different applications.

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® RGD, 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 INKREDIBLE 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® RGD 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 CO2 incubator for 24 hours before the time sweep testing.

Printability test

The printability of VitroINK® RGD 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® RGD. On INKREDIBLE+, VitroINK® RGD 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 lines were 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: $POI = \frac{1}{W_{line} * N_G * P}$, in which 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® RGD was determined by the highest POI value in each printing condition.

The printability of VitroINK® RGD 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.

Rheological Properties

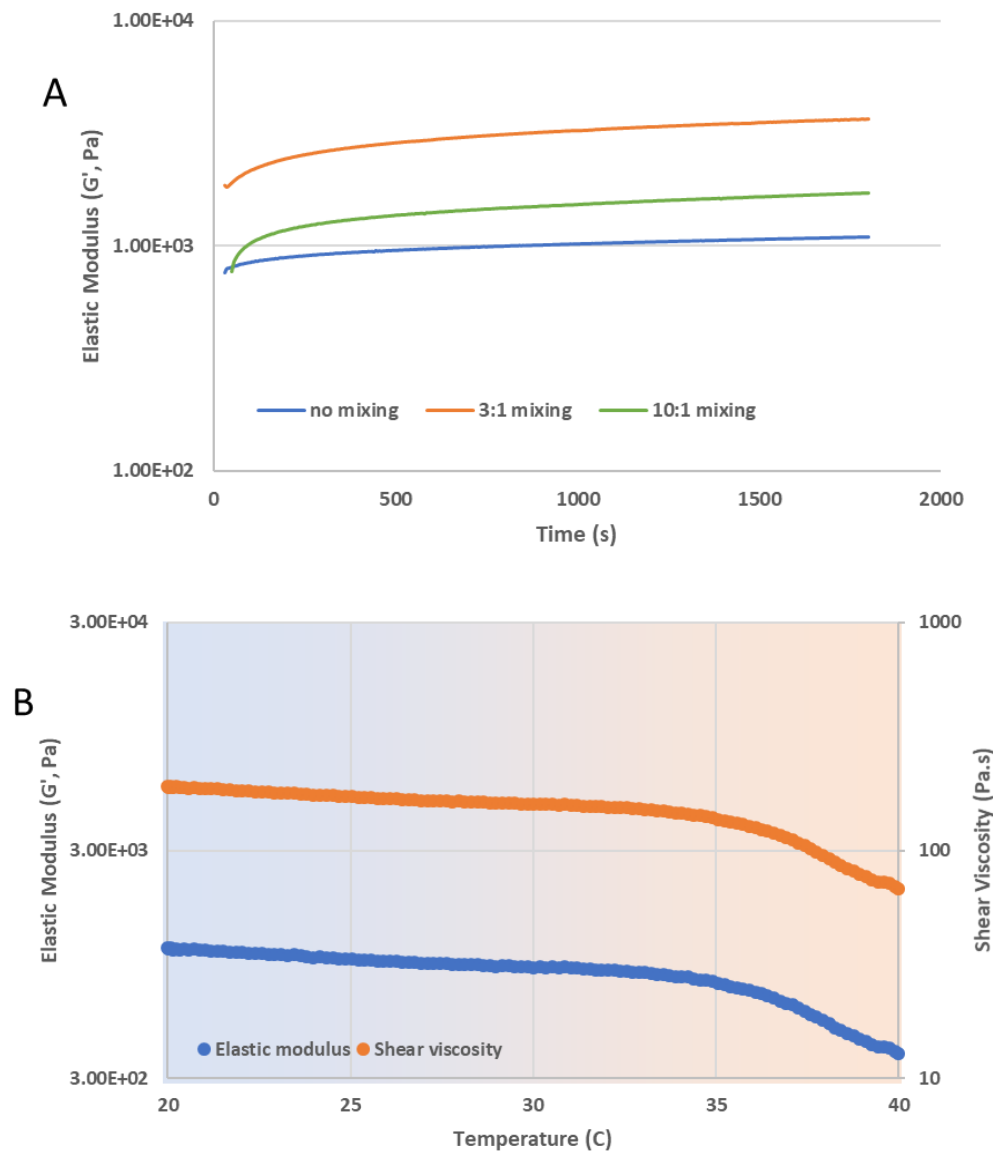


Figure 1. Rheological properties of VitroINK® RGD under the time sweep and temperature sweep tests. A) The time sweep test of VitroINK® RGD without or with 3:1 and 10:1 (v/v) mixing with DMEM medium. The elastic modulus was tested at room temperature and showed a feasible range for extrusion printing before incubation with cell culture medium. **B)** the elastic modulus and viscosity properties of VitroINK® RGD under the temperature sweep testing (20 to 40°C). The elastic modulus and viscosity are relatively stable at 20 to 35°C. When temperature higher than 36, 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.

