CHALMERS
UNIVERSITY OF TECHNOLOGY

A Cellulose Based Scaffold for Silk Protein

Possibilities for bone regeneration

Master's thesis in Product Development

OLIVIA SJÖBLOM

Department of Product and Production Development
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Gothenburg, Sweden 2016

MASTER'S THESIS 2016:06

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Cover:
Demonstrator for a mandible implant

Reproservice, Chalmers
Gothenburg, Sweden 2016

Abstract

This master thesis is an analysis of a new biomaterial of cellulose, Polylactic acid (PLA) and silk protein. The product for the material development is a 3D printed mandible implant with possibilities for bone regeneration. The silk protein can carry biological activity such as bone regeneration and develop bone while cellulose and PLA degrades in the human body.

The material has been mechanically tested and analysed on molecular level to evaluate potential change during the manufacturing process.

The project also includes verification of the possibilities for 3D printing of a mandibular implant and cellulose in particular.

Stakeholders for the product development are mapped and the path to CE certification is described.

Acknowledgements

I am grateful to my supervisors at Innventia, Elisabeth Sjöholm and at Chalmers, Andreas Dagman for the day-to-day guidance.

The knowledge and experience Elisabeth Sjöholm possesses has been invaluable for this master thesis and for my personal development in the field of chemical engineering.

Andreas Dagman has helped keep this master thesis project under control from day one. His input on product development and the framework for a thesis with multiple aims has been crucial to put it all together.

A special thank you to additional supervision from TechmarkArena 2016, in particular Fredrik Berthold for the mediation between chemical science and product development, Marie-Claude Béland for the inspiration and further interest in innovations of cellulose, Karin Edström for the unity of master thesis students and workshops with relevant associations and Henrik Pettersson for the help with laboratory equipment and for great answers to all my questions, mostly about the 3D printer.

Thank you to the interviewees for the contribution of their knowledge, a special thank you to My Hedhammar, Spiber for the contribution of silk protein and methods for testing.

Thank you to the testers and analysts for relevant data collection, a special thank you to Anders Reimann for the help to understand the Scanning Electron Microscope.

Abbreviations

PLA – Polylactic acid, a biodegradable thermoplastic
CPLA – a material mix of 70 percent PLA and 30 percent cellulose fibers
SEM – Scanning Electron Microscope
BSA – Bovine Serum Albumin
Dye – colouring substance
Woodfill – commercial filament of PLA and cellulose
CAD – Computer Aided Design

Table of Contents

1 Introduction

What if you could print a new organ or body part? What if a cancer patient with a malignant mandible tumour could get a new jaw with perfect fit and function? 3D printing may be the future tool to introduce bone regeneration. In the meantime this thesis will deliver an investigation on how a new biomaterial of cellulose and PLA may be used as a scaffold for bone regeneration through silk protein.

1.1 Background

This master thesis project has been performed at the Dept. Material Design, business area Biorefinery & Biobased Materials at Innventia. Innventia is a research institute with focus on innovations based on forest raw materials. Innventia is a no dividend organization with about 200 employees in close collaboration with other institutes, universities and the industry. Innventia is owned by and a part of RISE, Research Institutes of Sweden.

TechMark Arena 2016 is a project where Innventia assemble different master thesis projects to assess a specific area from different points of view. The focus is "Bridging the research-to-market-gap" and the area of 2016 is additive manufacturing. The project consists of students and supervisors with different backgrounds and competences to work multidisciplinary. This thesis deals with the question of how to use fibers of cellulose in combination with a thermoplastic polymer and protein from silk to create a new biomaterial suitable for implants.

1.2 Purpose

The purpose of this project is to examine if a cellulose-polylactic acid (PLA) blend and silk protein could be combined to make an implant that introduces biological activity in the human body. The cellulose fibers combined with PLA should work as a degradable scaffold and silk protein as a carrier of biological activity to stimulate bone growth.

