

## From Biomaterial to Organoid - Bioprinting for Practice

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**Abstract.** The current state of technology for 3D printing with biomaterials is based on the extrusion of viscous materials. Mostly, extrusion heads utilize pneumatic pressure systems or stepper motors to force the substrate onto a surface. These methods are well developed for high viscous materials. However, processing low viscous liquids may cause leakages in the system. This could be solved by applying continuous extrusion. Additionally, in order to process gelable substrates, such as gelatine and agar, tempered print heads in combination with a multi stage tempering system are required to prevent the system from clogging.

The ongoing work presented in this paper focuses on the development of an extrusion system, which should be able to process multiple viscosities of gelatine sequentially. In order to achieve this, several measurements to examine the properties, as well as the material parameters of different biomaterials are performed. In this process gel point, force resistance and elasticity are the factors of particular interest. Due to their ability to gel and their availability, the most relevant biomaterials are gelatine and agar. Using this data, an extrusion system involving a peristaltic pump, a heated tube and a nozzle, has been developed.

The next step envisaged is to calibrate the extruder based on the obtained data and finally to validate the printing process by printing simple geometric structures. Assuming that a positive evaluation is obtained, the printing system will be tested for printing first organic test structures from patient data using the examined biomaterials.

### Introduction

The progression in the field of medicine and the political stability had a strong influence on the quality of living within the last decades. Latest discoveries of vaccines, drugs, antibiotics and further medical products as well as the development in the field of surgery doubled the average human lifespan. At the very beginning of the 20th century the average age at death for female was 45.84 years and for males 43.65 years, whereas in 2008 the average ages were increases up to 79.79 for females and 73.58 for males. Accordingly, a permanent increase of age-related diseases was could be observed Those especially include cancer, diabetes and other similar medical conditions. [1]

Some of those diseases can lead to a cholecystectomy, the resection of the gall bladder. This can have many reasons, for example the development of a tumor, gallstones of the inflammation of a gall bladder, which are typical ailments caused by age or unhealthy lifestyle. Nowadays these are minimal invasive surgeries performed several thousand times per year within Germany and count as routine surgeries. Nevertheless, the survival rate is lower than expected, caused by different intricacies and the individual physical conditions within each patient, which have a very wide spectrum of possibilities. [2, 3]

Such training can be performed using medical models and dummies. Those models need to be processed to imitate the optical and haptic properties of the original patient organ. Hence, the most vital features are the solidity and colouring of several tissues contained in the human body. Those properties can be achieved by adding additives or modifying the biomaterials. Similar organ-like models are already manufactured via casting from collagen. However, the production of those models

generates high costs while being time-consuming and not customizable to the patients specifics. In order to enhance the availability of such models, 3d bioprinting can be used, where biomaterials can be applied to fabricate such organoids. With the help of this technology, hollow bodies could be realized, whereas casting is only able to generate fully filled structures, which does not meet the actual conditions of organs within the human body. [4]

## Requirements

The goal of the presented work is the development of a 3d printing system utilizing multiple compositions of biomaterials. Using additive methods stacking several layers of material upon each other, 3d printing is a method commonly applied in order to fabricate a product from computer-generated data. An expanding area of medical engineering is 3d bioprinting, whereas biomaterials are used for the fabrication of scaffolds for 3d cell culture. Gelatin is a reliable material mainly consisting of collagen. Collagen is the major structural protein within the cellular matrix of vertebrates. [5]

Furthermore, the processability, as well as properties can be influenced by different compositions of gelatin with stabilizers, crosslinking agents and pigments. The final organoid should simulate the actual organ as effectively as possible. For example, while being penetrated by electro scalpels, the material should sear and evaporate an odour of burned flesh instead of melting. This implies the possibility of processing several different compositions with multiple properties using the designed system. In order to achieve such functionality, multiple supplies of different materials are required. The system should also be able to continuously extrude in order to generate a highly detailed product with several properties.

The processing of the gelatin correlates to the desired physical characteristics, so the printing system needs to be adaptable to the different process parameters, like viscosity, of multiple material compositions. The extruder is based on a peristaltic pump, which can extrude continuous strands of the material at the gel point. Occurring pulsation should be reduced using a dampening system. In order to ensure a high stability of the whole printing system, the total weight of the extruder should not exceed 2 kg.

