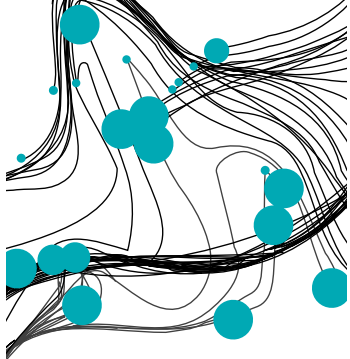
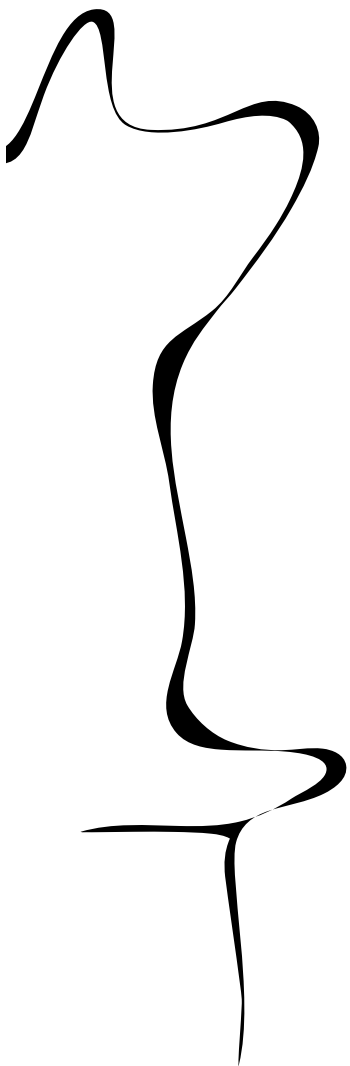




BSc Biomedical Engineering

The rheological evaluation of Xanthan Gum compositions for embedding 3D bioprinting

Bachelor thesis



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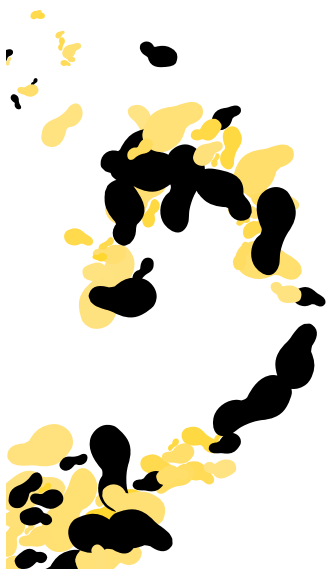
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Contents

1	Introduction	2
1.1	Research goal	2
2	Materials and methods	3
2.1	Embedding bath preparation	3
2.2	Ink preparation	3
2.3	G-code preparation	3
2.4	3D embedding bioprinting	3
2.5	Rheological evaluation	4
2.6	Sample visualization	4
3	Results	5
3.1	Rheological evaluation	5
3.1.1	Frequency sweep	5
3.1.2	Amplitude sweep	6
3.1.3	Flow sweep	7
3.2	Embedding 3D bioprinting	7
3.2.1	Macroscopic results	7
3.2.2	Microscopic results	9
4	Discussion	14
4.1	Rheological evaluation	14
4.2	Embedding 3D bioprinting	15
5	Conclusion	16
	References	17
A	G-code for 3D printing	19

1 Introduction

Every year 1000 of patients are in the waiting list for an organ transplant in the Netherlands alone[1]. Other options are researched to reduce the dependence on donors. One of them is tissue engineering. Tissue engineering is the scientific field which aims at fabricating artificial tissue constructs that can mimic the function of native human tissues and organs[2]. In addition to meeting the clinical demand for artificial tissues, the aim is creating models that can be utilized for drug testing and potentially replace clinical trials on animals for a personalized medicine approach[3].

3D Bioprinting is the branch of tissue engineering that attempts to fabricate tissue constructs[4] by using computer aided design (CAD). The combination of standardization through the machine aided process of 3D printing, and the customization potential by tweaking the g-code commands of the 3D printer, offers a suitable solution for the personalized and massive need of artificial tissues[4]. While there are a lot of different techniques, such as inkjet and laser assisted bioprinting, the most popular techniques are stereolithography (SLA) and extrusion bioprinting[4–8]. In SLA photocurable inks are polymerized under UV-light or Visible light, and either contain cells within them, or the resulting constructs are coated with cells post-print[8]. While the resolution is quite high since it is based on light patterning, the chemical composition of the resins as well as the low cell concentration per ml which are possible with this approach, hinder the fabrication of living tissue analogues[6].

In extrusion bioprinting a material is controllably deposited through a nozzle in a pattern in the x-y-z directions[5]. The material should be shear thinning, in order to liquify upon shear and recover in the absence of shear so it could retain its shape, in essence demonstrating a thixotropic behavior[9]. The material could be a hydrogel with cells, a material that can be coated with cells post-print or even a cell-only suspension, which all are within the category of bioinks[10]. Unfortunately, the bioinks that mimic well the properties of the extracellular matrix (ECM) are either 'too liquid' to keep their shape after deposition (low viscosity), or they need workarounds to demonstrate thixotropy. This can be done by crosslinking them post-deposition[11], heating or cooling them rapidly, or using photopolymerization where the same problems as in SLA techniques emerge[6].

Embedded bioprinting has come forward as a solution, where a support bath assists inks that are normally too 'weak' to print[11–13]. The support bath can either be a thixotropic hydrogel, such as xanthan gum (XG)[13], or a granular suspension[12]. In both cases the printing of weak hydrogel inks and their fidelity is improved[13, 14]. However the resolution of these techniques still depends mostly on the nozzle diameter of the 3D printer, and cell only inks present a range of challenges due to their 'particle in suspension' nature[14]. Recent developments have shown that, when properly formulated, cell only inks can create self-assembling tissues through the mechanism of diffusion packing[13].

Diffusion packing occurs when a water based Newtonian fluid particle suspension is deposited within a hydrogel embedding bath[13]. However the deposition mechanisms, and consequentially the print resolution, are severely affected by the nozzle architecture and the mechanical properties of the embedding bath[15]. Specifically in the case of XG, Salt concentration, liquid phase composition and pH can all affect its mechanical properties. Also during cell culture, pH and salt concentration are altered based on the cell type and the corresponding medium[16, 17].

1.1 Research goal

The aim of this thesis is to study the effect of XG embedding bath compositions related to the printing quality of suspension inks and the phenomenon of diffusion packing. Specifically, rheological evaluation is performed, using 1.5% XG embedding baths in Milli-Q water as control. Also different pH values and DMEM as the liquid phase of the XG embedding bath at the same w/v concentration will be tested. Then a single line 3D print is performed within that bath to evaluate the print fidelity.

2 Materials and methods

2.1 Embedding bath preparation

The embedding baths are prepared by adding Xanthan Gum (XG) (Sigma Aldrich, SLCF8454) in Milli-Q water (pH 6.998), for a target concentration of 1.5% w/v XG in solution. The solution is placed on a magnetic plate stirrer and left overnight at room temperature to homogenize. The above steps are repeated for embedding baths of different compositions, where the aqueous phase is DMEM (Gibco, 4.5 g/L D-Glucose, L-Glutamine), pH 2.00 buffer (WTW, Technical buffer, pH 2.00), pH 4.006 buffer (WTW, Technical buffer, pH 4.006) and pH 9.180 buffer (WTW, Technical buffer, pH 9.180). The pH of the embedding baths are validated using a pH meter. To remove gas bubbles from the XG solution, the XG solution is divided into two 50 mL centrifuge tubes and centrifuged at 300 rcf for 3 minutes. The solution can be stored in the fridge at 4 °C until usage. For a 3D print execution, a 35 mm NTCT Petri dish (Greiner bio-one, 627161) is filled with the appropriate xanthan gum composition up to 10 mm in height.