1.3 Aim

The first aim is to compile test results and a specification list for a possible biomaterial based on a cellulose- PLA blend (CPLA) and silk protein. The second aim is to produce a demonstrator for a mandible implant. The third aim is to define stakeholders and map the product development process for such a product.

1.4 Research Questions

The research questions are divided into the three aims presented in chapter 1.4 and then expanded into nine research questions.

1.4.1 Material properties

- What are the most important properties for a biomaterial that will be replaced by bone?
- What are the mechanical properties for 3D printed CPLA?
- How does the cellulose fibers change during material processing?
- Is there a viable adhesion between CPLA and silk protein?

1.4.2 3D Printing

- Can a bone-like structure be 3D printed?
- Can cellulose be 3D printed?

1.4.3 Stakeholders

- How does a product development process work for a MedTech product?
- Who are the stakeholders for such a product?
- What can the customer chain look like?

1.5 Delimitations

Boundaries have been set to define and limit the scope of the project. To be able to answer the research questions during the time of a master thesis all aspects of a new biomaterial and implant cannot be analysed.

1.5.1 Material properties

The project will include the materials cellulose, PLA and silk protein.
The focus will be on producing a mandible implant consisting of a scaffold to which proteins may be adsorbed in order to regenerate bone tissue.

1.5.2 3D print

The manufacturing method is additive manufacturing.
The 3D printer is an Ultimaker 2.

1.5.3 Stakeholders

The analysis of stakeholders and customer chain is focused on the Swedish market.

1.6 Deliverables

Deliverables are set to join the interests of the university (Chalmers), the industry (Innventia) and the student so that misunderstandings are eliminated. The deliverables of this project are listed below:

- An analysis on how cellulose, PLA and silk protein can be combined into a potential biomaterial
- Requirements for a scaffold/implant of cellulose and silk protein
- An analysis of the stakeholders for such a product
- A report
- A presentation at Chalmers
- A presentation at Innventia
- A demonstrator of a mandible implant

The demonstrator will be developed as a complement to the report, to physically communicate the purpose of the product and the area of use.

1.7 Product Development Process

The product development process is based on New Product Development (NPD). It differs from classic product development since it is more agile and focuses on multidisciplinary teams that are important in MedTech product development since the task at hand often is too complex to tackle within one discipline alone. NPD is iterative and in a close collaboration with customers to find why the product is necessary or why a change has to be made (Kahn 2013).

The stages of NPD are visualised in Figure 1. The initial idea stage it is often called the front-end process or the fuzzy front end since market application of new technologies can be dim. It is important in technology-push products to identify customer needs and establish target specifications (Ulrich och Eppinger 2012). The Research phase should give an understanding of the market, the client and the technology. The Development, Testing and Analysis are an iterative process that, with help of demonstrators, visualises how the product can be improved (Kahn 2013). This project had a given idea: the biomaterial. The research phase included a literature search and interviews to formulate a product and its development. The product was then developed, tested and analysed in several iterations to obtain feedback and to revise the conditions for testing. Further development, testing and analysis is required to be ready for the intro phase where a pro duct is introduced to the market.

Figure 1 - The New Product Development (NPD) Process

2 Theory

Initial theory includes information on the materials that are used for the final concept and in the experimental development. The theory continues with implants, bone regeneration, 3D printing, the product development process and stakeholders to give a relevant background to what the market look like today.

2.1 Materials

The materials presented are cellulose, polylactic acid and silk protein.

2.1.1 Cellulose

Cellulose fiber is a central product in the forestry industry and there is a great interest to find new applications as a consequence of the increased competition and a reduced market for traditional paper products. It coincides with the growing awareness about the need to replace fossil-based material with renewable resources and the aim for a sustainable society.

Cellulose is the most common natural polysaccharide and has shown biocompatibility as a tissue engineering material. Cellulose has poor biodegradability in the human body due to the lack of specific hydrolytic enzymes; instead derivatives are used as engineering material in implants (Kuznetsova, o.a. 2014). Pure cellulose was used during this project, as a proof of concept.

