EMS690U – Precision Nutrient Delivery for Tissue Engineering Bioreactors

Author: Jatinder Dhaliwal (ID: 220451019)

April 2025

Supervised by Professor Karin Hing

Acknowledgements

I would like to express my gratitude towards Professor Karin Hing, my supervisor, for her invaluable support throughout this project, providing constructive feedback where needed, and giving highly beneficial insights towards the theory of this project.

I am also grateful to Dr. Sujitha Kunalan and the PhD demonstrators, Aalia Rehman, and Yunpeng Fang, for instructing me on how to use the equipment in the lab and providing critical improvements to my designs, which were crucial to the achievements of this project.

Finally, I would like to thank my peers, Percy Tussamba, Xiang Li, Aisha Siddiqah, and Laith Ali, who worked alongside me on this project. Although we had different roles, each person exhibited excellent teamwork skills, allowing me to share thoughts and ideas in a comfortable environment.

Abstract

This project aimed to optimise a perfusion bioreactor system with the implementation of holders for the reservoirs and chambers. The role of these holders is to organise the system by allowing each component to be placed separately, accommodating the user operating the bioreactor, and improving repeatability of data by minimising errors caused due to disorder. It was found that Nylon-12 was a suitable material for this application, as it could withstand the post-processing method used in this project, autoclaving, as well as exhibiting biocompatibility. 3D printing using filament was the additive manufacturing technique used to form the models for each holder. A Nylon filament was tested but found to be incompatible with the 3D printers for various reasons, primarily due to temperature complications. Therefore, PLA filament was used instead; although PLA could not be autoclaved, the focus of the project was shifted to assess the design of each model. Both models suited the components in terms of geometry; however, the designs could not be tested in practice as they could not be sterilised. Consequently, it was decided that future work could be performed, exploring the use of alternative materials and implementing additional components to facilitate monitoring of the system.

Keywords: Tissue Engineering, Bioreactor, Holder, CAD, 3D Printing.

Table of Contents

List of Symbols and Abbreviations

Symbols/Abbreviations	Description
mm	Millimetre – Unit of measurement for length.
ºC	Degrees Celsius – Unit of measurement for temperature.
º	Degrees – Unit of measurement for angles.
kg	Kilogram – Unit of measurement for mass.
$USD	US Dollars – Currency
£	GB Pounds – Currency
2D	2-dimensional
3D	3-dimensional
PLA	Polylactic Acid
IR	Infrared
CT	Computed Topography
hiPSCs	Human-induced pluripotent stem cells
SLA	Stereolithography
SLS	Selective Laser Sintering
FDM	Fused Deposition Modelling
PEEK	Polyether Ether Ketone
ABS	Acrylonitrile Butadiene Styrene
PETG	Polyethylene Terephthalate Glycol
PET	Polyethylene Terephthalate

1.0 – Introduction

1.1 – Overview of Tissue Engineering

An active aim of tissue engineering is to synthesise functional tissue *in vitro*. This is achieved by cultivating cells that have been or are capable of differentiating into a desired cell type, under controlled conditions. Cells require specific environments to grow and proliferate, typically mimicking the physiological conditions of the human body [1].

Various factors, including nutrient supply, temperature and oxygen, must be optimal for cell growth [2]. Replicating these conditions is considerably challenging due to the dynamic nature of the body, which induces frequent change. The environment must leave very little margin for deviation due to the sensitivity of cells to their surroundings, reinforcing the arduous nature of growing tissue *in vitro*.

However, achieving this medical strategy would make bone grafting applications such as autografting, allografting, and xenografting redundant, which usually carry numerous risks, such as rejection, cross-species contamination, and limited tissue availability. Also, autografting results in two surgical operative sites, which may result in donor site morbidity, increasing the risk of disease [3]. Allografting may cause disease transmission between patients, further highlighting the risks of undergoing these treatments [4]. The various disadvantages associated with these methods incentivise the research of synthesising functional tissue *in vitro*.

1.2 – Bioreactors in Tissue Engineering

The role of bioreactors in tissue engineering is to closely simulate the *in vivo* physiological conditions to allow cells to grow and proliferate. By taking a sample of cells and placing them inside a bioreactor, the aim is for these cells to eventually grow into implantable bone tissue. Employing an efficient nutrient delivery system and maintaining a suitable temperature, oxygen level and pH, are essential for cells to survive outside of their natural environment. Consequently, these factors must be heavily regulated to ensure the correct cell growth process. Alongside this, sufficiently sterile conditions must be achieved, commonly through incubation, to avoid foreign agents contaminating the system [5].

Bioreactors have many advantages over traditional 2D cell culturing, which involves growing cells in a petri dish. A 2D cell culture does not mimic the natural structures of bone tissue as bioreactors do with 3D scaffolds, which can affect the morphology of the cells due to adapting to a different environment [6]. This change would ultimately result in altered functionality, limiting their use in implants for patients, whereas bioreactors could culture cells with the potential for clinical use. Another advantage of bioreactors, specifically perfusion systems, is the effectiveness of media and nutrient delivery, allowing for an even supply, leading to a higher survivability rate [7]. It is difficult to distribute nutrients without mechanically affecting the cells in 2D cell cultures, meaning that some cells may not survive. This, in turn, would increase waste material per run, reducing the cost-effectiveness of 2D cell culturing methods. A primary feature of bone cells is that they grow via mechanotransduction [8]. In 2D cell

culturing, it is difficult to provide mechanical stimuli similar to the physiological environment. Bioreactors can more effectively control environmental mechanics, triggering the correct differentiation pathway.

1.3 – Project Overview and Objectives

The objective of this project is to increase the precision and reproducibility of nutrient delivery in a perfusion bioreactor. To achieve this, fundamental roles for enhancing the function and design of a bioreactor are required. The first role involves finding the most accurate combination of tube diameters and volume of media delivered to achieve a given flow rate. This ensures the repeatability of results, allowing similar amounts of media to be delivered in each run. Another focus is to improve the reservoir and chamber holder design to accommodate ease of use, mitigating any potential faults when using the system. The third role includes assessing the performance of a syringe pump to introduce supplementary nutrients to the circulating media. This is done by testing the optimal positioning of the syringe in the system and taking samples of the mixed media to analyse how well the nutrients mix with the media. Another role is to create a valve for in-line sampling of the system. This should consider biosecurity, sample representation and positioning of the valve to avoid contamination and facilitate the monitoring of lipid mediators. The final role is to generate a 3D computational model of the chamber's porous granule constructs to simulate and analyse fluid flow behaviour.