2.2 Ink preparation

Light pink pigment powder (AMI, 586696) is added in a 50 mL centrifuge tube filled with Mill-Q water, aiming for a concentration of $\pm 13\%$ w/v powder to mixture. The pigment is added with a spatula to the tube, and then shaken until the powder is homogenously dispersed. In order to prevent sedimentation, before every 3D print execution, the tube is shaken vigorously for 1 minute. That way clogging due to sedimentation is prevented.

2.3 G-code preparation

To execute a print, a g-code is prepared in notepad++. The script is written for the printing of a single straight line of 1.5 cm (Appendix A). The E value is calculated by equation 1.

$$E = \frac{V_{print}}{S.A.plunger} \quad (1)$$

Where the V_{print} is calculated according to equation 2.

$$V_{print} = V_{ink} = \sqrt{(x_1y_1)^2 + (x_2y_2)^2} * \pi * r^2 \quad (2)$$

If there is not enough pigment visible in the print, the E value is adjusted (doubled, quadrupled) indicated to the result to optimize the print.

2.4 3D embedding bioprinting

A 3D printer (DR. INVIVO4D, ROKIT Healthcare) is used for 3D embedding bioprinting. The ink is loaded into a 10 mL syringe (BD,305959) and placed in the syringe extruder. A 90° bent 22G blunt needle (Needlez) and a straight stainless steel 22G needle (CELLINK) are fitted to the syringe. Then, the bed is levelled, depending on the nozzle. To make sure the nozzle is filled with the ink, the piston of the extruder is lowered until the ink flows out. Then the embedding bath is placed in the center of the printing bed. It is adjusted manually when switching between the straight and the bent needle. Finally, the print command is executed immediately to prevent clogging. In case of clogging, the syringe is removed, vigorously agitated until the ink homogenizes and then placed back in the printer module.

2.5 Rheological evaluation

A rheometer (Discovery HR20) is used for the rheological evaluation. For each embedding bath composition a frequency sweep, an amplitude sweep and a flow sweep were performed. A 20 mm diameter parallel plate (Peltier) is used for all the measurements. All the experiments are executed at 25 °C. The measurement gap is 1000 μm with a trim gap offset of 25 μm . The XG composition is loaded into a 10 mL syringe (BD, 305959) and then extruded onto the lower parallel plate of the rheometer. The upper parallel plate is lowered to the trim gap to remove the excessive sample at the edges. To investigate the visco-elastic behaviour in the linear visco-elastic region (LVR), a frequency sweep is measured using the oscillation frequency. The frequency sweep measurements are executed at 3% strain, at a frequency from 0.01 Hz to 100 Hz, with 10 points per decade[18]. The amplitude sweep measurements aim to determine the turnover point of the loss and storage modulus. The amplitude sweep measurements are executed at a constant frequency of 1.0 Hz, with strain from 0.1% to 1000%, with 10 points per decade[18]. The flow sweep is performed in order to determine the viscosity of the XG composition and to see if it has shear thinning properties. The flow sweep measurements are executed at a shear rate from 0.1 1/s to 100 1/s, with 10 points per decade[18]. All the data is processed using the TRIOS software and plots were created using Origin software.

2.6 Sample visualization

Macroscopic pictures are obtained via cellphone camera, as well from the top as from the side. Microscopic pictures are obtained using an EVOS FL (ThermoFisherScientific). All the pictures are made with the bright filter, using transmitted light, and using the 2x and the 4x magnification. Microscopic pictures are made of the entry point, some points in the middle, and the end point of the printed line. Processing of the images are done in ImageJ.

3 Results

In order to identify if there is a significant difference between printing quality and embedding bath composition, rheological evaluation and 3D printing of particle suspension inks were performed using different liquid phases for the bath compositions, and different nozzles for the 3D printing.

3.1 Rheological evaluation

The effect of pH on the rheological behavior of XG embedding baths was determined by performing a frequency sweep (Figure 1), an amplitude sweep (Figure 2) and a flow sweep (Figure 3). Also the difference between different media, DMEM vs. MilliQ water, was determined.

3.1.1 Frequency sweep

The frequency sweep (Figure 1) shows the mean and storage modulus against the angular frequency. Figure(1) shows that in every XG composition, the storage modulus is higher than the loss modulus. At approximately an angular frequency of 100 rad/s, the XG become strain dependent and with increasing strain the storage modulus will surpass the loss modulus. Therefore only the LVR is seen until an angular frequency of 100 rad/s.

In Figure (1.A) a difference is seen between DMEM and Milli-Q water. Specifically the storage modulus and loss modulus is higher of XG with DMEM, especially at a lower angular frequency, than XG with Milli-Q water. Also it is noticeable that the loss modulus of XG with DMEM stays relatively constant with increasing angular frequency, while there is a slightly increase visible in the loss modulus of XG with Milli-Q water.

In Figure(1.B) a difference is seen between different pH of the XG compositions. All the pH buffers, pH 2.00, pH 4.006, and pH 9.180, have a storage and loss modulus which is higher than the XG composition with Milli-Q water. Between the different pH buffers, a difference is seen in both the storage modulus and loss modulus. Both storage and loss modulus increase with increasing pH. It results in the lowest storage and loss modulus for the pH 2.00 buffer and the highest storage and loss modulus for the pH 9.180 buffer. The effect of the pH on the storage modulus is higher at lower angular frequencies while the effect of the pH of the loss modulus is higher at higher angular frequencies.

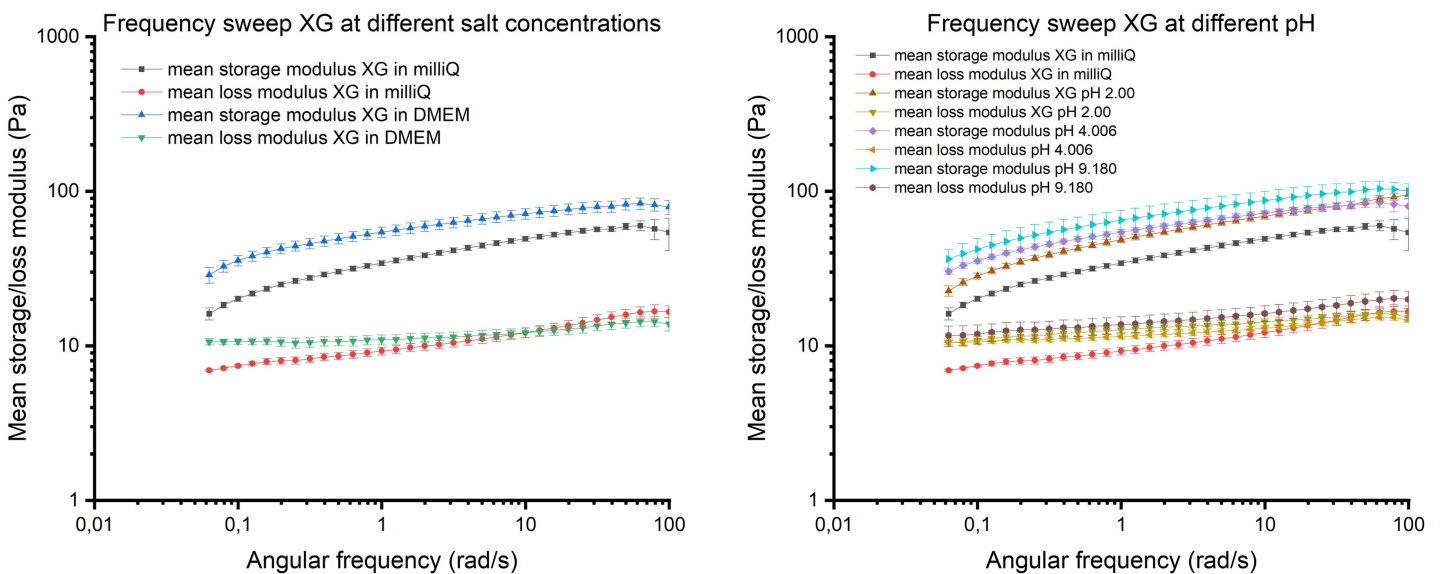


Figure 1: Frequency sweep of 1,5% w/v XG embedding baths at A.) different salt concentrations and B.) different pH buffers