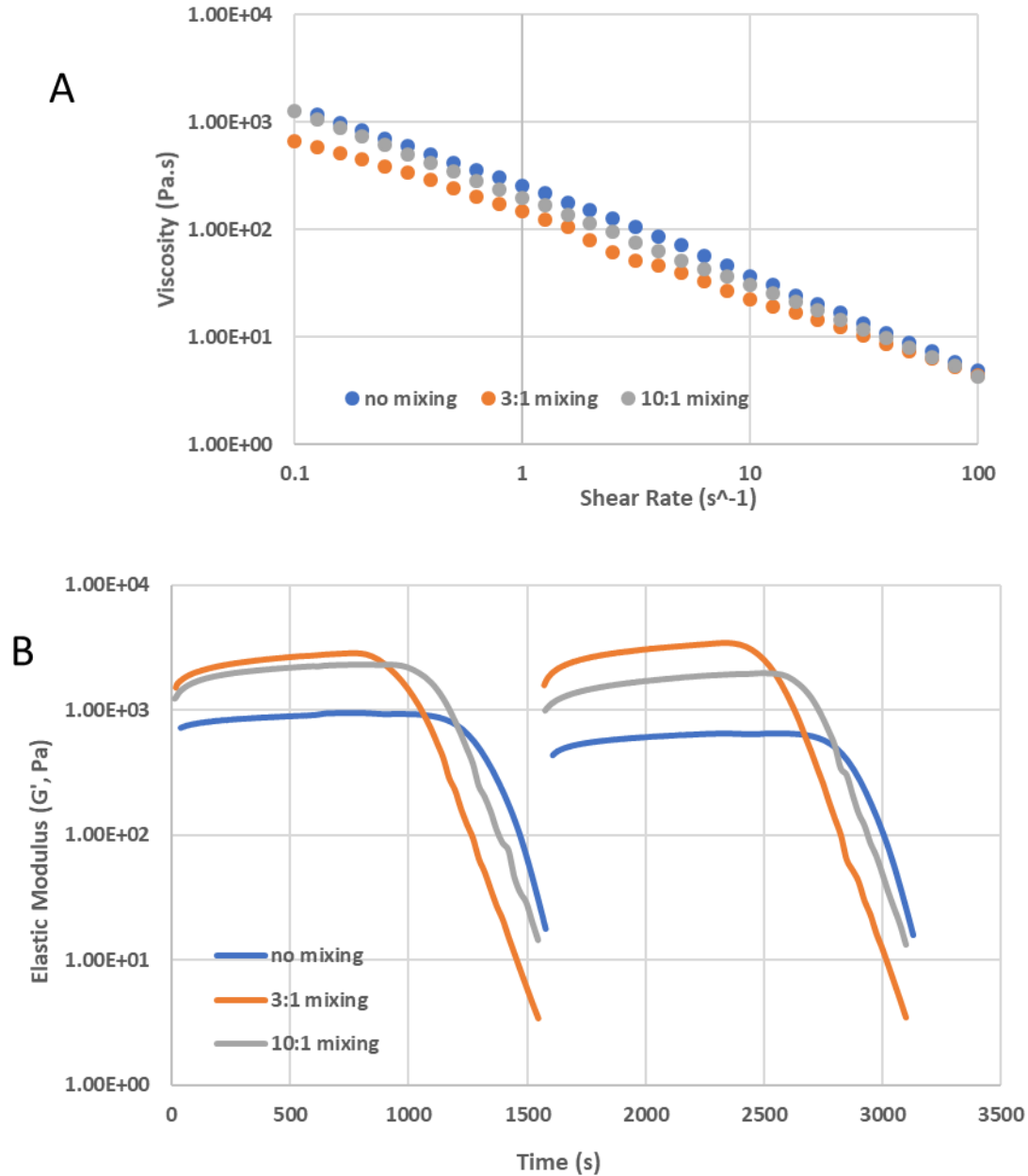


Figure 2. Shear-thinning and rapid recovery property of VitroINK® RGD. A) The shear-thinning property of VitroINK® under the shear rate from 0.1 to 100 HZ. **B)** the amplitude sweep test show the shear thinning and rapid recovery property of VitroINK® under the shear strain change from 0.1 to 500% in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

