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Effect of temperature, viscosity and surface tension on gelatine structures produced by modified 3D printer

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Abstract. In the present study, gelatine scaffolds were manufactured by using modified 3D (3 Dimensional) printing machine and the effect of different parameters on scaffold structure were investigated. Such as; temperature, viscosity and surface tension of the gelatine solutions. The varying of gelatine solutions (1, 3, 5, 10, 15 and 20 wt.%) were prepared and characterized. It has been detected that, viscosity of those solutions were highly influenced by temperature and gelatine concentration. Specific CAD (Computer Assistant Design) model which has 67% porosity and original design were created via computer software. However, at high temperatures gelatine solutions caused like liquid but at the lower temperatures were observed the opposite behaviour. In addition to that, viscosity of 1,3,5 wt.% solutions were not enough to build a structure and 20 wt.% gelatine solution too hard to handle, because of the sudden viscosity changes with temperature. Even though, scaffold of the 20 wt.% gelatine solution printed hardly but it was observed the best printed solutions, which were 10 and 15 wt.% gelatine solutions. As a result, 3D printing of gelatine were found the values of the best temperature, viscosity, surface tension and gelatine concentration such as 25-35 ℃, 36-163 cP, 46-59 mN/m and 15 wt.% gelatine concentration respectively.

1. Introduction

It was observed that the life quality of the humans has increased with the researches about tissue engineering and regenerative medicine, which aims producing biocompatible structures mimicking biological organs, tissues or systems. Scaffolds, which were manufactured for this purpose, provides cell like matrix and they need to meet some mechanical and design features, which contributes functionality of the structures [1]. Consequently, various materials and methods have been used to obtain the printing scaffolds. Lately, 3D printing technology came forward cause of ability of producing scaffolds designed similar to the structures found in the human body. This technology allows controlling the aimed scaffold by layer-by-layer. Also porosity, pore size and pore morphology are adjustable by designing CAD (Computer Assistant Design) models with some computer software [2]. With the ability of to control of design of scaffolds and fabrication of complex shapes, this technology became crucial in tissue engineering. Some of the 3D printing applications in tissue engineering are renovation of anatomic defects, rebuilding of organs with 3D microarchitecture and scaffolds for stem cell differentiation [3].

Gelatine is a natural material and this water soluble protein obtained from collagen by denaturation of it. Due to its biocompatibility and biodegradability, gelatine has come to an important place for the regenerative medicine applications [1]. Also it contains biological mark, which promotes cell adhesion and proliferation [2]. As a result of that, for the researches working in tissue engineering, this material, which has biological origin, derived from collagen, has become an appealing choice [4]. But aqueous solutions of the gelatine form a hydrogel structure, which behaves like solid, when it gets below their critical solution temperature of 25-35 ℃ [5]. On the contrary, too much heat application can cause them to behave like liquid. For these reasons, temperature of the solution should be kept in a stable state. This thermo depended behaviour can be used to build layers on one while retaining the shape of printed structures [6].

In this study, we focused to investigate the behaviour of the gelatine solution and printability of it by changing its viscosity, surface tension and density. These changes influenced by temperature differences and gelatine concentration of the solutions. Viscosity and surface tension of the solutions were measured at temperature range between 25-55 ℃. Following by that gelatine scaffolds with specific design printed with modified 3D printer. As a result, very suitable printing conditions for the scaffolds were obtained.

2. Materials and methods

Aqueous solutions of the gelatine were prepared by adding 1, 3, 5, 10, 15 and 20 wt.% gelatine to distilled water. Solutions were stirred with magnetic stirrer with heat application until gelatine fully dissolved. After dissolving, viscosity and surface tension were performed between the temperature range of 25-55 ℃. Viscosity measurement of the solutions were fulfilled via viscometer (Brookfield, DV-E, USA). Desired temperature achieved by circulating water inside the machine. Density of the solutions were obtained by a standard density bottle DIN ISO 3507-Gay-Lussac (Boru Cam, 10 ml., Turkey). For surface tension measurements digital densitometer (Sigma, 703D, Finland) was used and the Du Nouy ring method was conducted. Surface tension of the water used for solutions preparation was 71mN/m.