3.1.2 Amplitude sweep

The amplitude sweep (Figure 2) shows the storage modulus and the loss modulus against the oscillation strain. Figure (2) shows the same as in the frequency plots that in every XG composition, the storage modulus is higher than the loss modulus. At approximately an oscillation strain around 100%, the XG becomes strain dependent and with increasing strain the storage modulus will surpass the loss modulus. This applies to both Figure (2.A) and Figure (2.B).

In Figure (2.A) a difference is seen between the storage modulus of DMEM and Milli-Q water. The storage modulus of XG with DMEM is higher than the storage modulus of XG with Milli-Q water. In contrast, the loss modulus is nearly identical for XG in both DMEM and Milli-Q water. Figure (2.A) also shows that the storage and loss modulus for both XG compositions are relatively constant before the crossover point. Slightly before the crossover point, the storage modulus starts to decreasing and the loss modulus starts to increasing, with increasing oscillation strain.

Figure (2.B) shows the amplitude sweep of XG at different pH. In the figure, it is seen that XG with Milli-Q water has the lowest storage and loss modulus of all. Between the different pH buffers, a difference is seen in both the storage modulus and the loss modulus. The storage modulus is the highest for the pH 9.180 buffer. The storage modulus of the pH 2.00 and pH 4.006 buffer are almost identical, although the pH 4.006 buffer is slightly higher than the pH 2.00 buffer. The loss modulus shows the same effect as the storage modulus. The loss modulus increases with increasing the pH where pH 9.180 shows the highest loss modulus and pH 2.00 shows the lowest loss modulus of all the pH buffers.

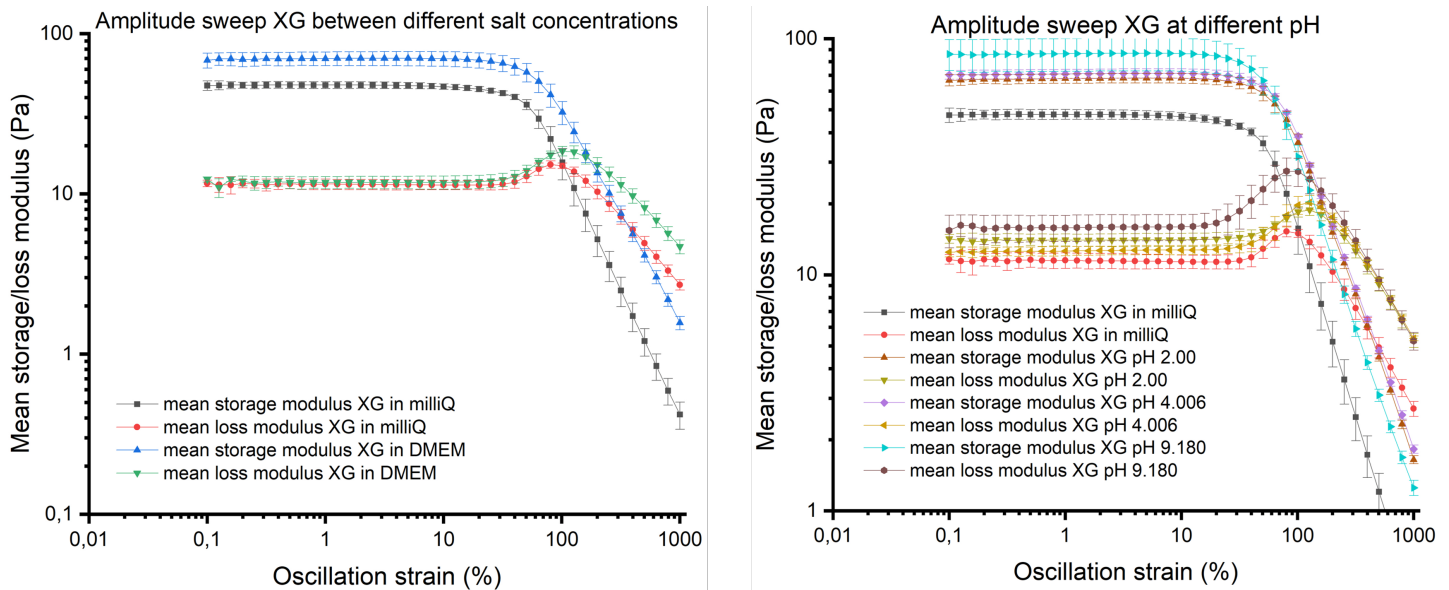


Figure 2: Amplitude sweep of 1,5% w/v XG embedding baths at A.) different salt concentrations and B.) different pH buffers

3.1.3 Flow sweep

The flow sweep (Figure 3) shows the relationship between the mean viscosity and the shear rate to determine how the viscosity changes as shear rate increases. Both Figure (3.A) and Figure (3.B) shows a decrease in viscosity across all XG compositions, as the shear rate increases. Also in both figures the XG with Milli-Q water shows the lowest viscosity.

Figure (3.A) shows a difference between the XG with DMEM and the XG in milliQ. The viscosity of XG in DMEM is higher than XG in milliQ. Also the difference is bigger at lower shear rates than higher shear rates.

Figure (3.B) shows a difference between milliQ and the pH buffers. XG in MilliQ has the lowest viscosity, but the viscosity of the XG compositions with various pH buffers are almost identical. There is a slight difference in viscosity between the pH buffers, where pH 4.006 is the highest, followed by pH 2.00, with pH 9.180 showing the lowest viscosity. This minimal difference diminishes as shear rate increases. At higher shear rates the viscosity of the pH buffers are almost identical.