## Results

At first, multiple measurements to examine the printing parameters were performed utilizing a rotational viscometer of the type RheoPLUS. Exemplary, some of these results are shown in Figure 1, Figure 2 and Figure 3. In order to determine the gel point the dynamic and loss modules  $G'$  and  $G''$  need to be investigated. The dynamic module describes the elastic parts of the material, whereas the loss module depicts the viscous parts. When the dynamic module rises above the loss module, the elastic parts predominate the viscous, the low viscous material hardens and the solution solidifies. Hence at the crossing section of both modules, the gel point is located. Those crossing sections were determined for multiple gelatin and agar solutions, where concentrations of agar within a range of 0,5% to 3% and of gelatin with 10% and 20% were applied.

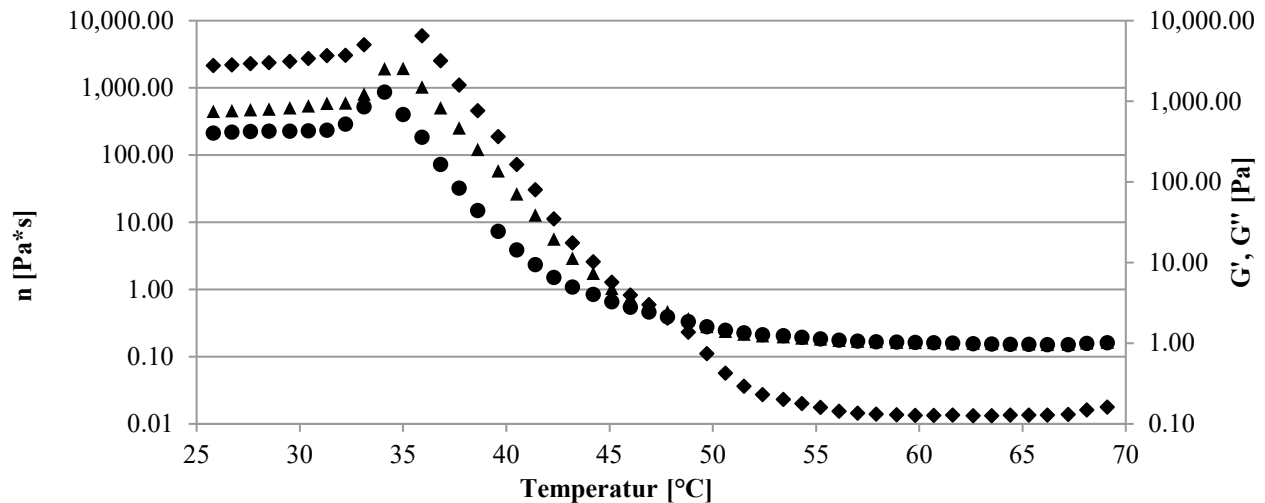


Figure 1: Example of a rheometric measurements of a 3% Agar solution. All measurements were performed within a range of 25°C to 50°C with an amplitude of 10%, a circular frequency of  $\Omega = 0,16$  rad/s and a cooling rate of 1°C/min. Viscosity -  $\blacktriangle$ ,  $G'$  -  $\blacklozenge$ ,  $G''$  -  $\bullet$

Figure 1 shows an example of such a measurement of a 3% agar solution. In order to process the material at the gel point, the extruder must be capable of handling the viscosity within the area of the crossing section. All gel points were compared to their respecting concentrations and a linear correlation could be examined.