Cellulose needs to be combined with other components to be processed by additive manufacturing since it is not a thermoplastic material and cannot be heated without losing its properties. In the present project, polylactic acid (PLA), a biodegradable thermoplastic aliphatic polyester, will therefore be added to work as a softener. The cellulose and PLA (CPLA) are combined into a load-bearing scaffold that will degrade while adsorbed silk protein will build up a new structure of bone.

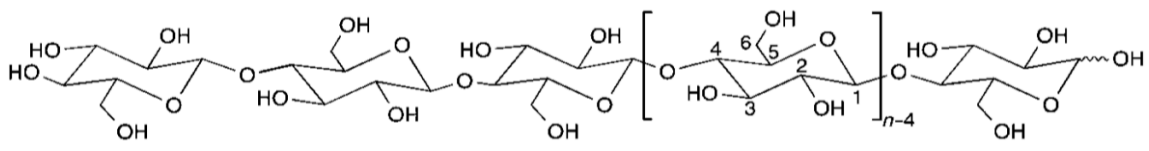

Figure 2 - Structure of cellulose (Klemm, o.a. 2005).

2.1.2 POLYLACTIC ACID (PLA)

PLA is a biodegradable thermoplastic polymer derived from corn starch or sugar canes. It degrades into lactic acid and is decomposable by the human body. Large amount of lactic acid is harmful to the body but is used in sutures and coating for pills (Ramot, o.a. 2016). The amount of lactic acid released from an implant can be reduced by slow release of the acid and optimized geometry that minimizes the amount of material required. Refinement of PLA and its copolymers can minimize the side effects from an inflammatory reaction.

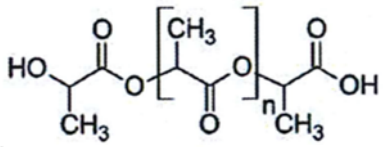

Figure 3 - Structure of polylactic acid (PLA) (Ratner, o.a. 2013)

2.1.3 PROTEINS

The used proteins are:

1. Bovine serum albumin (BSA), a protein concentration standard in lab experiments
2. Silk protein from the silkworm Bombyx mori (B. Mori)
3. Silk protein from the silkworm Antheraea assama (A. assama)

Silk is a fiber from protein, naturally produced by worms and spiders. Silk is used as a biomaterial because of its favourable mechanical properties, the ability to carry biological activity into the human body as well as its biocompatibility and stability in a physiological environment (Vepari och Kaplan 2007). The main advantage for silk protein is its long repetitive sequences of amino acids alanine and glycine that are flexible. Silk protein has a larger tendency to change structure while other proteins focus on a specific structure and to keep it (Hedhammar 2016).

The silk protein is diluted into a liquid and layered onto a cellulose-based surface. The idea is to introduce specific activity for different areas of a mandible. For example to add anti-inflammatory activity where the mandibles bone will interact with teeth or soft tissue.

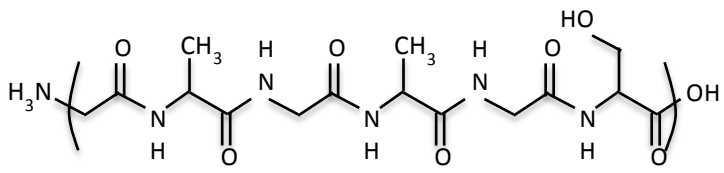

Figure 4 - Structure of silk protein (Kaplan 2015).

2.2 Mandibular implants

There are three major causes for a patient to need a mandibular implant (Pettersson 2013):

1. A birth defect of the mandible or a part of the mandible
2. A broken mandible due to impact force
3. Mandibular cancer where the tumour need to be replaced by new bone

The golden standard for reconstructions with mandibular implants is free vascularised flaps, bone with soft tissue and vessels, usually taken from the shank or hip depending on where the preferable blood vessels are. For large defects or a malignant tumour the soft tissue is often radiated or in other way damaged so new soft tissue is needed. Titanium rails can be used if vascularised flaps would not be possible due to the new vessels being unfunctional. Titanium rails are then attached in the remaining mandible with screws. Neither of these solutions can be customized for perfect fit like a 3D printed implant can. In contrast, an original mandible can be copied with 3D scanning technique and then 3D printed.