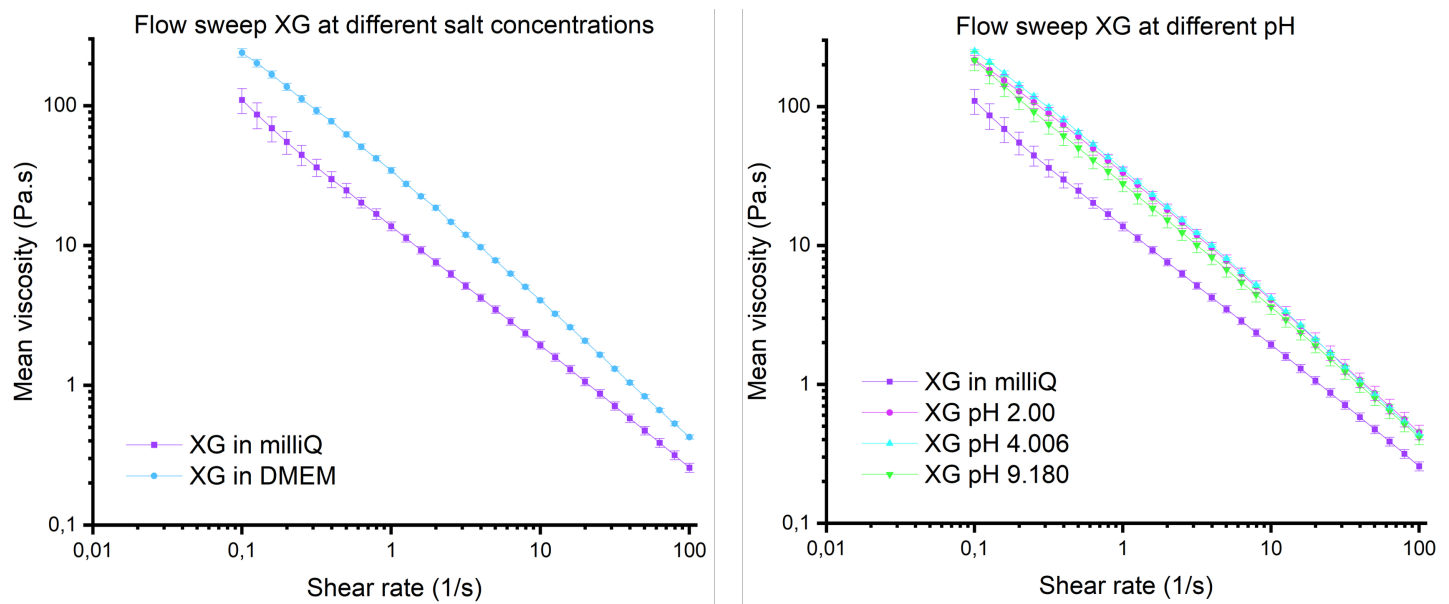


Figure 3: Flow sweep of 1,5% w/v XG embedding baths at A.) different salt concentrations and B.) different pH buffers

3.2 Embedding 3D bioprinting

The effect of the XG bath composition on the 3D printing was determined by performing the prints, with both a straight and 90° degree bent needle, using Milli-Q water as control (Figure 5). Different pH values and media (DMEM) are tested and evaluating via microscopy and macroscopic pictures (where it was possible).

3.2.1 Macroscopic results

Figure (4) shows the difference between a 3D print with a straight needle and a bent needle. The biggest difference between the two, is that the straight needle does not show a horizontal straight line, while printing with a 90° bent needle does show a horizontal straight line with concentrated pigment particles along the extruded line. Although, the macroscopic pictures of the straight needle shows a dense straight upward line with a lot of pigment particles in the end point of the extruded line. This is not present in the prints with the bent needle. Also, the prints made with a bent needle shows that the extruded line is bigger in the beginning and becomes smaller towards the end of the extrusion path. The same trend

persists in all other XG compositions that were tested, upon macroscopic evaluation. Optical properties are influenced by the different compositions. XG with Milli-Q water (Figure 4.A) is the best to visualize, while XG with DMEM (Figure 4.B) and the pH buffers (Figure 4.C-D-E) are not due to its colour and opaqueness.

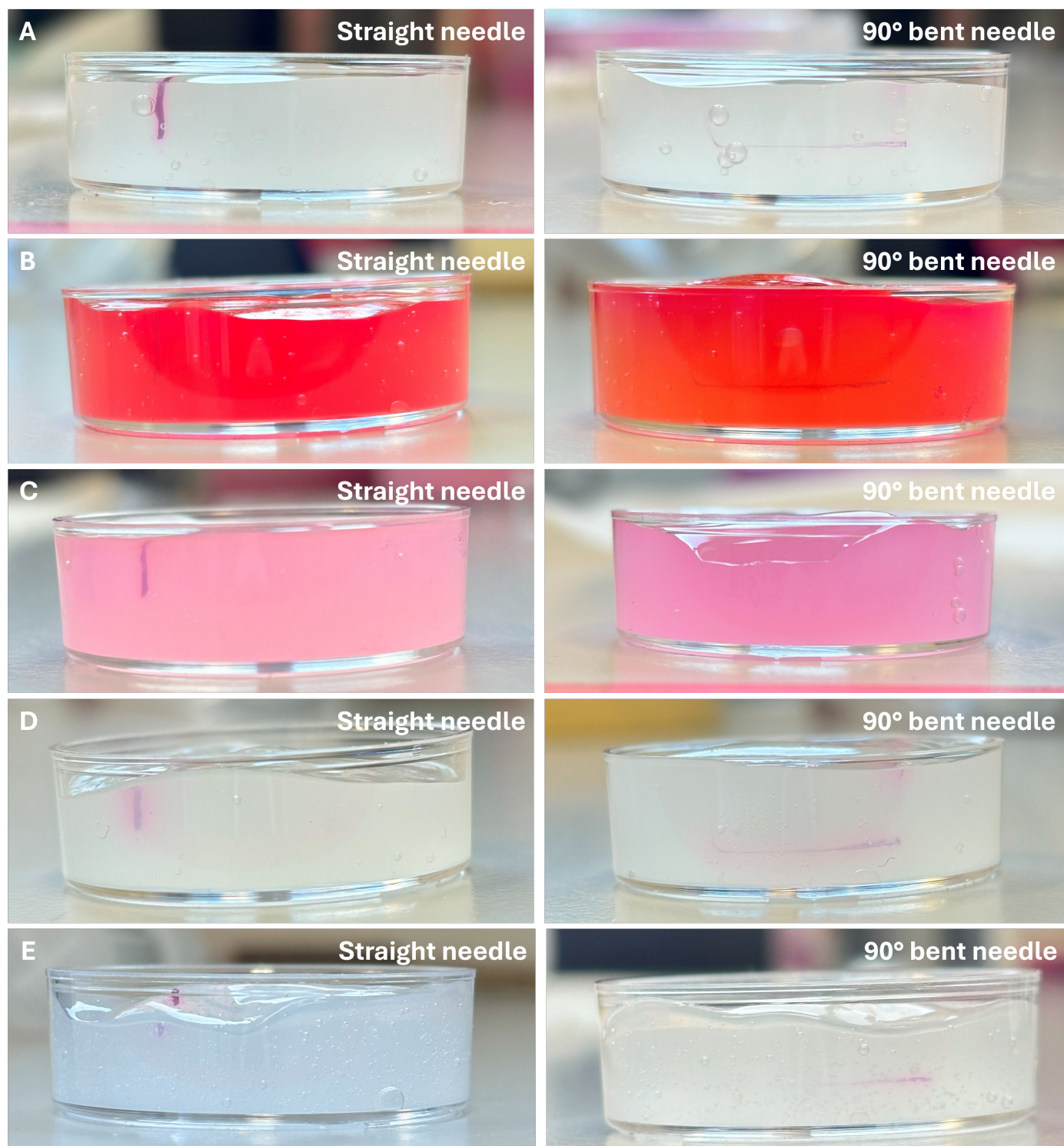


Figure 4: Macroscopic pictures taken with a mobile of prints with a straight and a 90° bent needle in 1.5% w/v embedding baths of XG with A.) Milli-Q water B.) DMEM C.) pH 2.00 buffer D.) pH 4.006 buffer and E.) pH 9.180 buffer. The blue scale bar is 0.5 cm.

3.2.2 Microscopic results

The same samples shown above were imaged microscopically, as described in section 2.6. The light intensity varies with different magnifications and different XG compositions, due to the difference of optical properties. Images were taken with a 2x magnification and a 4x magnification. Multiple photos are taken in sequence to show the print from entry to exit point.