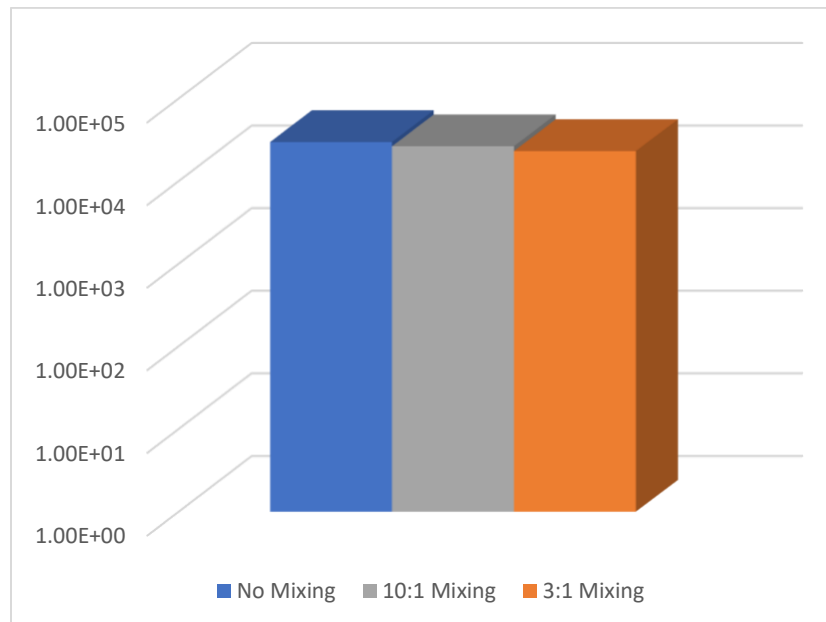


Figure 3. Gel strength of VitroINK® after adding cell culture medium and overnight incubation. The VitroINK® RGD was mixed with the cell culture medium in different ratio and submerged in the medium for overnight incubating.