Prepared solutions loaded into 10 ml syringe and polyethylene tube was used to for streaming of solution. To obtain the desired temperature, tube was wrapped with resistance and voltage applied to resistance to get heat. Before mounting syringe to syringe pump, one side of the tube connected to syringe, while other side of the tube connected to print head. At the tip of the printer head, tube connected with stainless steel needle via luer lock adaptor. When connection completed, syringe mounted into syringe pump (Inovenso, IPS-12, Turkey) and solution was purged for printing. After printing, surfaces of the samples were investigated by using optical microscope (Olympus, BX51M, Japan).

Ultimaker 2+ (Ultimaker, Netherlands) was used for this study, but for the purpose of the present study, original print head of the printer was dismantled from the machine and a new print head was designed and printed with another Ultimaker 2+ (Figure 1). Print speed parameter changed over the device, while flow rate and temperature parameters changed from the outside. For this study, 8-10 ml/h flow rate used and print speed changed between 20-50%. Design of the sample was obtained via Solidworks CAD software. Values of dimensions of layers of the design selected as 30 x 20 x 0.7 mm (Figure 2). Samples designed to have 67% porosity. The reason of the using samples, which have high porosity rate, is that high porosity increase the potential of the application of the scaffolds clinically [1].

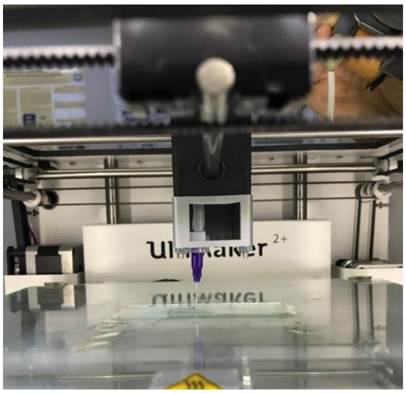

Figure 1. Modified print head, luer lock and stainless steel needle.

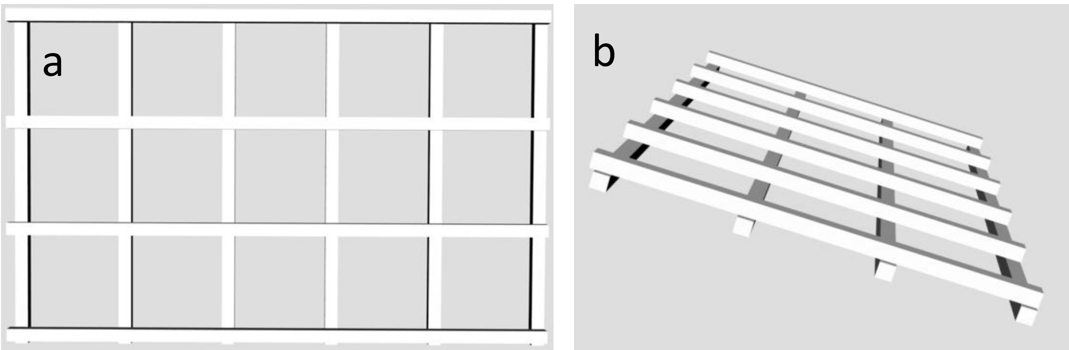

Figure 2. CAD design of the samples. a) top view of the design b) side view of the design.

3. Results and discussion

To investigate the effect of solution density, all of the solutions were putted in density bottle. As a result, it was observed that density change with a slight difference (Table 1). Increasing gelatine concentration influenced the viscosity and the surface tension more than the density.

Table 1. Density of the solutions.