Xanthan Gum with Milli-Q water

Figure (5) shows the microscopic view of the embedding XG bath with Milli-Q water. Figure (5.A) shows a clear straight line with dense pigment particles. The same trend as in Figure (4) with using a 90° bent needle, where the line is a little thicker and more visible towards the beginning of the extrusion path, is also visible in Figure (5.A). The pigment becomes less dense and the printing resolution decreases. The black spots with a white spot in the middle represent the bubbles in the embedding bath.

Figure (5.B) shows that with a straight needle there are pigment particles visible alongside the extrusion path, although it is not visible to the eye. It shows much fewer and not dense pigment particles. Also, different layers are visible, due to upwards flowing particles. This is seen during the possibility of focusing on different particles at the same spot of the line. The big black spot on the endpoint in Figure (5.B), represents the vertical pink line with a lot of pigment particles seen in Figure (4) in the macroscopic pictures of the straight needle.

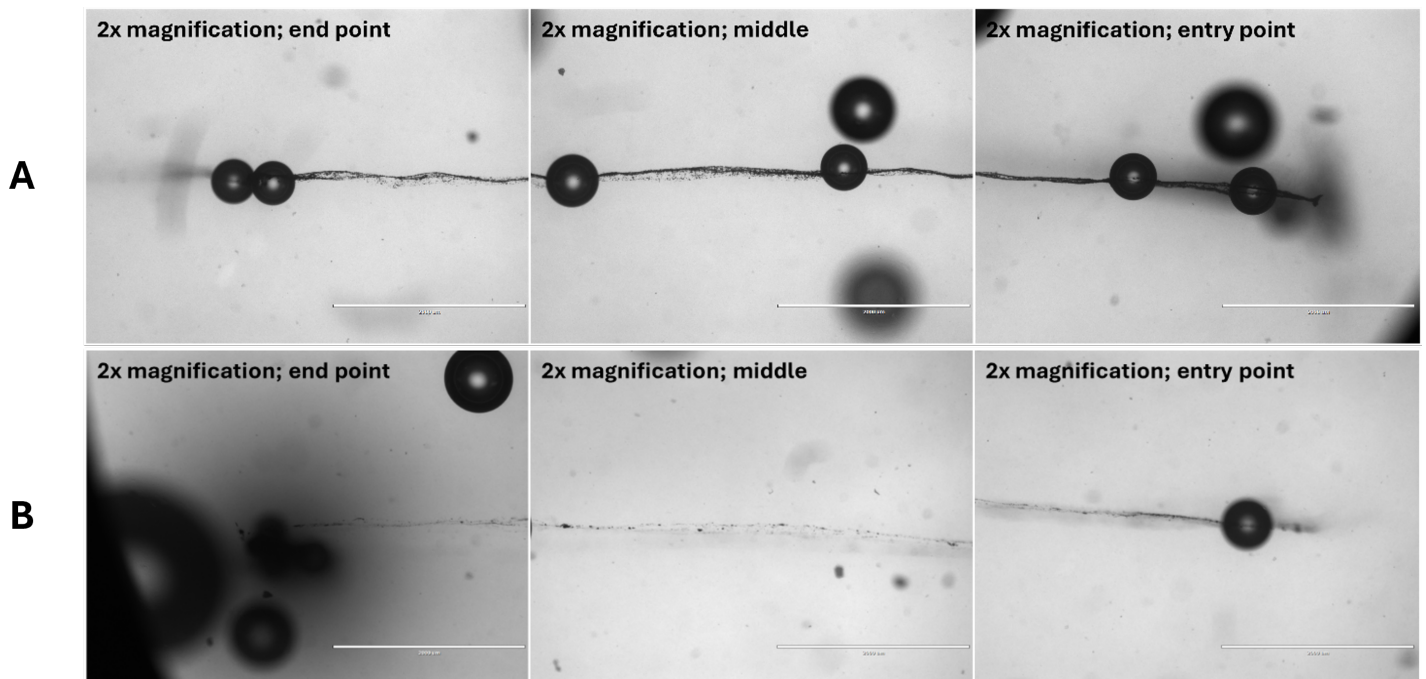


Figure 5: Microscopic pictures taken with the EVOS FL of prints in an embedded bath of XG with Milli-Q where A.) shows the result of a print with a 90° bent needle and B.) a print with a straight needle. The white scale bar in the lower right corner of every picture is 2 mm.

Xanthan Gum with DMEM

Figure (6) shows the microscopic pictures of XG with DMEM as an embedding bath. The same result as in Figure (5) is seen, that a 90° bent needle prints a straight line with dense pigment particles (Figure 6.A), while a straight needle does not show a straight line along the extrusion path and has much fewer pigment particles present (Figure 6.B). Compared to the Milli-Q, the embedded line of XG with DMEM (Figure 6.A) shows a thicker line than the embedded line in XG with Milli-Q (Figure 5.A). The print with a straight needle in XG with Milli-Q (Figure 5.B) shows less pigment than XG with DMEM as an embedding bath (Figure 6.B). Also, the big black spot in the endpoint, which represents the upward flow of particles creating a thick vertical line, is smaller in XG with DMEM (Figure 6.B) compared to XG with Milli-Q (Figure 5.B).

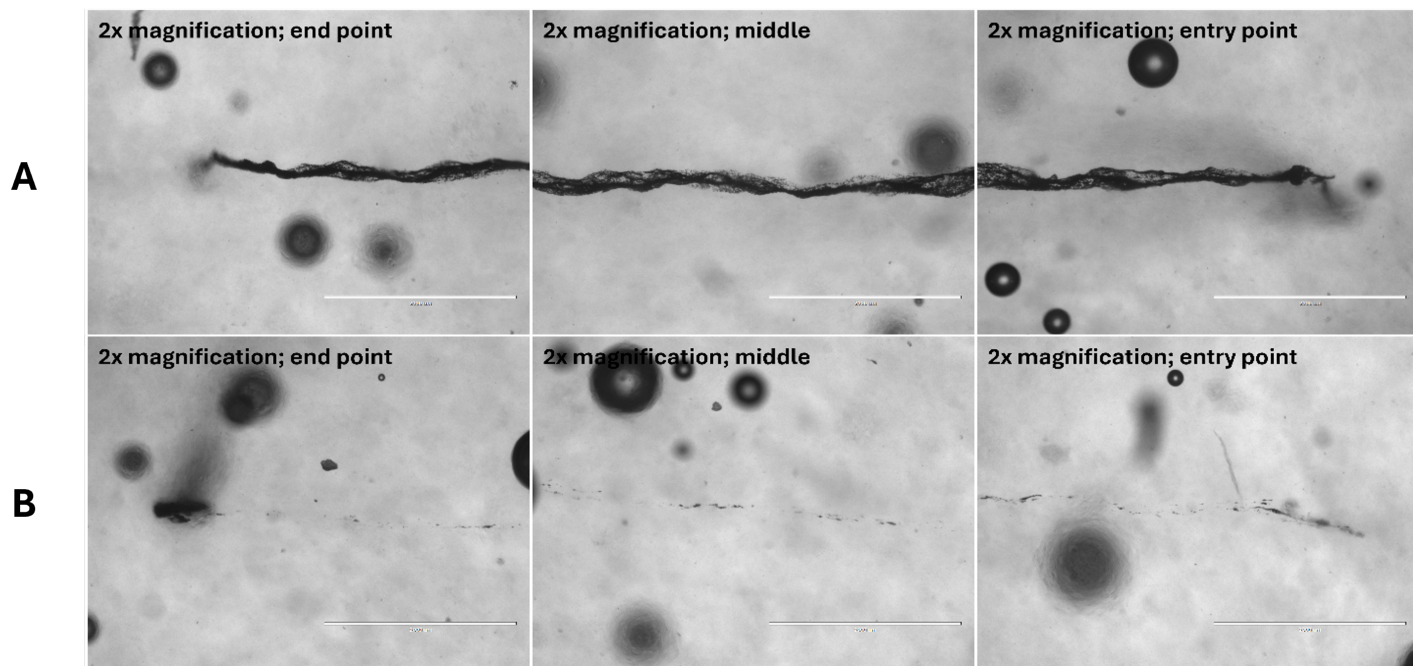


Figure 6: Microscopic pictures taken with the EVOS of prints in an embedding bath of xanthan gum with DMEM where A.) shows the result of a print with a 90° bent needle and B.) a print with a straight needle. The white scale bar in the lower right corner of every picture is 2 mm.