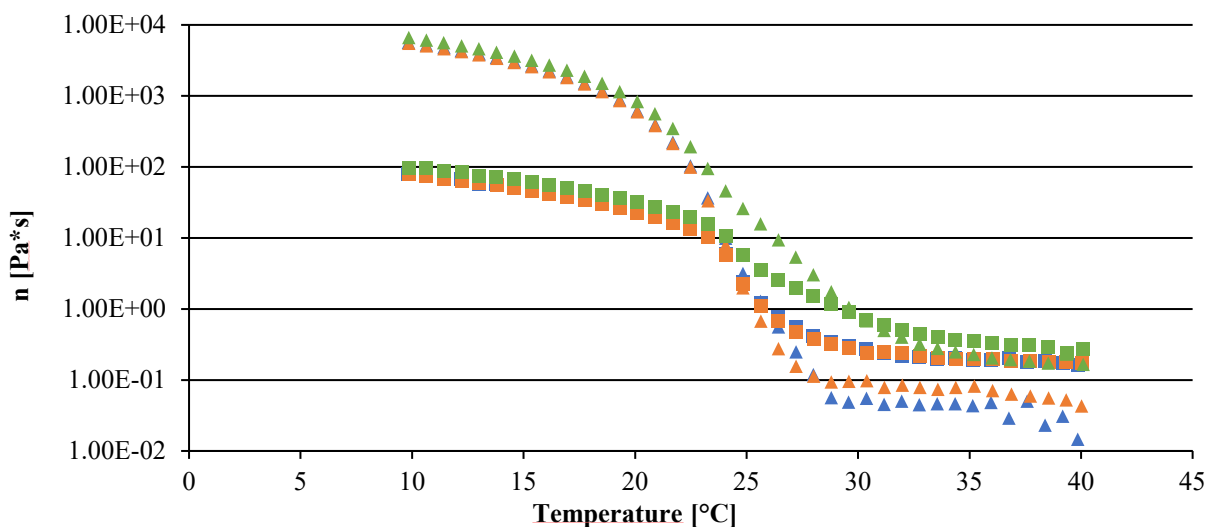


Figure 2: Measurements of dynamic  $G'$  and loss modulus  $G''$  of a 10% gelatin solution. These measurements were performed with a range from 25°C to 60°C, cooling rate of 1°C/min, an amplitude of 0,1% and a circular frequency of  $\Omega = 5$  rad/s.  $G'$  -  $\blacktriangle$ ,  $G''$  -  $\blacksquare$ , Blue - 1 min, Orange - 30 min, Green - 120 min after dissolving

Figure 2 displays multiple measurements of a 10% gelatin solution, which were measured after different times of extraction from the bulk. Recognizable is the shift of the gel point depending on the time of extraction. This could be caused by the vaporization of the solvent, in this case water, whereby the real concentration distinguishes from the desired concentration and increases within the bulk solution. This is a problem to which the extruder system needs to be adapted.

In order to further analyze the stability of the gelatin, multiple measurements were performed whereas a 20% gelatin solution was tempered within ten cycles and different ranges of temperature. These results are shown in Figure 3. Remarkable is the constant increase of viscosity after each cycle. This correlates to the loss of water from the sample through evaporation. Because of the main

component of collagen, which is a biopolymer, the gelatin could also denature after being heated above 42°C. This could also lead to further deviation of the data.