When considering an optimal design for bioreactor reservoir and chamber holders, a critical factor is the biocompatibility of the materials used in the process. As the bioreactor will be placed inside an incubator along with cells, the materials must not contaminate the system, which would result in cell death or abnormal growth. Another aspect of improving holder design is to create a structure which will limit disruption of the system. A relatively large-scale design, such as a reservoir holder, may be prone to interfering with the system by pinching tubes, which could alter flow rates, thus decreasing the accuracy of results and affecting cell growth. Another point to address is to consider data collection from the system; therefore, the holders could be designed with distinct markers differentiating each part of the system, reducing the probability of error and, in turn, obtaining repeatable data.

The rest of this report is organised as follows. Section 2 provides a literature review. Section 3 displays the design process and results. Section 4 explains the results and the justifications of each decision made throughout the project. The final section presents the conclusions gathered from the project based on current literature and the results obtained.

2.0 – Literature Review

2.1 – Initial Requirements

The basis of this literature review was to research materials that were used in bioreactor design applications, which could be integrated into this project. A few prerequisites for the materials include:

biocompatibility, availability in filament form with a 1.75 mm diameter, and melting at a temperature of 280ºC or below. Furthermore, the material must be able to withstand autoclaving, which is a common post-processing method to eliminate contamination sources by using saturated steam under high pressure [9]. Some articles provided information on alternative sterilisation techniques and the addition of components to improve the bioreactor's functionality.

2.2 – Review of Literature Articles

The first article proposed the use of Teflon to machine a perfusion bioreactor. The reservoir vessel used a gas-permeable membrane, indicating that the rate of oxygen diffusion was relatively high, which was advantageous for the cell culture. Cells were uniformly distributed across the scaffold, meaning that this perfusion bioreactor could be proficient in accommodating thicker scaffolds. When compared to a static culture, it was seen that there was a significant decline in the peripheral cell density for the static culture, reinforcing the effectiveness of the perfusion bioreactor. However, a key factor mentioned was that the equipment was sterilised using plasma-phase hydrogen peroxide processing. Since the only sterilising method available in this project is autoclaving, Teflon was unlikely to be the material of choice [10].

The following article highlighted the importance and potential of 3D printing in the manufacture of bioreactors. A relevant point that was emphasised is the lack of 3D printable materials suitable for sterilising via autoclaving. An example used was temperature-stable PLA, which could be used to manufacture bioreactor vessels without difficulty. However, only coarse structures made from PLA are autoclavable, limiting its use in bioreactor design, as tissue engineering applications require much finer structures, which could deform when exposed to autoclaving. Therefore, autoclavable materials that are also 3D printable could integrate into standard workflows when considering bioreactor design [11].

One literature paper underlined the drawbacks of current invasive methods of measuring organ growth in a bioreactor, which could introduce infection to a long-term organ culture. Non-invasive methods such as near-infrared (IR) imaging and micro-computed topography (CT) have been researched. IR allows for imaging in real-time, preventing the removal of the organ from the bioreactor, and in turn, decreasing the risk of contamination. CT has a similar advantage; however, since it is based on X-ray penetration, it has a limited resolution of soft tissue. Despite this, a contrast agent made from iodine salt could be used to enhance the resolution, resolving this setback. Though these techniques could potentially eliminate the need for sampling or removal of cells or organs, their effectiveness could be reduced in this project, as the bioreactor is kept in a sterile incubator, making it more difficult to utilise external equipment. Nevertheless, the incorporation of environmental sensors and non-invasive monitoring in bioreactors could significantly develop conditions to closely mimic *in vivo* cell growth [12].

Another study tested the effects of various thermoplastics on a culture of human induced pluripotent stem cells (hiPSCs). None of the thermoplastics adversely affected the viability or potency of the stem cells, suggesting that the materials exhibited biocompatibility. However, Nylon-12 was the only material which did not deform during autoclaving. Additionally, it is a 3D printable material, meaning that Nylon-12 bioreactor vessels can be fabricated using a range of additive manufacturing techniques, including resin-based stereolithography (SLA), selective laser sintering (SLS) and fused deposition modelling (FDM) [13].

While not from a literature paper, a general price comparison for Nylon and PLA filament was found, which stated that Nylon is typically $USD 65-75 per kilogram, while PLA filament is approximately $USD 20-30 per kilogram [14]. Therefore, it was decided that most of the test runs focusing specifically on the design would be printed using PLA filament to reduce material costs.

After evaluating the information provided from the literature review, it was evident that Nylon-12 was the most suitable material for the final design, meeting all of the aforementioned requirements. Table 1 below shows a comparison of material properties relevant to this application, to reinforce the material selection process. Various materials similar to PLA and Nylon were considered for this project.

Table 1 – Material selection based on relevant material properties.

Material	**3D Printable**	**Biocompatible**	**Autoclavable**
PLA	Yes	Yes	No
Nylon-12	Yes	Yes	Yes
PEEK	Yes	Yes	Yes
ABS	Yes	Yes	No
PETG	Yes	Yes	No
PET	Yes	Yes	No

Table 1 shows an abundance of materials which are both 3D printable and biocompatible. However, they are differentiated in this specific use by whether they can be autoclaved. PEEK is apparent to be a viable option for this project; however, there was not much application of this material in the literature. Due to Nylon-12 proving to be autoclavable in a similar bioreactor application found in the literature review, there was sufficient evidence to justify the use of this material for the final designs of each holder.

3.0 – Design Process and Results

3.1 – Project Planning

Once sufficient information had been gathered from the literature review, a Gantt chart was created to plan out the individual tasks and regularly track progress throughout the project, shown in Figure 1.