Xanthan gum with different pH buffers

The results of the prints under different pH conditions are shown for a 90° bent needle (Figure 7) and a straight needle (Figure 9). Comparing the two nozzle types, the same phenomenon of printing quality is observed. A print with a 90° bent needle creates an embedded line with dense pigment particles, while a print with a straight needle does not create a visible line and has a low concentration of pigment particles. Comparing the different pH in Figure (7), XG with pH buffer 4.006 (Figure 7.C) shows the thickest line with the highest pigment particle concentration alongside the extrusion pattern. The embedded line in XG with pH 9.180 buffer (Figure 7.D) shows a similar result as the embedded line in XG with Milli-Q (Figure 7.A). However, the embedding bath with pH 9.180 presented many more gas bubbles than the one with Milli-Q. Also, a significant difference is seen at pH 2.00 (Figure 7.B), where a lot of bubbles are present alongside the line, even though the experiment was repeated while ensuring that no gas is present in the nozzle and ink cartridge.

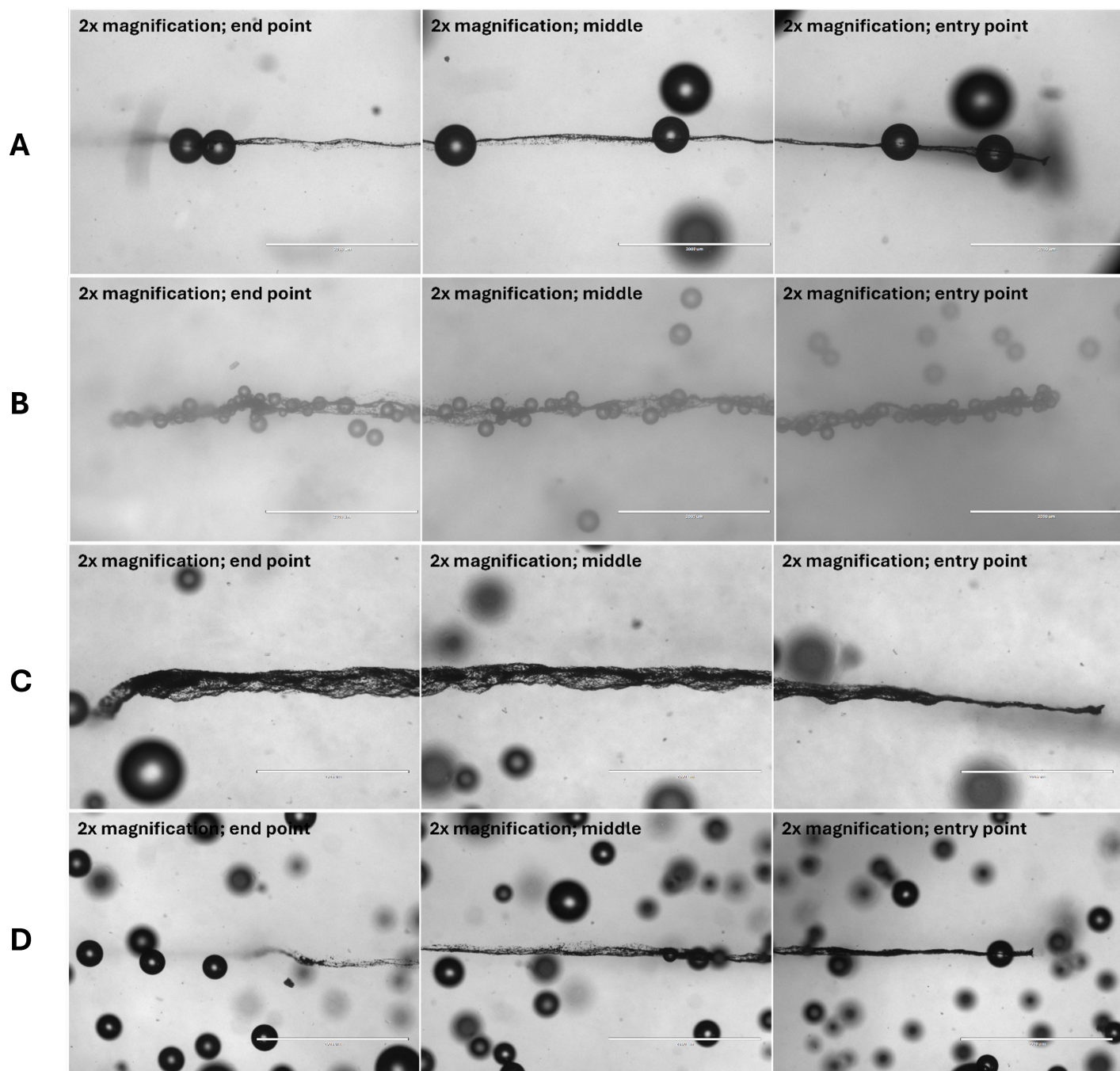


Figure 7: Microscopic pictures taken with the EVOS FL of prints with a 90° bent needle in an embedding bath of XG with different pH buffers where A.) shows the result of the reference print with Milli-Q B.) with pH buffer 2.00 C.) with pH buffer pH 4.006 and D.) with pH buffer 9.180. The white scale bar in the lower right corner of every picture is 2 mm.

Due to the peculiar morphology of the printed lines in Figure (7B-C), closeups are presented in Figure (8).

Figure (8) is a higher magnification to see the difference between XG compositions with different pH. Diffusion packing could be seen in Figure (8.C). However, in figure (8.A) the line contains bubbles that can not attributed to the experimental handling. Also, in figure (8.B) the line is wider than observed in the other XG compositions.

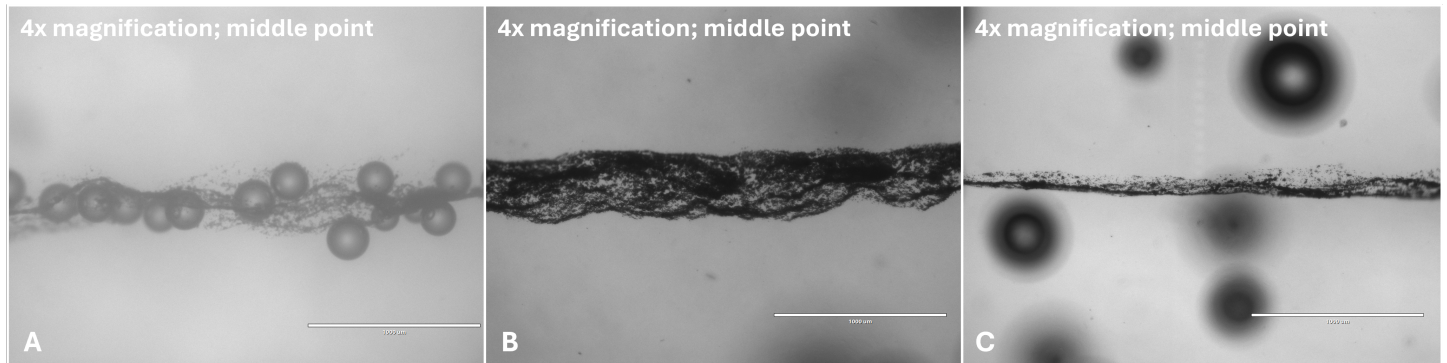


Figure 8: Microscopic pictures taken with a 4x magnification with the EVOS FL from the middle part of the embedded line to show the difference between XG compositions with A.) pH 2.00 buffer B.) pH 4.006 buffer and C.) pH 9.180 buffer.