Research and development within reconstructive maxillofacial surgery in Sweden focuses on bone regeneration with mesenchyme stem cells, material development to replace titanium and how to grow bone in a more aerated location in the, body e.g. a muscle, and then inserted as a mandibular reconstruction (Andersson 2016) (Pettersson 2013).

2.3 Bone regeneration

In theory it is preferable to use the human regenerative abilities instead of adding external material to reconstruct soft tissue or bone due to the risk of rejecting a foreign object, i.e. autoimmune response. It is vital for tissue engineering and bone regeneration to mimic nature and use the intelligence of the human body. In some cases it can even be better to let the body regenerate on its own with minimal interference from additives rather than to use materials and chemicals that can interfere with the regenerative process (Place, Evans och Stevens 2009).

2.3.1 Bone structure

For optimal bone growth it is important that the regenerative cells have enough circulation and supply of oxygen. It is therefore important with a porous scaffold that mimics existing bone structure. A porous structure gives the regenerative cells a better chance to grow into a 3D structure for maximal strength and load bearing properties, to disrupt fibrosis and promote angiogenesis (Ratner, o.a. 2013). Topology optimization can be used to create a natural porosity with organic shapes and still optimize for specific loading condition. Topology optimization calculates where material can be removed and creates a pattern with Finite Element Method. The pattern creates a 3D structure that is optimised for a given load condition and can be 3D printed (Almeida och Bártolo 2013).

At Massachusetts Institute of Technology (MIT) they develop bone regeneration with bone growth factors that is slowly released in nanogram quantities. The scaffold in their case is a porous, nanostructured poly(lactic-co-glycolic acid) (PLGA) membrane (Shah, o.a. 2014). Figure 5 shows the bone growth after a couple of weeks.

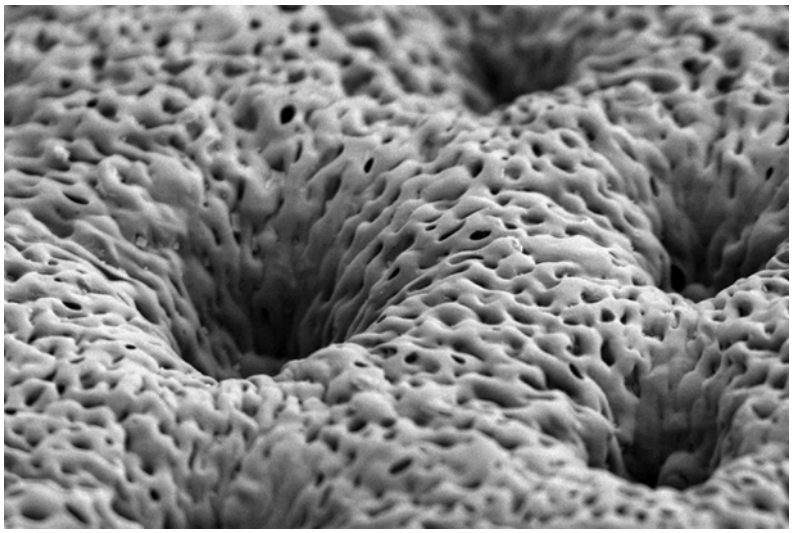

Figure 5 - Bone regeneration on poly(lactic-co-glycolic acid) (Shah, o.a. 2014).

At University of Pavia they develop programmable 3D silk bone marrow. It has generated functional platelets ex vivo with modelled silk, functionalized with surface coating of stem cells or entrapment of an extracellular matrix, seen in Figure 6.