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Printability test

VitroINK® RGD with INKREDIBLE+

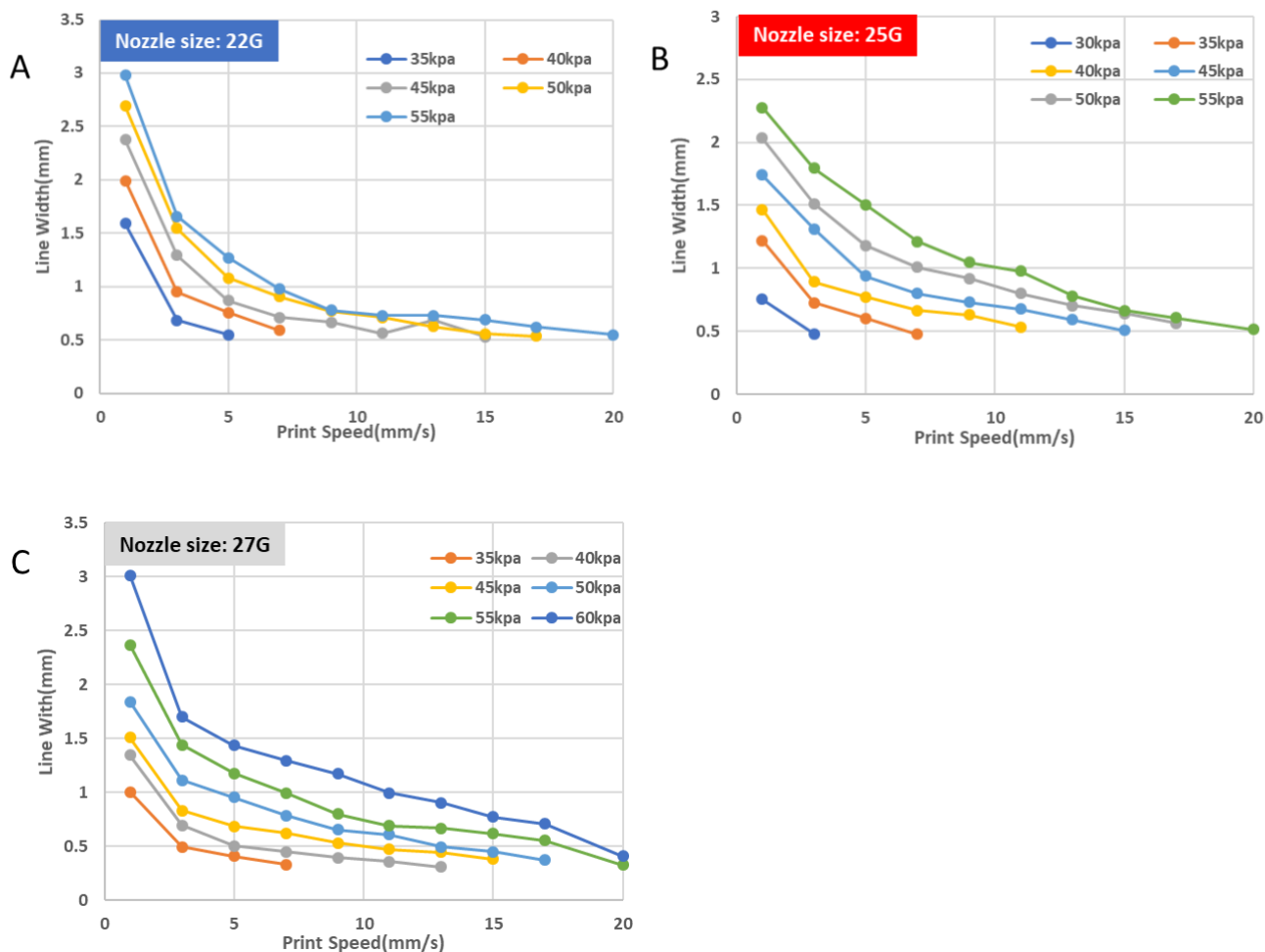


Figure 4. Printed line width of VitroINK® RGD with 22G, 25G, 27G nozzle at different pressures and print speeds. The line width decreases as the print speed increases. At lower printing pressures, lines can only be printed continuously under low print speed.