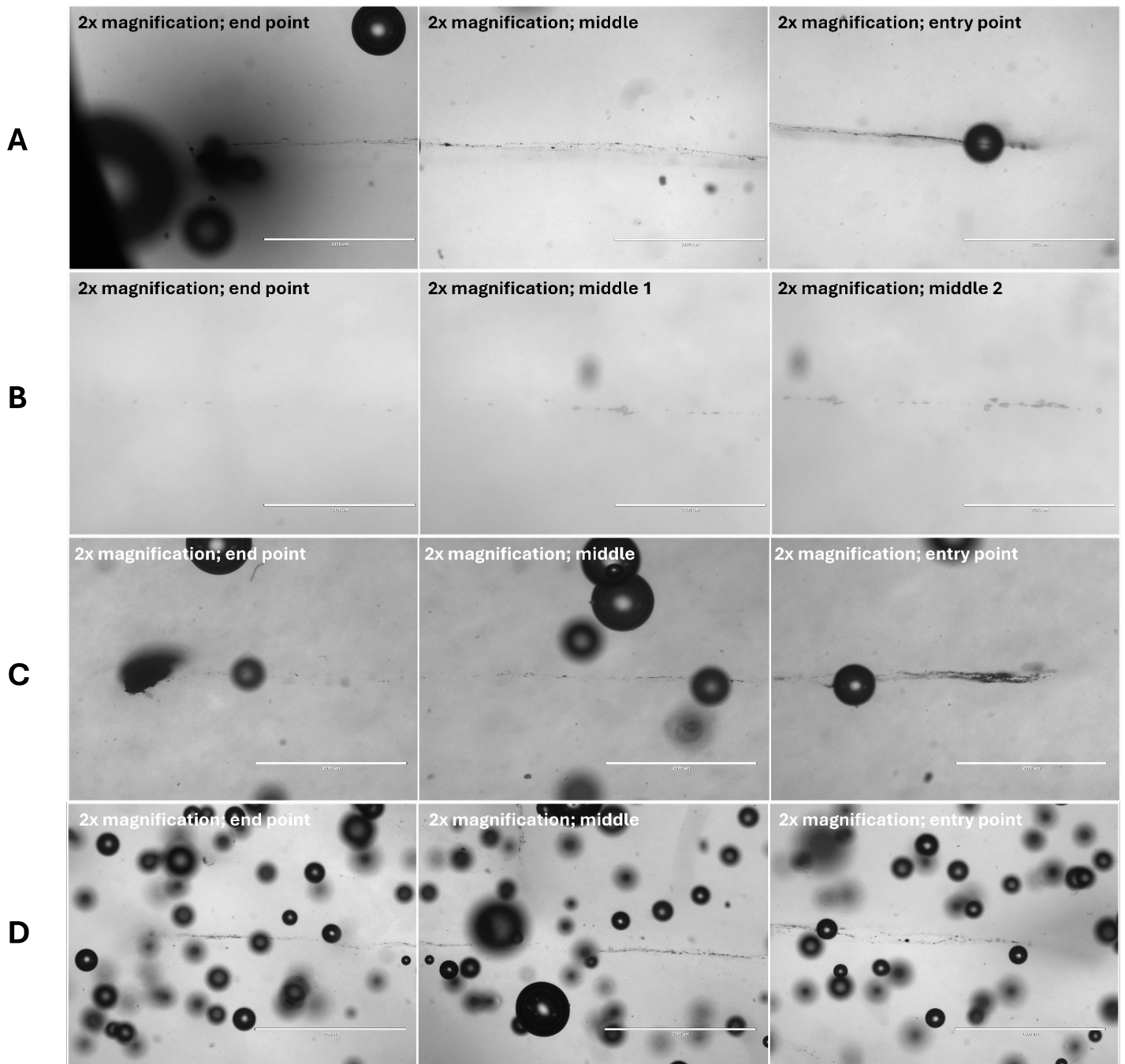


Figure 9: Microscopic pictures taken with the EVOS FL of prints with a straight needle in an embedding bath of xanthan gum with different pH buffers where A.) shows the result of the reference print with Milli-Q B.) with pH buffer 2.00 C.) with pH buffer pH 4.006 and D.) with pH buffer 9.180. The white scale bar in the lower right corner of every picture is 2 mm.

Figure (9) shows the results of the microscopic pictures of the prints with a straight needle. No significant difference was observed in the print quality using the straight nozzle across all experimental conditions (Figure 9).

4 Discussion

4.1 Rheological evaluation

The results of the rheological evaluation offer a good insight into the rheological properties of XG embedding baths with different liquid phases, which were Milli-Q, DMEM and different pH buffers. First, the frequency sweep (Figure 1) was determined by presenting the mean storage modulus against the angular frequency. The mean storage modulus indicates the elastic solid-like behavior and the loss modulus is related to the viscous liquid-like behavior[19]. In the frequency data of all XG compositions, the storage modulus is higher than the loss modulus, which indicates that the embedding baths have a predominantly elastic behavior at a strain of 3% in the linear visco-elastic region (LVR). This is needed to maintain the structural integrity of the embedded lines during 3D printing. Also, storage and loss modulus are higher at XG in DMEM (Figure 1.A), specifically at lower angular frequencies. This could suggest that DMEM enhances the elastic-like behavior, due to the presence of ions and other components in the culture medium. An embedding bath with higher elasticity is better for supporting the printed lines. Also, the pH has a significant impact on the storage and loss modulus (Figure 1.B). Both storage and loss modulus become higher while increasing the pH, where pH 2.00 result in the lowest storage and loss modulus of all pH buffers and pH 9.180 the highest. So the rigidity of XG embedding baths is lower under acidic conditions and higher under basic conditions. Maybe this is related to the molecular structure of XG.

To determine the LVR and the crossover point between the elastic-like and liquid-like behavior, the amplitude sweep was determined. The amplitude sweep presents the storage and loss modulus against the oscillation strain (Figure 2). The amplitude sweep results indicate that at approximately an oscillation strain around 100% the embedding XG baths become strain-dependent. When XG becomes strain-dependent, it will lose its elastic behavior and result in more liquid-like behavior. This affects the support of the embedding XG baths, because it is less able to withstand mechanical stresses during 3D printing. This will result in losing the ability to support the printed lines. Comparing XG with DMEM and Milli-Q water, the same trend as in the frequency sweep (Figure 1.A) is observed. XG with DMEM has a higher storage modulus which indicates that embedding XG baths with DMEM may offer better resistance to deformation under strain, improving the rigidity of the bath. This could result in better printing quality. Also the pH buffers (Figure 2.B) shows the same trend as in the frequency sweep. An increase in the pH values increases the storage modulus. This could suggest again that molecular interactions between acid and bases could influence the mechanical support during printing. A higher pH, in this case at pH 9.180, could provide better support of the printed lines which improves the print quality.