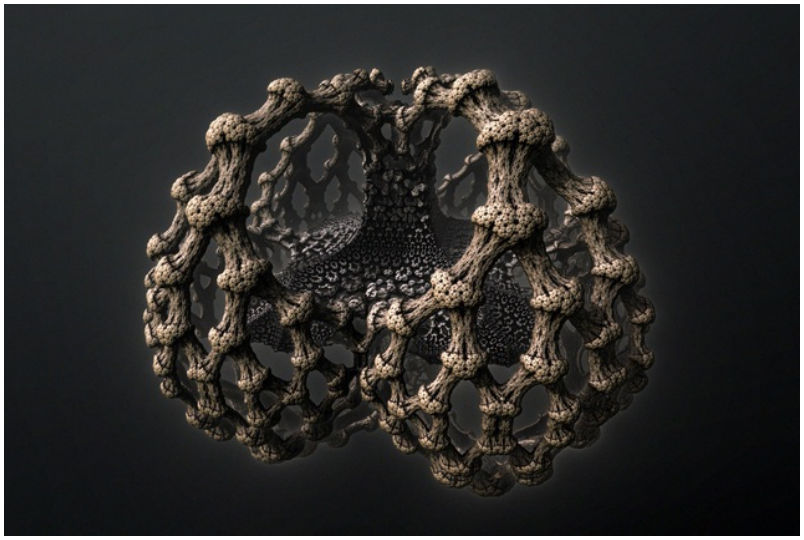

Figure 6 - Programmable 3D silk bone marrow (Christian A. Di Buduo 2015).

2.4 3D printing

The possibilities for customised products are near endless with 3D printing techniques. Examples on techniques that are used today are Stereolithography, Digital Light Processing, Fused deposition modelling, Selective Laser Sintering, Electronic Beam Melting and Laminated object manufacturing. Customised products are important in the MedTech industry, especially for implants and prostheses since such products often will replace a unique part of the body. In dental or orthopaedic surgery it is increasingly valuable with 3D scanning and Computer Aided Design (CAD). 3D scanning, CAD and 3D printing can all work together to mimic a body part that needs to be replaced. There are tools like topology optimization to calculate and evaluate how to make an implant or prosthesis even better than the original body part.

The market for 3D printers has exploded and now there are bio printers that can print gel, biomaterial or several materials at once. The difficulty is to be able to sterilise the 3D printed product properly. In Sweden there is only one commercial filament to 3D print that is biocompatible and that material can only be in contact with skin for 30 days. Other products are in research state or in clinical trials.

A 3D printer can also be useful for the concept of a mandible implant whereas protein can be added to certain zones of a scaffold to introduce different biological activity for areas of interest.

The printer used to make the material in this project is an Ultimaker 2+, a basic 3D printer that tested the printability of cellulose and PLA (Ultimaker, ULTIMAKER 2+ SPECIFICATIONS 2016), see Figure 7.

Ultimaker has its own software that is compatible with their 3D printers. Cura software is a 3D printing slicing software where a CAD model with a .STL file is converted into a .gcode file needed to be able to print. The program slices the CAD model into layers that the 3D printer then mimics and prepares the model with over 200 settings like nozzle size, printing speed and material flow (Ultimaker, CURA SOFTWARE 2016).

Figure 7 - An Ultimaker 2+, the 3D printer used during this master thesis project

3 Experimental

The experimental chapter describes the materials and methods used in the laboratory as well as the interviews performed.

3.1 Material Properties

Methods to identify if the scaffold material could fit as a mandible implant concerning the strength of the 3D-printed material, property changes during manufacturing and possibilities for bone regeneration with protein adhesion.

3.1.1 Mechanical Properties

The mechanical properties were measured with MTS FlexTest 60. To calculate stress and strain and thereby calculate the tensile strength, compression strength and impact strength these equations are required:

$$\boldsymbol{\varepsilon = \Delta L / L_0} \qquad \boldsymbol{strain}$$

$$\boldsymbol{\sigma = P / A_0} \qquad \boldsymbol{stress}$$

3.1.1.1 Tensile strength

The tensile strength was tested according to the standard ISO 3167 that includes the dimensions of a standardised test specimen. The specimens were exposed to a load cell of 10kN until failure.