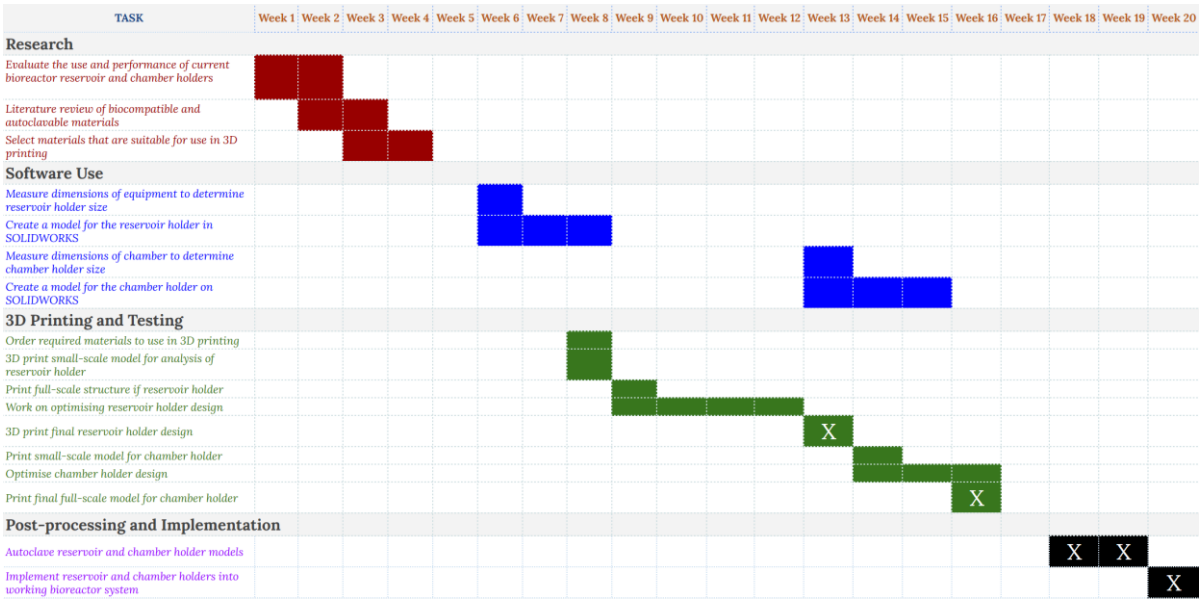

Figure 1 – Gantt chart used to track project progress. The boxes marked "X" indicate tasks that were overrun, and boxes filled in black indicate incomplete tasks.

3.2 – Reservoir Holder Design

3.2.1 – Initial Design Phase

The existing setup of the bioreactor reservoir holders was analysed. Due to size limitations, the holder could not store a reservoir, and the overall design had no organised structure, meaning that this was an area of improvement. The original setup is displayed below in Figure 2.

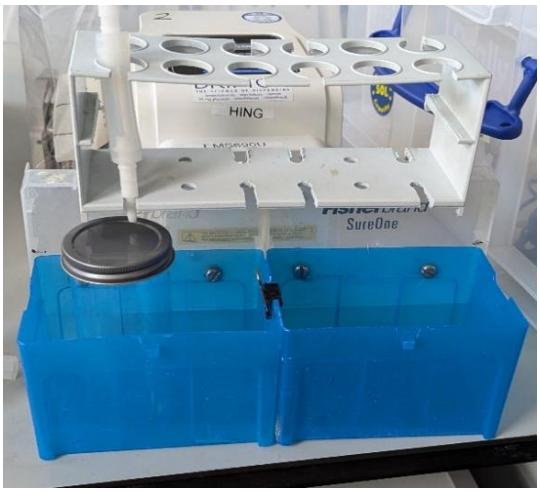

Figure 2 – Original Setup of Bioreactor Reservoir Holders

Subsequently, the first approach was to create a basic design to allow the reservoir to fit inside the holder. SOLIDWORKS was used to create a CAD model for the initial design. The structure involved a simple cuboid shape with length, width and height of 75 mm, 75 mm and 150 mm, respectively. A circular cavity with a diameter of 70 mm, slightly greater than that of the reservoir, which was approximately 65 mm in diameter, was created to allow the reservoir to fit inside. This model is shown in Figure 3.

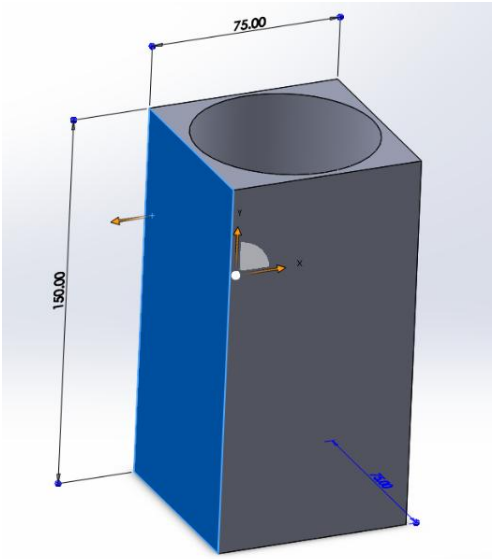

Figure 3 – Initial improved design for reservoir holder made in SOLIDWORKS, with units in mm.

3.2.2 – 3D Printing

This design was 3D printed using the Creality Ender 3D printer, which used fused deposition modelling to melt filament and print one layer at a time to complete the model. The model in SOLIDWORKS was saved as a .3mf file and opened using the Creality software. In Creality, certain parameters, such as the scale factor, support structures and orientation, could be altered. This model was printed at 30% scale to test the precision of the 3D printer, as well as provide a reference for any future prints. An infill of 10% was selected as it was unnecessary to entirely fill a structure which could hold together using less material. The orientation and position of the model were adjusted so that no support structures were required, which would otherwise include an additional step of breaking off the supports after the model had been printed. Once all the settings had been changed, the model was sliced on Creality, and a G-code was generated for the model. This G-code was saved to an SD card, which was inserted into the 3D printer. The bed and head temperatures of the printer were set to accommodate the PLA filament that was pre-loaded into the printer. Although Nylon-12 was the selected material, it was decided that prototype models would be made of PLA, due to the wider availability and lower cost, as mentioned in the literature review. Also, PLA is derived from renewable biomass, allowing safe use in most biomedical applications [15]. The melting temperature of PLA is typically between 190-220ºC, unlike Nylon, at 220-270ºC, which reduces energy consumption, hence saving costs over time [16]. The bed temperature was set to 60ºC and the head temperature to 200ºC, meeting the required temperatures for the specific PLA filament to print the model. Finally, the G-code for the initial design was selected, and the print was started. This method was followed for the future prints made throughout this project.

Figures 4a and 4b below show the Creality Ender 3D printer and the printed model of the initial reservoir holder design, respectively.

(a) (b)

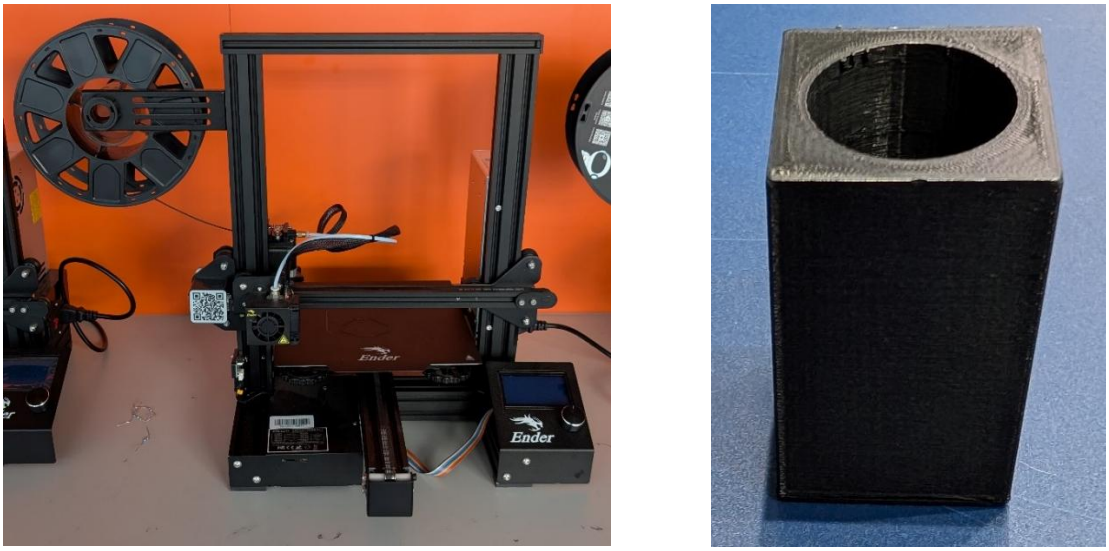

Figure 4 – Creality Ender 3D Printer (a) and initial 3D printed model at 30% of original size (b).