The viscosity was determined by measuring the flow sweep (Figure 3). In all XG compositions, a decrease in viscosity is seen with increasing shear rate. This property is typical for shear-thinning fluids[20]. This is a good characteristic for embedding 3D bioprinting because it could achieve shape fidelity during printing[bron]. During extrusion of the material the XG embedding bath regains its viscosity quickly when the nozzle is passed to support the printed line. Comparing all the XG compositions, Milli-Q shows the lowest viscosity across all shear rates. This suggests that this composition result in the lowest structural support, although extrusions will be easier in this composition. The other compositions, DMEM and pH buffers, almost identical in viscosity. These are higher than Milli-Q and should be better for improving print quality and supporting the printed material. On the other hand, it could be possible that it needs higher extrusion pressures. Comparing the pH buffers (Figure 3.B), lower shear rates show a slight difference in viscosity but at higher shear rates this difference diminishes. So at lower shear rates the pH could affect the flow properties but while printing it shouldn't make a big difference because higher shear rates predominate.

4.2 Embedding 3D bioprinting

The results of the embedding 3D bioprinting provide information of how the different bath compositions influence the printing quality. In the macroscopic analysis (Figure 4) a drastic difference is seen between printing with a straight or a 90° bent needle. With a 90° bent needle, it is possible to print a straight embedded line with concentrated pigment particles while it is not possible with a straight needle. This may occur because of the alignment of the flow direction with the plane of the print. When using a straight needle the particles could flow upwards when the needle flows through the embedding bath. Looking at the optical properties, XG in Milli-Q water is the best to visualize the print, but it has a lower viscosity and storage modulus which results in a decrease of the support of the print. The prints in other XG compositions are worse to visualize macroscopically due to their opaqueness and colour, but they have higher viscosity and storage modulus according to the rheological evaluation which can make it better for structural supports of the print.

The microscopic analysis provides more information of the printing quality using different XG compositions. Figure (7) shows that also in the microscopic view a clear straight line is visible with more concentrated pigment present than printing with a straight needle (Figure 9). Comparing XG with Milli-Q and DMEM, XG with DMEM shows a thicker line than with Milli-Q. There are more concentrated particles present in the embedded line of XG with DMEM (Figure 6).

The difference in pH buffers of XG compositions also show a peculiar result in printing quality (Figure 7). Diffusion packing for example, which is seen in embedding baths of XG, is only visible in the pH 9.180 buffer and the control. It can be seen that the fluid diffuses to the outside while the particles become concentrated in the middle[13]. Also in the pH 2.00 buffer there are bubbles present alongside the extrusion path. This is peculiar because it is not seen in the other compositions. Also pH 4.006 creates a thicker line which is not totally explainable.

It is not possible to draw a conclusion that the pH effects immediately the effect of diffusion packing. Further investigation is needed as to why in pH 4.006 the morphology of the line is so different.

5 Conclusion

The aim of this thesis was to study the effect of 1.5% w/v XG embedding bath compositions related to the printing quality of suspension inks and the phenomenon of diffusion packing. Specifically the rheological properties were evaluated.

From the rheological evaluation it could be concluded that of all XG compositions, the storage modulus is higher than the loss modulus, which indicates that the embedding baths have predominantly elastic behavior. This is preferable for embedded 3D printing to maintain the rigidity of the printed material. From the flow sweep it could be concluded that all XG compositions have shear thinning properties, which is also preferable during the 3D printing. Also, there is a slightly difference between the different pH buffers, where the increase of pH shows an increase in storage modulus and thus the rigidity of the embedding bath.

From the 3D printing it can only be concluded that printing with a straight nozzle does not form a straight embedded line, while with a 90° bent needle it is possible to print a straight embedded line with concentrated particles. But comparing the rheological results with the 3D printing, it is hard to draw a clear conclusion between them. Peculiar results are seen in the XG compositions with different pH buffers. Diffusion packing can only be seen in the pH 9.180 buffer, but it is not possible to relate it to the rheology.

To get a total understanding of the relation between pH and the print fidelity, and also relate it to the rheological properties, further research is necessary.

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Appendices

A G-code for 3D printing

```

1  ;(**** start of start-gcode for Edison Bio NK 1.57.68.0 BI-P1-01-170320-0010 ****)
2  G21; set units to mm, setting it to metric
3  G90; set positioning to absolute, instead of relative. Effect on the arc and calibration
4
5  ;M104 S0 T0 (set extruder temperature, no parameters set turns off the heater, S
    determines degrees celsius, T unknown)
6
7  G92 Z0; (Sets position to the value of X/Y/Z to zero, no motion occurs !!potential for
    mistakes here)
8  G1 Z20 F200; (X Y Z E F slots, at least one parameter must be used, linear move while
    printing. E is amount of extrusion, F feedrate)
9
10 G28 X Y F500 (home XY axes maximum)
11 G28 Z F500 (home Z axis minimum)
12 M1010 S1 (C clamp activation, dont know dont touch)
13
14
15 ;G1 Z20 F1500 (Weird movement to Z=20, i dont know why its there)
16 ;G1 X32 Y17 F1500 (moving at a calibration point its ignored in this case)
17 ;M109 S0 T0 ; (wait for extruder to reach temperature, useful only in filament module)
18
19
20
21
22 ;M106 P4 S255                ; (uv led 1, P for part, s for intensity)
23 ;M106 P3 S255                ; (uv led2 same as above)
24
25
26
27 ;M1015 P1 F600.0 E=10.0; (weird thing about plotting feedrates, dunno if useful, used in
    right module with temp control)
28
29
30 G92 E0 (Set E to 0 again, thus all values above 0 will be treated as extrudable volume)
31
32 T2                ; (T1 right extruder, T2 left extruder.Switch to the first
    extruder effectively tool change, in dual nozzle print this is vrey important)
33
34
35
36 ;(**** end of start-gcode ****)
37
38
39 ;Edislicer 3.7.29 (Aug 16 2019 08:55:27)
40 ;config.smallSkinSpeedHalfInfillSpeed = 0 6
41 ;Nozzle 2 infill line-width(NozzleSize) = 0.4(0.4)
42 ;
43 G1 Z20.000 F600 (Nozzle Hop)
44 ;Layer count: 8
45 M73 P0 ( sets the progress rate, this needs to be updated at least once mid print,
    LAYER: 1 )
46 ;LAYER:1
47 M106 S255 (Fan On, but useless in hydrogel extrusion/hot melt)
48 G0 F600 X60 Y50
49 G0 F600 Z2
50 M203 Z7          ;Z axis max speed 7 mm/sec, this must be a peculiarity of the specific
    machine connected to M1010)
51 ; (PRINTING COMMANDS HERE)
52
53 G0 F600 X60 Y50
54 G1 F600 X45 Y50 E1,25720176
55
56 ; (WRAP UP)
57 G92 E0
58 G0 Z23.600 F600 (Nozzle Hop)
59 M73 P100
60
61
62 ;(**** start of end-gcode for Edison Bio NK 1.57.68.0 BI-P1-01-170320-0010 ****)

```

```
64 M73 P100 ; end progress
65
66 M104 T0 S0 ;extruder heater off
67
68 M106 P4 S0 ;uv led1
69 M106 P3 S0 ;uv led2
70 M1010 S0 ; TM
71
72 ;(**** end of end-gcode ****)
73
```