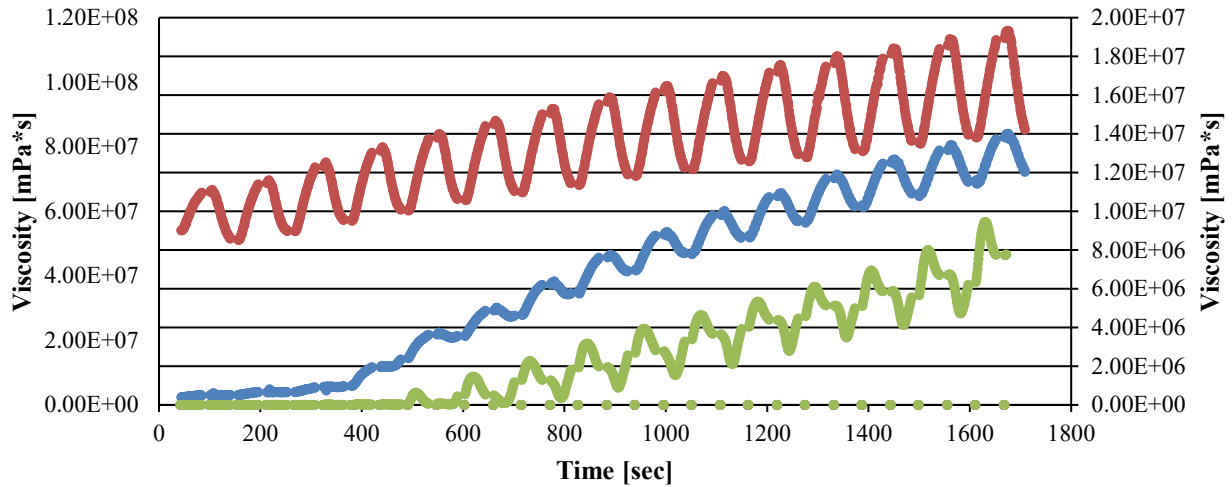


Figure 3: Measurement of stability of a 20 % gelatin solution with different max temperatures. The compositions were heated and cooled within ten cycles ranging from 25°C to 40°C (Blue), 25°C to 50°C (Red) and 25°C to 60°C (Green) with a heating/cooling rate of 10°C/min, an amplitude of 0,1% and a circular frequency of  $\Omega = 6,3$  rad/s

Furthermore, in order to simulate the different tissues, several concentrations of gelatin were validated in regard of their shore hardness. Therefore, a tool for measuring shore hardness 00 was applied. The results are shown in Figure 4. This data can be compared to different tissues and be used to calibrate the printing system to recreate those tissues. Similar measurements were carried out with agar.

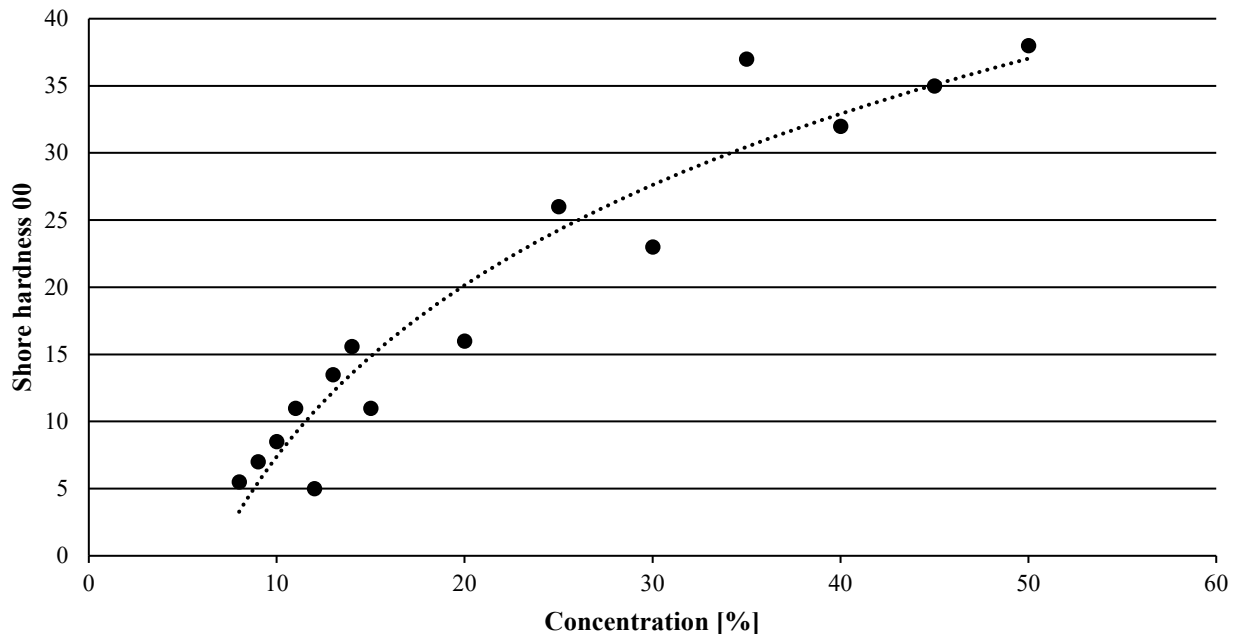


Figure 4: Correlation of shore hardness 00 to concentration of pure gelatin

Those have shown, that the lack of elasticity given by the characteristics of being a glycan prevents the method from generating steady data. As a reference material, ballistic gel was used. This material is an anorganic gel based on silica. Those gels are mostly used for ballistics experiments to examine firearms behavior and the ammunition's effect on synthetic organic tissue. Stability measurements for the two ballistic gels are shown in Figure 5. Noticeable is the superior stability compared to the

organic gelatin as seen in reference to Figure 3. This could enable these gels as anorganic materials to be support materials for the organic gel and further enhance the stability of the final product.