Once the first model had been printed, the focus was on exploring which material suppliers Nylon-12 filament could be ordered from. During this process, the second 3D printer used in this project, the Prusa i3 MK3 3D printer, was available to use. The main advantages of this printer over the Creality Ender 3D printer were the significantly reduced printing time, which provided more time for analysis and further prints, and the use of multiple printer heads, which could allow multiple materials to be implemented into the design. Additionally, the removal of support structures could be made less difficult by selecting a material that has a lower strength than the material of the model itself. Nevertheless, both 3D printers were used throughout the project to ensure maximum efficiency of printing. The printing process in both printers was practically identical; however, the Creality printer used the Creality software, while the Prusa Slicer software was utilised for the Prusa printer to slice and obtain a G-code. To test the functionality of the newly available printer, the PLA filament was loaded to print the initial model at full size. A minor advantage of the Prusa printer over the Creality printer was that it has preset materials in its settings, meaning that the printer bed and head temperatures could be automatically adjusted for a specific filament. An image of the Prusa i3 MK3 3D printer can be seen in Figure 5 below.

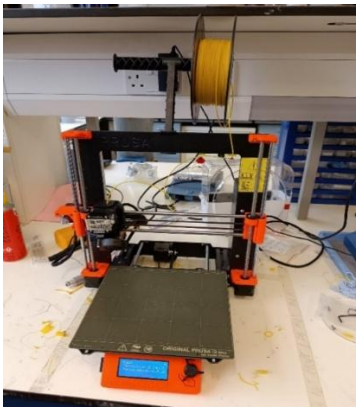

Figure 5 – Prusa i3 MK3 3D Printer.

When testing the dimensions of this model, it was found that the reservoir could not fit inside the holder. This issue was speculated to have resulted from the printer resolution, where it may have printed the circular cavity at a smaller diameter, hence not allowing the reservoir to fit. However, this was resolved in future prints by increasing the diameter of the model in SOLIDWORKS to ensure that the space was large enough for the reservoir. Due to increasing the diameter of the cylindrical cut, the dimensions of the cuboid were increased by the same scale factor to maintain consistency throughout the design.

Using the Farnell website, Nylon filament at 1.75 mm diameter, which met the size requirements for both 3D printers, was ordered. However, the website did not clarify which type of Nylon the filament was made from. Despite this, it was decided that the effectiveness of 3D printing and autoclaving models made from the Nylon filament could be investigated. A screenshot of the Nylon filament is displayed below in Figure 6.

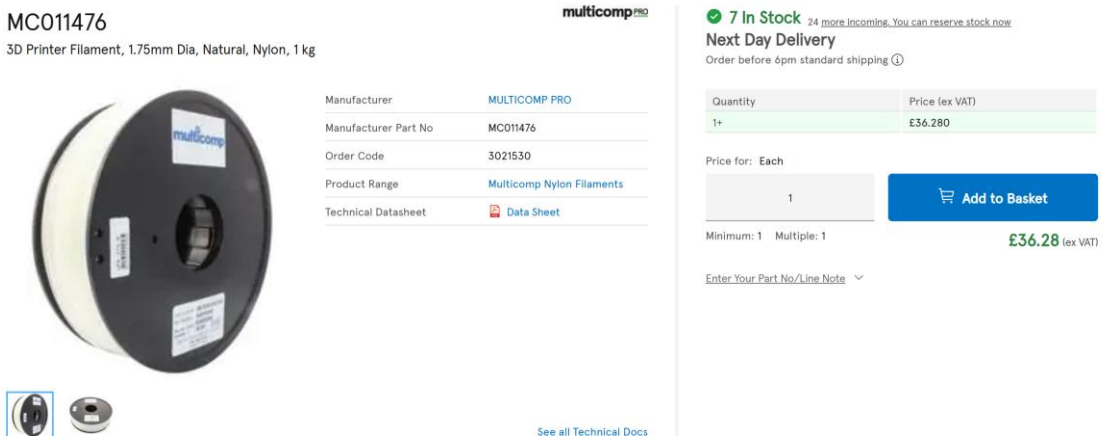

Figure 6 – Nylon filament ordered from the Farnell website.

3.2.3 – Optimisation of Reservoir Holder Design

After the order had been placed, the next stage was to optimise by considering the other requirements of the reservoir holder. Firstly, the initial model made it difficult to observe the media; therefore, a simple solution was to extrude a cut on one of the sides, through which the media could be seen.

Since four reservoir holders were required in the bioreactor system, the focus was now shifted to organising the components, which was achieved by implementing attachment mechanisms to the holders. A dovetail arrangement was decided upon, as it was a simple connection that could be designed in SOLIDWORKS without difficulty. When determining the dimensions of the dovetail, it was imperative that the tail, the wedge-shaped part, was smaller than the socket to allow a secure fit. This enabled the detachment of the parts if needed. Figures 7a and 7b below show the dimensions of both the tail and socket.