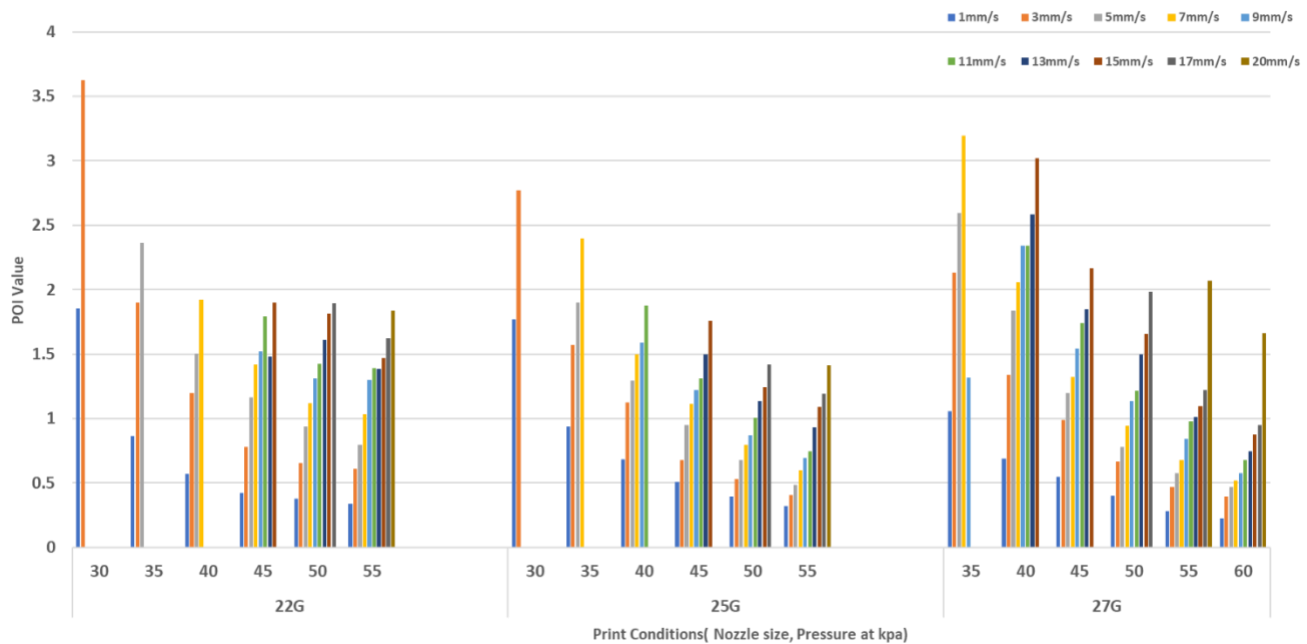
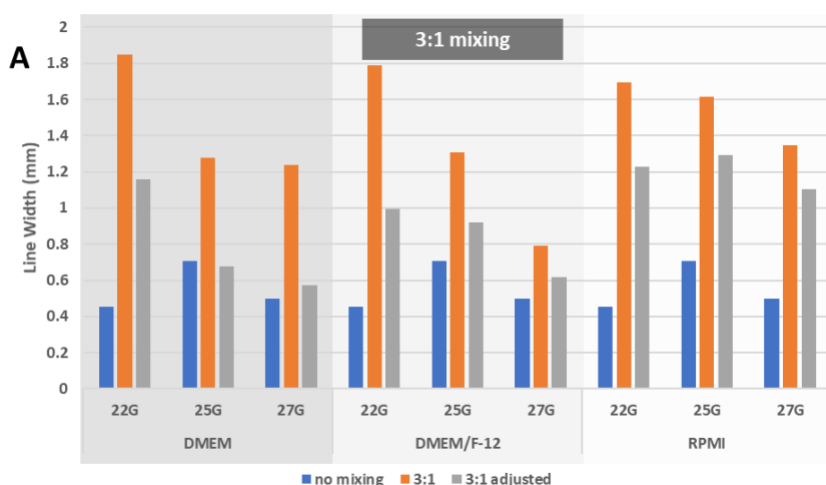
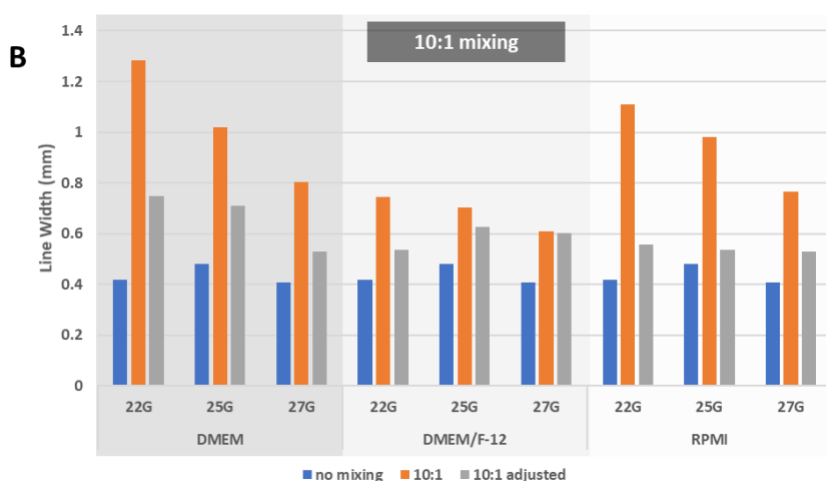


Figure 5. POI value of VitroINK® RGD with 22G, 25G, 27G nozzle at different pressures and print speeds. The printing conditions give the highest POI value of different nozzles are 30 kpa at 3 mm/s for 22G and 25G and 35 kpa at 7 mm/s for 27G. Under a fixed pressure, the faster print speed gives a higher POI value.

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	5
10:1	Pressure (kpa)	25	35	35
	Speed (mm/s)	3	3	5
10:1 adjusted	Pressure (kpa)	25	35	35
	Speed (mm/s)	5	7	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 increases 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 RGD under different pressure, print speed and nozzles