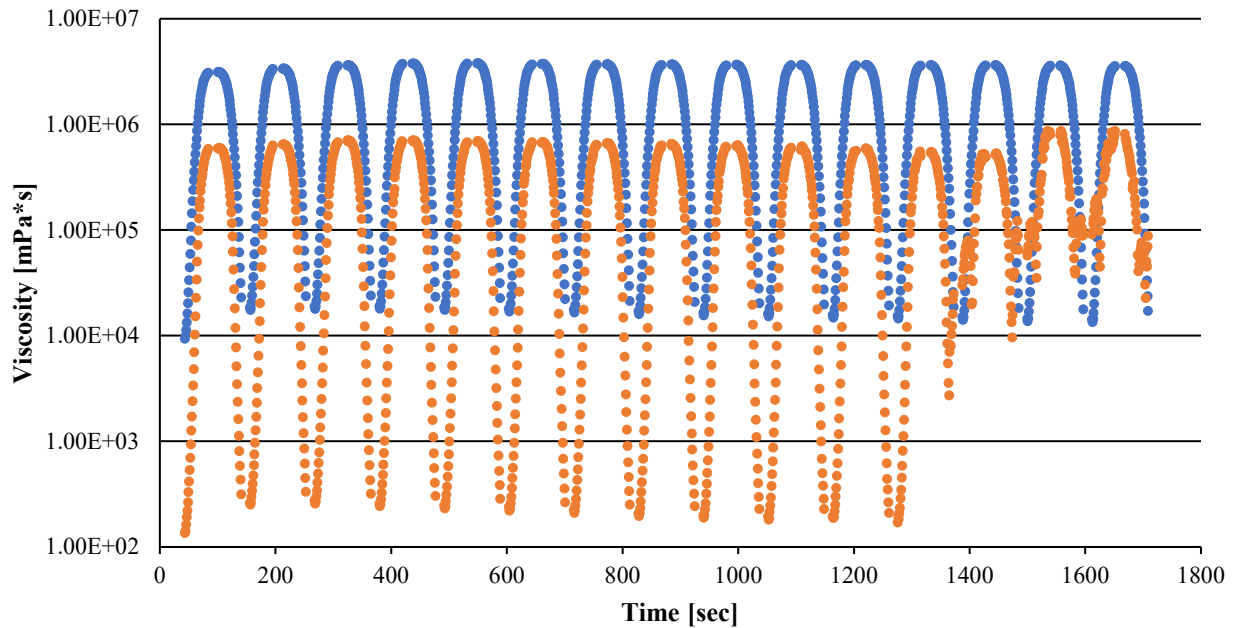


Figure 5: Rheometric measurement of stability of two different ballistic gels. Both materials were heated and cooled within ten cycles ranging from 25°C to 100°C with a heating/cooling rate of 10°C/min, and amplitude of 0,1% and a circular frequency  $\Omega = 6,3$  rad/s. Blue – tough, Orange - soft

Based on the generated data, a 3d printing system was designed. This system was based on a mechanical extrusion system (e.g. a peristaltic pump), an extrusion nozzle was developed by the working group Systems Integration of the Technical University of Applied Sciences Wildau.

## Summary

The aim of the presented ongoing work is to develop a continuous extrusion system for the 3d printing of different biomaterials. This extruder should be adapted to the material parameters needed to extrude the materials without leaking or clogging. This was realized by developing a temperature regulation system utilizing a PID controller, different temperature zones and a peristaltic pump. The final objective is to print organ like test structures based on patient data. Those structures should emulate the haptic and optical behavior of real tissues and organs. Eventually, multiple measurements were performed to determine the printing parameters of two different biomaterials, gelatin and agar. Those measurements have shown, that the stability of gelatin within an open storage system is too low for longer printing processes, while agar lacked the elasticity needed to simulate organic tissue. Currently, the extruder concept is improved and optimized while being integrated into an already existing 3d printer. Furthermore, additional measurements are performed to further determine and optimize the printing parameters of the gelatin. Therefore, several additives will be investigated in respect of their effects to the printability, stability and enhancing characteristics on the biomaterials.

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