(a) (b)

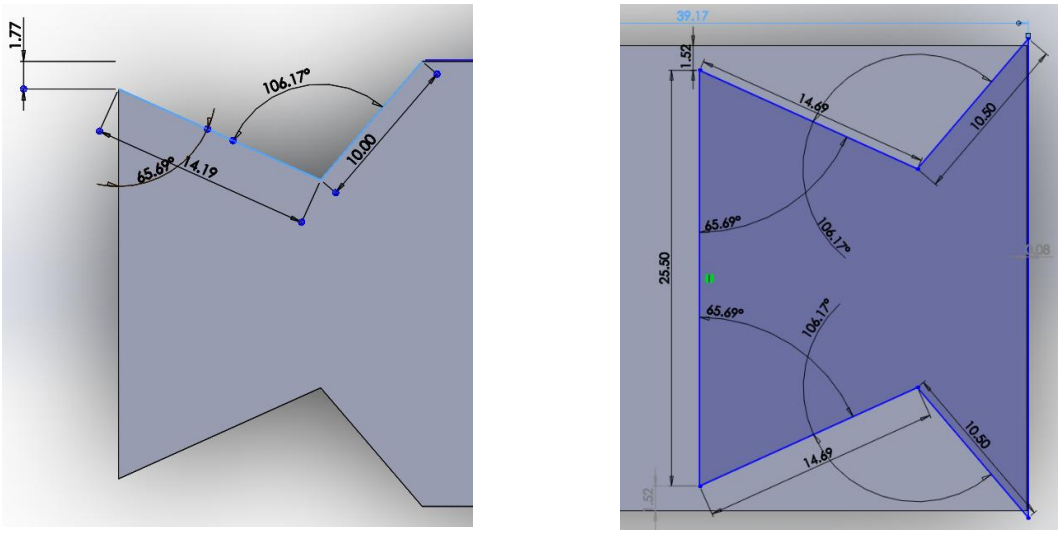

Figure 7 – Dimensions of tail (a) and socket (b) in dovetail arrangement, with length units in mm.

Subsequently, three separate designs for the holder were created – one holder with the tail on one side, another holder with the socket on one side, and two holders with a tail and socket on either side. This allowed the holders to fit side-by-side, keeping the system organised. Figure 8 displays an assembly of the parts made in SOLIDWORKS.

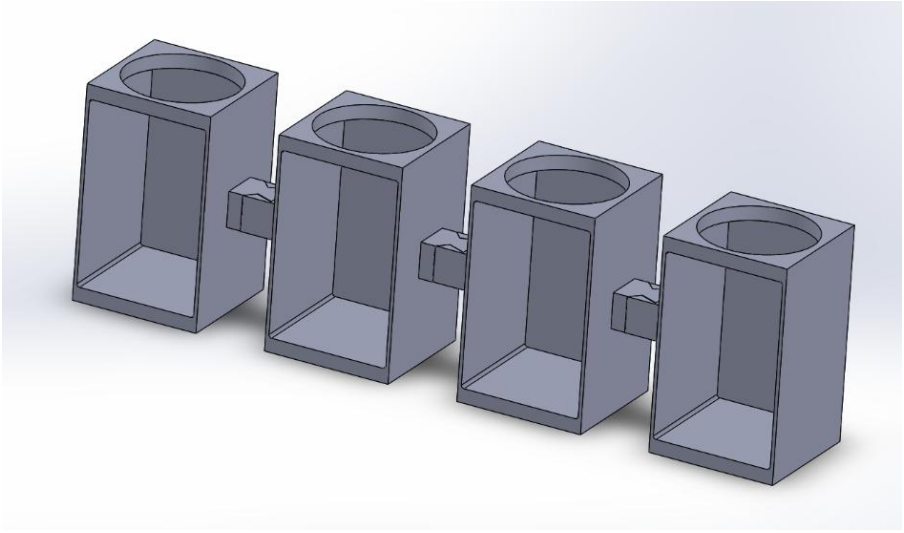

Figure 8 – Assembly of reservoir holder parts made in SOLIDWORKS.

When printing the parts for the updated design, various errors occurred with each test run. Firstly, the model would occasionally warp from the bed, resulting in a bent surface of the reservoir holder. Secondly, specifically on the Prusa printer, any large models, including the holder, could not adhere to the bed surface sufficiently for the duration of the print. To solve this, an even layer of glue was spread onto the printer bed to allow the model to stay in place. Another problem stemmed from the support structure printing too densely in some areas, such as the cut on one of the holder's walls, making it difficult to remove. Addressing this required precise adjustments of the support structure in Creality or Prusa Slicer, such as the shape and infill of the supports. In some runs, the filament ran out, so it was

decided that the model size would be scaled down to reduce material usage during testing. Adjusting the scale size proved to be quite challenging, as if the model was printed too small, the dovetail parts would not have the correct shape due to the printer resolution. This was found when a scale size of 30% was tested. Therefore, the future reservoir holder models were printed at a 50% scale to maintain structural integrity. Another issue with the printer resolution involved the dovetail connection being unable to fit, despite adjusting the dimensions in SOLIDWORKS so that the parts could join. As a result, the dimensions were changed even further for a guaranteed fit. Figures A to D in the Appendix section show images of each test run and illustrate each error.

At this stage, the Nylon filament had arrived and was tested with the updated design. Many problems occurred with this filament, some examples including the Nylon filament burning, the filament getting stuck in the printer head, thus rendering the print incomplete, and strands of filament hanging off the printed model. To counter these setbacks, the Nylon filament was no longer used and was instead replaced with the original PLA filament, shifting the attention to design instead of practical application.

A minor error appeared where the update file for the Prusa printer resulted in an incorrect calibration in the X-axis, preventing any prints. This issue was not able to be completely resolved at the time; however, another Prusa printer was available for use, so this error did not significantly impact the project.

Although these drawbacks had overrun a few tasks in the latter half of the project, as shown in Figure 1, four full-scale PLA reservoir holders were printed, with the dovetail arrangement connecting as intended, shown in Figure 9 below.

Figure 9 – Full-scale reservoir holder model.

3.3 – Chamber Holder Design

3.3.1 – CAD Model

The next and final stage of the project was to create a holder for a chamber of the granule construct. Having acquired the necessary steps on using the 3D printers, this process was made a lot simpler. Additionally, the chamber holder was considerably smaller than the reservoir holder, allowing for more prints in a given timeframe. The chamber dimensions were provided on a handout, and the model was created in SOLIDWORKS. The envisioned design was a simple box that could snap shut with a lid. An

image of the chamber holder design in SOLIDWORKS, as well as the mechanism for the lid, can be seen in Figures 10a and 10b, respectively.

(a) (b)

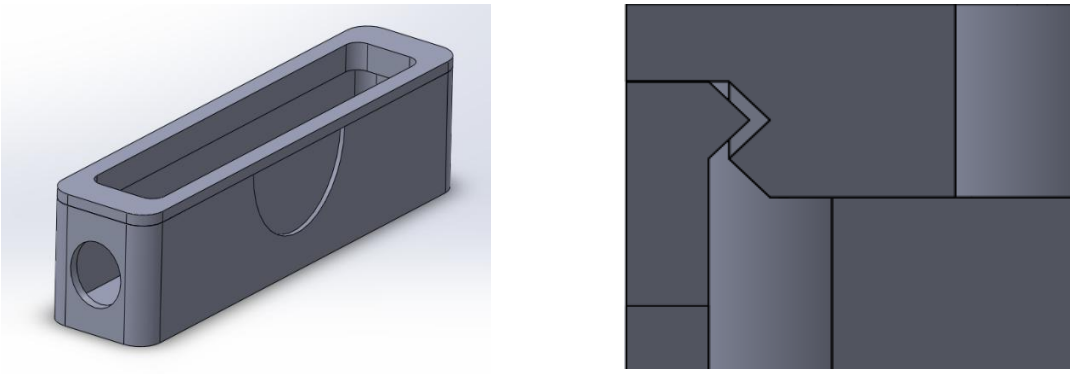

Figure 10 – Chamber holder design made in SOLIDWORKS (a), Snap-box mechanism (b).