Print Speed(mm/s)	1	3	5	7	9	11	13	15	17	20
30kpa										
22G	0.8182±0.023	0.4178±0.007	NA	NA	NA	NA	NA	NA	NA	NA
25G	0.7537±0.022	0.4812±0.040	NA	NA	NA	NA	NA	NA	NA	NA
27G	0.5303±0.029	0.2752±0.014	NA	NA	NA	NN	NA	NA	NA	NA
35kpa										
22G	1.590±0.057	1.293±0.019	0.6845±0.011	0.5498±0.021	NA	NA	NA	NA	NA	NA
25G	1.219±0.028	0.7278±0.215	0.6021±0.047	0.4769±0.018	NA	NA	NA	NA	NA	NA
27G	1.0001±0.012	0.4960±0.019	0.4078±0.006	0.3312±0.006	NA	NA	NA	NA	NA	NA
40kpa										
22G	1.9912±0.004	0.9502±0.003	0.7548±0.047	0.591±0.005	NA	NA	NA	NA	NA	NA
25G	1.4673±0.012	0.8910±0.063	0.7238±0.020	0.5665±0.006	0.4291±0.005	NA	NA	NA	NA	NA
27G	1.346±0.009	0.6919±0.010	0.5034±0.005	0.4500±0.010	0.3951±0.010	0.3586±0.018	0.3069±0.014	NA	NA	NA
45kpa										
22G	2.3575±0.057	1.293±0.019	0.8693±0.034	0.6645±0.035	0.6829±0.083	0.5313±0.061	NA	NA	NA	NA
25G	1.742±0.083	1.312±0.043	0.9381±0.020	0.7980±0.012	0.6769±0.021	0.5933±0.032	0.5050±0.032	NA	NA	NA
27G	1.506±0.009	0.8303±0.007	0.6856±0.011	0.6233±0.022	0.5324±0.010	0.4722±0.008	0.4458±0.003	0.3798±0.019	NA	NA
50kpa										
22G	2.689±0.009	1.545±0.015	1.079±0.038	0.9027±0.025	0.7717±0.012	0.7104±0.020	0.6269±0.010	0.5572±0.046	0.5335±0.008	NA
25G	2.035±0.145	1.512±0.075	1.008±0.070	0.8812±0.034	0.7429±0.013	0.7049±0.025	0.6034±0.010	0.5273±0.006	NA	NA
27G	1.840±0.105	1.114±0.038	0.9523±0.007	0.7838±0.019	0.6528±0.017	0.6089±0.017	0.4938±0.018	0.4474±0.021	NA	NA
55kpa										
22G	2.9787±0.047	1.6587±0.035	1.2685±0.023	0.9787±0.015	0.7764±0.009	0.7273±0.010	0.7289±0.023	0.6872±0.056	0.6217±0.043	0.5498±0.025
25G	2.2753±0.046	1.7936±0.021	1.5038±0.013	1.2139±0.019	1.0478±0.018	0.9787±0.006	0.7820±0.054	0.6656±0.065	0.6086±0.030	0.5154±0.030
27G	2.3655±0.190	1.4415±0.026	1.1752±0.046	0.9947±0.015	0.8005±0.011	0.6906±0.028	0.6666±0.026	0.6144±0.052	0.5527±0.020	0.3257±0.015
60kpa										
22G	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
25G	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
27G	3.0145±219	1.6990±0.035	1.435±0.046	1.2921±0.021	1.1723±0.010	0.9931±0.065	0.9008±0.073	0.7693±0.066	0.7075±0.005	0.4059±0.013