Gelatine (wt.%)	Density (g/cm^3)
1	1.028 ± 0.021
3	1.031 ± 0.025
5	1.039 ± 0.023
10	1.045 ± 0.031
15	1.051 ± 0.034
20	1.0518 ± 0.035

As mentioned earlier, it has been observed that the temperature change and gelatine concentration greatly influence the viscosity. In this study, the viscosity of the solutions are obtained in temparature range of 20-55 °C, for the solutions viscosity has been found as follows, for 1 wt.% gelatine 0.88-1.85,

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for 3 wt.% 2.20-20.00 cP, for 5 wt.% 3.75-27 cP, for 10 wt.% 9.78-120 cP, for 15 wt.% 25-430 cP and for 20 wt.% 56-480 cP (Figure 3). This behaviour is harmanious with Arrhenius Law, which suggests that logarithmic decrease of viscosity with increasing temperature. Schuurman *et al.* suggest that, increasing the viscosity of the solution will affect the connection of pores and ability to print wider pores. As a result it will be much easier to print scaffolds with larger pores [1]. In parallel with that, scaffolds produced with gelatine solutions have higher viscosity revealed more stifness than the others. But 20 wt.% gelatine solution at room temperature blocked the needle. So while 10 wt.% and 15 wt.% gelatine could printed between 25-35°C, 20 wt.% gelatine need more temperature.

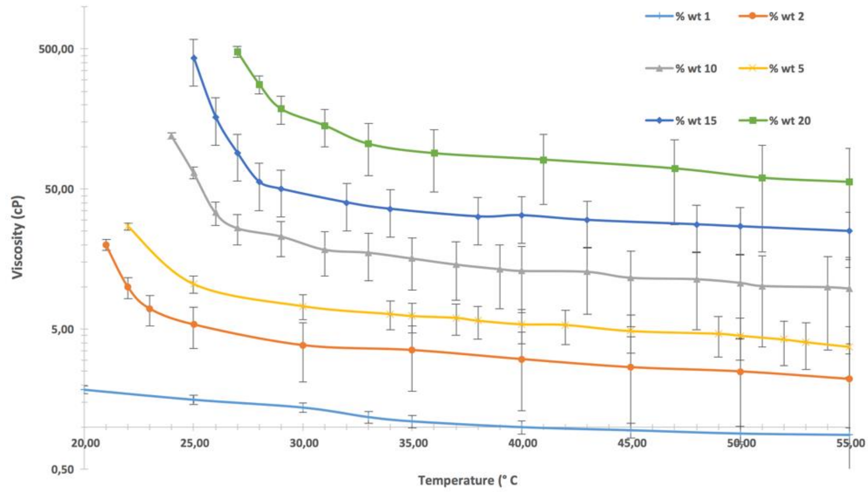

Figure 3. Viscosity of the solutions with logarithmic y-axis.

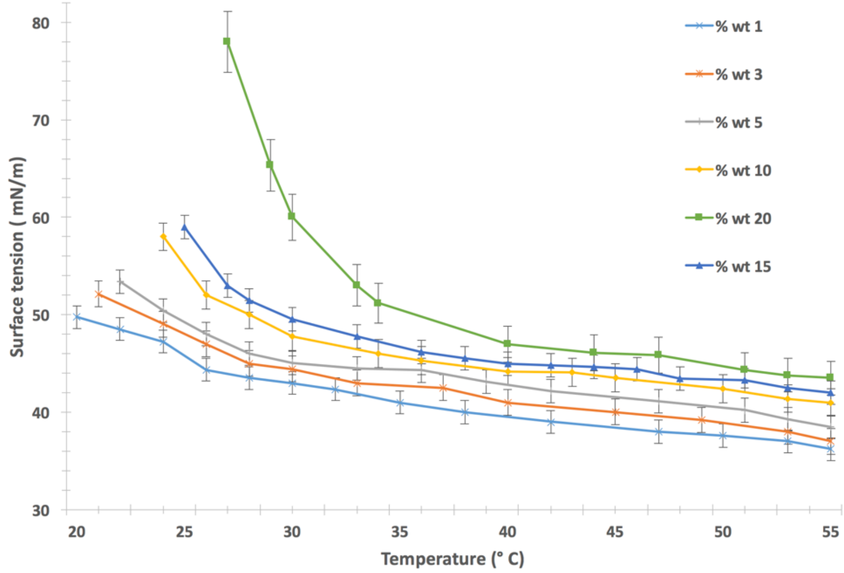

Figure 4. Surface tension of the solutions.