3.3.2 – Final Design Phase

When printing the chamber holder, the support material required was minimal, meaning that it could be removed easily. However, the lid and base were printed as one structure and could not be separated. This was likely due to both parts being saved onto a single file, resulting in Creality combining the two parts. Although this is the case, the holder still functioned as intended, and the chamber fit securely inside. Similar to the reservoir holder, a trivial error occurred where filament strands were left after the print; nevertheless, these were easier to remove than the Nylon filament. The chamber holder was printed on the Creality printer and can be seen in Figure 11 below.

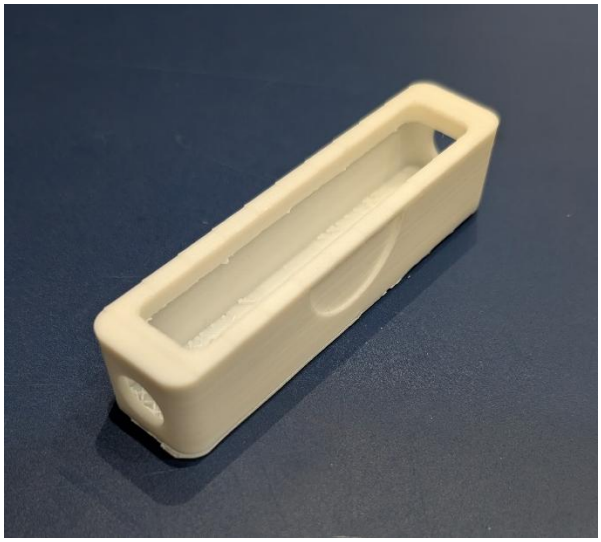

Figure 11 – 3D printed model of chamber holder.

4.0 – Discussion

4.1 – Evaluation of Project Objectives

Overall, this project was partly successful, as models for both a reservoir and chamber holder were created. The designs met most of the prerequisites, including organising the system, biocompatibility,

and catering for data collection. However, the Nylon filament could not be used due to errors within testing, resulting in the use of PLA filament. These models could not be utilised in standard practice, as PLA is unable to be sterilised via autoclaving. Therefore, the models instead served as an assessment of the suitability of the design.

4.2 – Software and 3D Printing Evaluation

SOLIDWORKS was used throughout this project as it was a relatively user-friendly software that could create complex designs with little difficulty. Both the Creality and Prusa Slicer applications were compatible only with .3mf files, which SOLIDWORKS models could be saved as, simplifying the printing process. The abundance of features and tutorials available for SOLIDWORKS deemed it the most suitable software for this project.

With regards to the 3D printers used, the first limitation was the size restrictions for printed models. This was not an issue for the chamber holder, but the reservoir holders had to accommodate the printers as a result. However, this was not a significant drawback, as a means of connecting the reservoirs via a dovetail arrangement was implemented, meaning four separate structures could be printed and linked together. This did increase printing time significantly, but simultaneously reduced material waste by printing a single part and identifying errors, rather than printing four parts which could not be used.

The second limitation was the selection of materials the 3D printers were compatible with. The printer bed and head temperatures could increase up to a threshold, reducing the amount of materials available for use. Additionally, the filament had to be 1.75 mm in diameter and biocompatible, which caused further complications. Nylon-12 filament was available from other suppliers, but the diameter restriction inhibited the use of these, meaning that the Nylon filament from Farnell was the only option.

Concerning the errors that occurred during 3D printing, most were likely a result of the printers, rather than the filament itself. For instance, the warping of the reservoir holder may have been due to the low temperature of the printer bed compared to the melted filament, meaning that when it landed, it shrank due to thermal deformation [17]. As mentioned previously, a layer of glue was applied to the printer bed on some prints as the model had trouble adhering to the surface. When applying the glue, the layer needed to be spread evenly; otherwise, the surface of both designs would be uneven, potentially affecting the remainder of the print.

There was not much difference in the quality of the prints from the two printers. However, it was noted that the Prusa printer produced support structures that were easier to remove than the Creality printer, possibly due to greater precision. Modifying the density of the support structures was a difficult task, as it required careful adjustments so that the supports would not collapse under the model's weight during printing. It could be argued that had the Nylon filament worked, the model could be autoclaved, removing excess supports; however, this was not an option due to using PLA filament. Eventually, a threshold mark was found that minimised the support for the final constructs.

The cause of the minor issue with the update file on the Prusa printer was unknown. However, research showed that users had a similar issue, meaning that it was likely an oversight in the update. Although this was an obstacle, the printers were successful in creating functional models.

The resolution of the printers hindered the project in various ways, in particular, the dovetail attachment. The dimensions on SOLIDWORKS for the tail and socket had a tolerance of 0.5 mm, which, theoretically, should have allowed a tight yet smooth fit. However, the printer released filament of 1.75 mm diameter, which may have resulted in overlapping the tolerance, hence the two parts not joining. As a result, the dimensions were changed further to allow the tail to fit.

The problems with the filament were mostly caused by the incompatibility of the Nylon with the printers, even though the initial requirements, such as melting temperature and diameter, were met. The filament being stuck may have been due to an insufficient printer head temperature. However, when the temperature was increased, the Nylon filament burned, indicating too high a temperature. A range of temperatures from 230-260ºC, as provided on the data sheet, was used, which led to further ambiguity. It should be noted that in some test prints, the temperature automatically changed during the filament melting process, which may have caused this problem. Also, unlike the PLA filament, there was no preset bed and head temperature on the Prusa printer; therefore, the temperature was manually adjusted, which may have resulted in errors.

4.3 – Design Evaluation

The dovetail arrangement for the reservoir holder was decided upon as it was a simple design that could easily attach and detach the holders if needed. The modular design allowed for these holders to be used in various types of test runs, with a different number of reservoir holders required. Other connection methods, such as using screws and bolts, were considered; however, this would require more complex designs to accommodate these fasteners, which may have been affected by printer resolution. For the chamber holder, the intended snap-box design was decided to keep the chamber in place. However, although the final print combined the lid and base, the chamber could still fit securely inside, due to the two holes created on either side, through which the chamber was fed. Subsequently, both models were successful in meeting the design objectives of the project – accommodating ease of use, creating organisation in the system and, in turn, simplifying data collection.