Mean line width (mm) ± standard deviation

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VitroINK® 3D with ALLEVI2

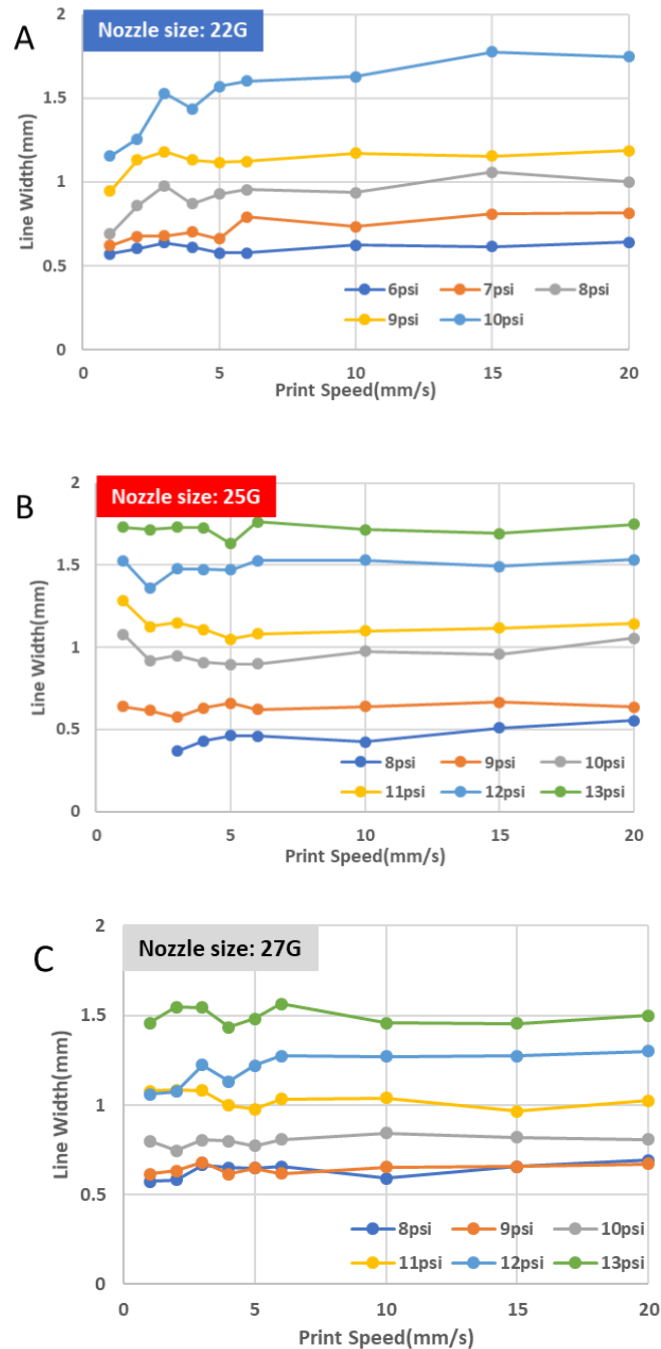


Figure 7. Printed line width of VitroINK® 3D with 22G, 25G, 27G nozzle at different pressures and print speeds. The line width remains relatively stable as the print speed increases.

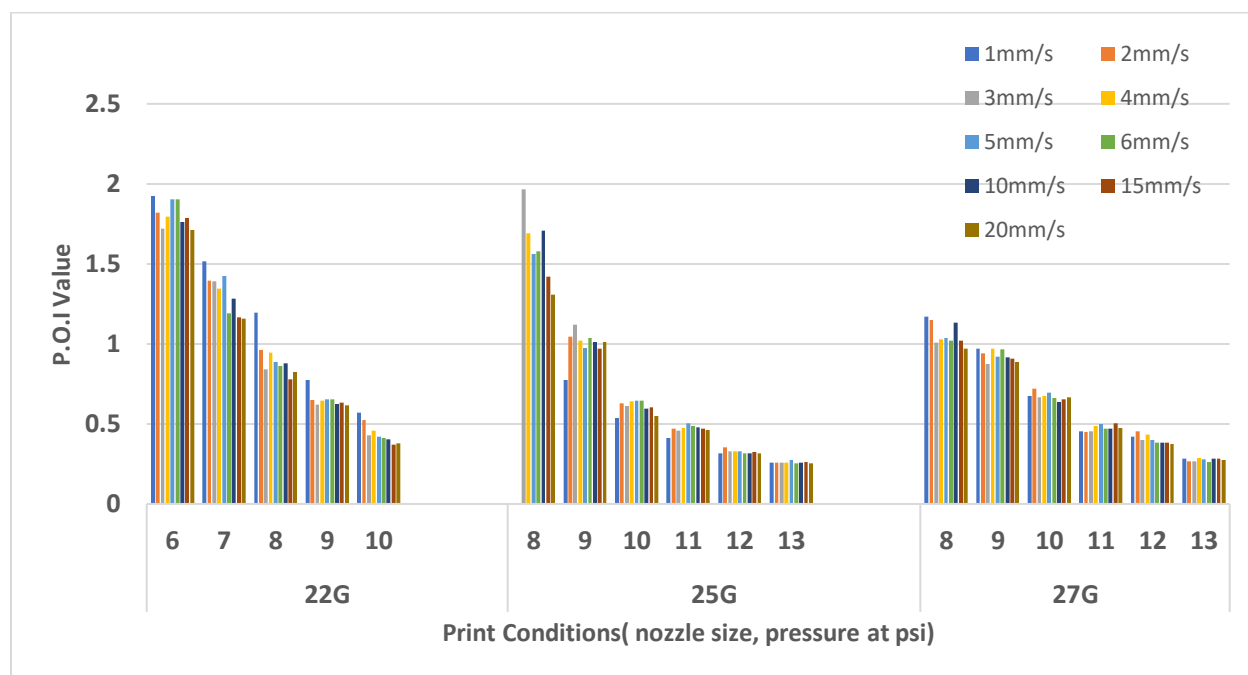


Figure 8. POI value of VitroINK® 3D with 22G, 25G, 27G nozzle at different pressures and print speeds. For all three nozzle sides, print speed at 1 or 3 mm/s give the highest POI value at low pressure.