Similar to viscosity, surface tension of the solutions also has been affected with temperature and gelatine concentration. No study was found in the literature related to surface tension and 3D printing of gelatine structures. It is clearly seen that the surface tension is directly proportional according to the viscosity and the surface tension is inversely proportional according to the temperature. Surface tension of the solutions have been found as follows; for 1 wt.% 36.2-49.77 mN/m, for 3 wt.% 37.0-52.13 mN/m, for 5 wt.% 38.5-53.4 mN/m, for 10 wt.% 41.0-58.0 mN/m, for 15 wt.% 42.0-59 mN/m and for 20 wt.% 43.5-78 mN/m (Figure 4). It has been shown that, higher surface tension cause difficulty to building second layer on first layer. Therefore, desired stifness and the rigid structure of the samples can not be printed.

As a result, optimal gelatine concetration for printing was found as 10 and 15 wt.% with the working temperature at 25-35°C . The 20 wt.% gelatin solution was found with printable as 35-45°C. Investigation of the printed samples were obtained via the optical microscope (Figure 5). Images show that, the 15 and 20 wt.% gelatine have much more smooth and uniform surface than 10 wt.% of gelatine sample.

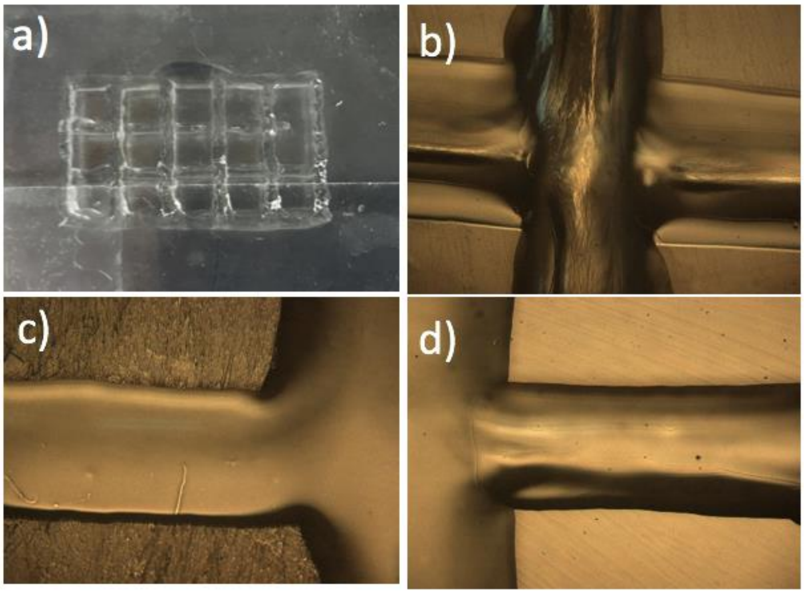

Figure 5. Camera image of gelatine structure and optical microscope images of the gelatine scaffolds taken at 5x magnification. a) gelatine scaffold b) 10 wt.% c) 15 wt.% d) 20 wt.%.

4. Conclusion

The gelatine solutions were printed succesfully via modified 3D printing device. It has been found that stifness of the scaffolds were highly dependent on viscosity, surface tension, temperature and concentration of the solutions. It can be seen that, 10 wt.% of gelatine sample was printed easily according to the lower viscosity. But 15 and 20 wt.% gelatine samples were accomplished much more smooth and uniform surface compared with the low concentrations.

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