In terms of material selection, PLA was a suitable filament to use for all test runs, as it was compatible with both printers and could print models with low difficulty. In addition, its recyclability due to being a biodegradable polymer, low energy consumption due to low melting temperature, and wide availability significantly increased the sustainability of this project. The selection of Nylon was a suitable option based on the reviewed articles; however, more research could have been performed for alternative materials that could withstand autoclaving, allowing a greater range of materials to be used and increasing the options available for a final design which could be used in practice.

Depending on resources and time, optimisations such as installed sensors or cameras for real-time imaging could be implemented. As the bioreactor is incubated, it is difficult to periodically view the system's status due to contamination risks. Therefore, real-time monitoring could further reduce contamination sources and assess the media at more frequent timepoints, increasing the reliability and repeatability of results.

5.0 – Conclusions

5.1 – Key Findings

This project aimed to improve a perfusion bioreactor system with the addition of reservoir and chamber holders. The primary objective was to facilitate ease of operation by organising the system. Simplifying the data collection process was considered by creating designs that differentiated each part of the system, mitigating potential errors from users, which, in turn, could lead to more repeatable data.

Nylon-12 was the proposed material based on the literature review. However, a suitable Nylon-12 filament was not available, resulting in the use of an alternative Nylon filament. This filament resulted in various errors, including warping of the model surface, burning of the filament and filament becoming stuck. It was deduced that this was caused predominantly by the 3D printer changing temperatures, despite selecting a fixed temperature before printing. Consequently, it was decided that PLA filament would be used to create the final models to assess the design.

Both a reservoir and chamber holder model were 3D printed using either the Creality Ender 3D printer or the Prusa i3 MK3 3D printer. These models would not be able to be used in practice, as the bioreactor equipment is sterilised via autoclaving, which would result in damaging the PLA constructs. Nevertheless, the design specification of this project was achieved.

5.2 – Future Work

Regarding any future work, more research could be conducted to explore alternative materials that could withstand autoclaving, as well as exhibiting biocompatibility. In terms of improvements to be made on the design, given further time and resources, real-time imaging techniques through the use of sensors or cameras could be implemented to assess the media and flow rate in the bioreactor at more frequent timepoints while minimising contamination risks, which could increase reliability and repeatability of data.

References

1. Rouwkema J, Gibbs S, Lutolf MP, Martin I, Vunjak-Novakovic G, Malda J. In vitro platforms for tissue engineering: implications for basic research and clinical translation. Journal of Tissue Engineering and Regenerative Medicine. 2011 Feb 24;5(8):e164–7.
2. Rademakers T, Horvath JM, Blitterswijk CA, LaPointe VLS. Oxygen and nutrient delivery in tissue engineering: Approaches to graft vascularization. Journal of Tissue Engineering and Regenerative Medicine. 2019 Jul 30;13(10):1815–29.
3. Perez JR, Jose J, Mohile NV, Boden AL, Greif DN, Barrera CM, et al. Limb salvage reconstruction: Radiologic features of common reconstructive techniques and their complications. Journal of Orthopaedics. 2020 Sep 1;21:183–91.
4. Paschos NK, Vasiliadis HS. Allograft versus autograft for reconstruction of anterior cruciate ligament rupture in adults. Cochrane Database of Systematic Reviews. 2018 Sep 27;
5. Somers SM, Grayson WL. Protocol for the Use of a Novel Bioreactor System for Hydrated Mechanical Testing, Strained Sterile Culture, and Force of Contraction Measurement of Tissue Engineered Muscle Constructs. Frontiers in Cell and Developmental Biology. 2021 Apr 13;9.
6. Kapałczyńska M, Kolenda T, Przybyła W, Zajączkowska M, Teresiak A, Filas V, et al. 2D and 3D Cell Cultures – a Comparison of Different Types of Cancer Cell Cultures. Archives of Medical Science. 2016;14(4).
7. Egger D, Krammer M, Hansmann J, Walles H, Kasper C. Dynamic cultivation of human stem cells under physiological conditions. BMC Proceedings [Internet]. 2015 Dec [cited 2025 Apr 25];9(S9). Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC4685432/
8. Stewart S, Darwood A, Masouros S, Higgins C, Ramasamy A. Mechanotransduction in osteogenesis. Bone & Joint Research. 2020 Jan;9(1):1–14.
9. ehs.research.uiowa.edu. (n.d.). Autoclaving Guidelines | Environmental Health and Safety. [online] Available at: https://ehs.research.uiowa.edu/biological/autoclavingguidelines.
10. Sailon, A.M., Allori, A.C., Davidson, E.H., Reformat, D.D., Allen, R.J. and Warren, S.M. (2009). A Novel Flow-Perfusion Bioreactor Supports 3D Dynamic Cell Culture. Journal of Biomedicine and Biotechnology, 2009, pp.1–7. doi: https://doi.org/10.1155/2009/873816.
11. Achleitner, L., Winter, M. and Satzer, P. (2024). 3D printed autoclavable biocompatible biodegradable bioreactor vessels with integrated sparger made from poly-lactic acid. Journal of Biotechnology, 391, pp.33–39. doi: https://doi.org/10.1016/j.jbiotec.2024.06.001.
12. Bijonowski, B.M., Miller, W.M. and Wertheim, J.A. (2013). Bioreactor design for perfusion-based, highly vascularized organ regeneration. Current Opinion in Chemical Engineering, 2(1), pp.32–40. doi: https://doi.org/10.1016/j.coche.2012.12.001.

13. Licata, J.P., Slupianek, H., Rizokulova, S., Gerstenhaber, J.A. and Lelkes, P.I. (2024). Biocompatibility of 3D printed plastics for use in bioreactors. Bioprinting, [online] 40, p.e00347. doi: https://doi.org/10.1016/j.bprint.2024.e00347.
14. 3D Filament Cost [Internet]. Makeshaper. Available from: https://www.makeshaper.com/3d-printer-filament-price-cost
15. BioPak. What is PLA? | BioPak [Internet]. www.biopak.com. 2022. Available from: https://www.biopak.com/uk/resources/what-is-pla
16. Acres T. 3D Printing Materials Melting Temperature Chart : What's the Melting Point of Different Plastic 3D Printing Materials [Internet]. 3D Printernational. 2022 [cited 2025 Apr 25]. Available from: https://www.3dprinternational.com/blogs/news/printing-materials-melting-temperature-chart-whats-the-melting-point-of-different-plastic-3d-printing-materials?srsltid=AfmBOopbnkA2aIRL6-JfPXBh9IIA4la4cJtpVGgvfs1E_boBLMa9e4ua
17. Designing for Printing Part 2: Why 3D Printed Parts Warp and How to… [Internet]. markforged.com. Available from: https://markforged.com/resources/blog/3d-printed-part-warping