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Table of Printed line width of VitroINk RGD 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	0.5704±0.007	0.6042±0.053	0.6386±0.065	0.6122±0.022	0.5788±0.026	0.5768±0.025	0.6243±0.019	0.6143±0.036	0.6412±0.011
25G	NA	NA	NA	NA	NA	NA	NA	NA	NA
27G	NA	NA	NA	NA	NA	NA	NA	NA	NA
7 Psi									
22G	0.6217±0.064	0.675±0.010	0.6777±0.026	0.7009±0.029	0.6613±0.008	0.7912±0.037	0.7352±0.012	0.8086±0.027	0.8139±0.016
25G	NA	NA	NA	NA	NA	NA	NA	NA	NA
27G	NA	NA	NA	NA	NA	NN	NA	NA	NA
8 Psi									
22G	0.6893±0.178	0.8577±0.064	0.9792±0.051	0.871±0.130	0.9285±0.055	0.9555±0.060	0.9375±0.058	1.059±0.055	1.002±0.095
25G	NA	NA	0.3687±0.006	0.4289±0.022	0.4637±0.032	0.4595±0.008	0.4267±0.022	0.5108±0.038	0.5541±0.017
27G	0.5741±0.093	0.5836±0.047	0.666±0.058	0.6523±0.078	0.6486±0.045	0.6576±0.045	0.5921±0.079	0.6581±0.045	0.6919±0.048
9 Psi									
22G	0.9454±0.090	1.131±0.076	1.182±0.028	1.132±0.069	1.118±0.069	1.124±0.078	1.173±0.046	1.156±0.048	1.186±0.045
25G	0.6391±0.052	0.6159±0.038	0.5747±0.058	0.6317±0.044	0.6608±0.019	0.6217±0.016	0.638±0.061	0.665±0.074	0.637±0.033
27G	0.6164±0.027	0.6338±0.055	0.6814±0.040	0.6164±0.044	0.6497±0.034	0.6180±0.017	0.6528±0.032	0.6576±0.099	0.6729±0.028
10 Psi									
22G	1.156±0.067	1.257±0.081	1.530±0.083	1.438±0.035	1.572±0.032	1.602±0.026	1.629±0.100	1.776±0.175	1.748±0.055
25G	1.078±0.184	0.9211±0.104	0.9481±0.061	0.9074±0.056	0.8963±0.022	0.8995±0.044	0.9771±0.048	0.9576±0.017	1.056±0.072
27G	0.7981±0.023	0.7474±0.015	0.8055±0.045	0.7981±0.026	0.7783±0.026	0.8097±0.014	0.8435±0.021	0.8208±0.010	0.8071±0.019
11 Psi									
22G	NA	NA	NA	NA	NA	NA	NA	NA	NA
25G	1.526±0.053	1.359±0.010	1.478±0.042	1.474±0.029	1.472±0.013	1.527±0.043	1.530±0.068	1.492±0.064	1.532±0.030
27G	1.061±0.021	1.074±0.063	1.223±0.041	1.133±0.006	1.222±0.063	1.275±0.035	1.27±0.022	1.275±0.027	1.301±0.013
12 Psi									
22G	NA	NA	NA	NA	NA	NA	NA	NA	NA
25G	1.526±0.053	1.359±0.010	1.478±0.042	1.474±0.029	1.472±0.013	1.527±0.043	1.530±0.068	1.492±0.064	1.532±0.030
27G	1.061±0.021	1.074±0.063	1.223±0.041	1.133±0.006	1.222±0.063	1.275±0.035	1.27±0.022	1.275±0.027	1.301±0.013
13 Psi									
22G	NA	NA	NA	NA	NA	NA	NA	NA	NA
25G	1.730±0.024	1.716±0.035	1.729±0.025	1.729±0.066	1.631±0.053	1.763±0.049	1.717±0.040	1.694±0.060	1.748±0.036
27G	1.458±0.008	1.546±0.112	1.545±0.014	1.435±0.073	1.481±0.038	1.565±0.013	1.459±0.067	1.454±0.048	1.500±0.070

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