Appendix

(a) (b)

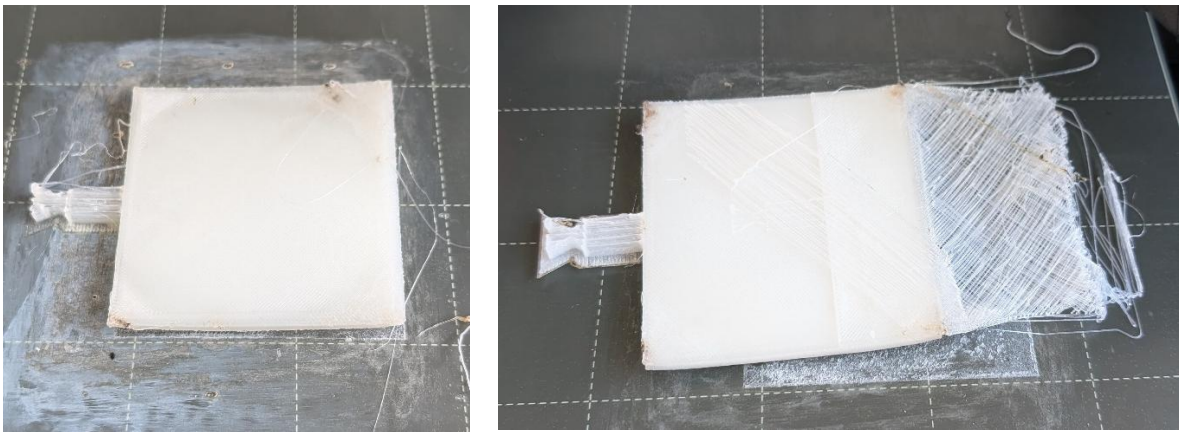

Figure A – Warping and burning of reservoir holder surface using Nylon filament (a), Shifting of model during 3D printing as a result of not adhering to the printer bed (b)

(a) (b)

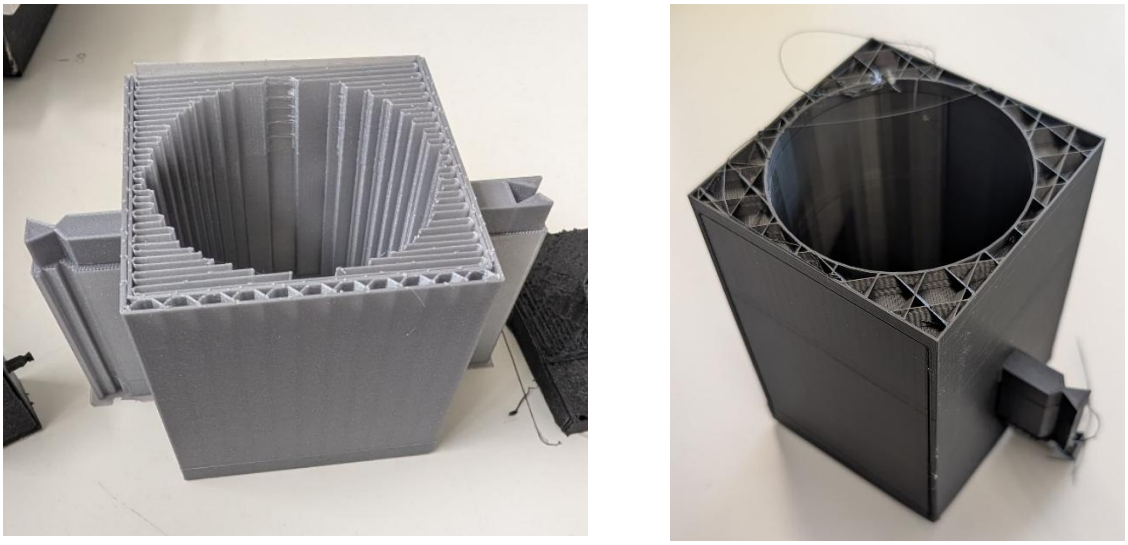

Figure B – Filament running out during 3D printing, causing incomplete models (a), (b).

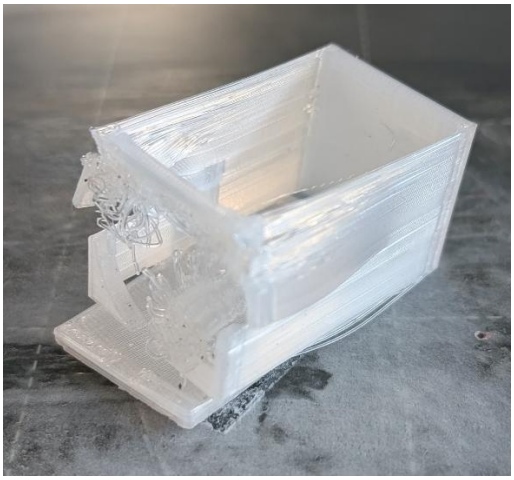

Figure C – Filament becoming stuck resulting in failed print.

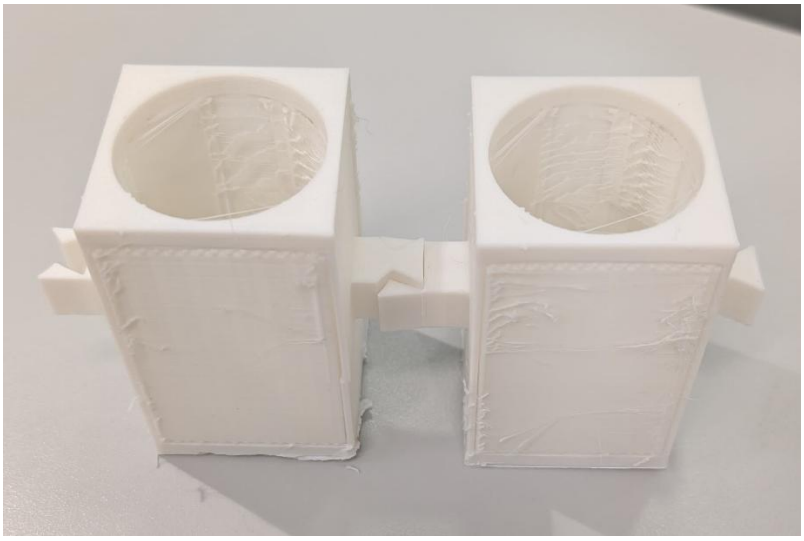

Figure D – Resolution of 3D printer preventing dovetail from fitting, support structure of wall too dense, many strands of filament remaining after print.