3.1.1.2 Compression strength

The compression tests were performed according to the standard ASTM D695, ISO 604 with the specimen dimension of 25.4x12.7xx12.7 mm. The specimens were exposed to a load cell of 9kN at 4mm/min.

3.1.1.3 Impact strength

The Charpy impact test was performed according to the standard ISO 179, ASTM D6110 with the specimen dimension of 80x10x4 mm. A Zwick Pendulum impact tester HIT5P performed the test with a pendulum of 5 J. The absorbed energy is then calculated with the cross section of the specimen to get the mean toughness [J/m^2].

3.1.2 Size and Functionality of Cellulose Fibers

Particle tests were conducted to measure the size and size distribution and functionality of the cellulose fiber materials in CPLA and the difference between unextruded, 2x- and 4x-

extruded material. The PLA in CPLA were removed with chloroform since only the cellulose component of the scaffold material was of interest.

The size of the cellulose fibers were measured with a L&W FiberTester (Code 912, inventory no. FP99987). The tester was operated by Agneta Molin, MSc, Physical Testing, Papermaking & Packaging at Innventia. The results are presented in chapter 5.1.2.1 and in Appendix 8.1.

Fourier Transform Infrared Spectroscopy (FTIR) spectra were recorded using a Varian 680-IR FTIR spectrometer, equipped with a deuterated triglycine sulfate (DTGS) detector. The system was operating in attenuated total reflection (ATR) mode. An ATR crystal of ZnSe, having a contact area of Ø2 mm and a penetration depth of 2 µm, was used. Background and sample spectra were scanned using a spectral resolution of 4 cm^{-1} and a spectral range of 4000 cm^{-1} – 650 cm^{-1}; 32 scans were collected. Spectra were ATR and baseline corrected using Varian Resolution Pro software. Spectra were normalised at 1160 cm^{-1}. Jasna Stevanic, Analyst, Biorefinery Processes and Products at Innventia operated the spectrometer. The results are presented in chapter 5.1.2.2 and in Appendix 8.2.

3.1.3 PROTEIN ADHESION

Protein was added to the scaffold material. Two different methods were used to evaluate protein adsorption, in order to verify successful protein adsorption to the scaffold material. A Scanning Electron Microscope (SEM) was used for the indication of protein through mapping of nitrogen and sulphur. The other method was protein staining which was done using two different dyes, in order to visualise adsorbed protein

3.1.3.1 Indication of protein through mapping of nitrogen and sulphur

Nitrogen and sulphur are elements in proteins. To map these elements with SEM can indicate where on a surface a protein exists. If the specimen is a hydrophilic material containing water, like cellulose, the mapping is done in partial vacuum to prohibit that the specimen gets damaged. Here follows instructions for SEM:

- Choose partial vacuum (VP-SEM) to minimize disturbance from the electron beam.
- Put the specimen on a specimen stub, preferably the 51 mm, check the total height. The pictures will be clearer if the height is measured correctly since the focus of the electron beam depends on it. The focus of the beam has an accuracy of 1 nm.
- Take a photo of the specimen stub with specimen to be able to navigate.
- Apply voltage acceleration (VACC) and variable pressure (VP).
- Check that the scan has the right height to focus with Stage – EDX z(10).
- Start the scanning, set auto focus and then manually improve the picture of the specimen.

- Slow 1/Fast 3 differs when the camera moves, to navigate choose Fast or Reduced view and then choose Slow when the area is found and focus for favourable resolution.
- Choose the same magnification for all specimens enable comparison. Export image – add to report.
- Exit test session: Select specimen EXC – air. Remove the specimen when able to open the chamber of the SEM.

Instructions for mapping:

- Start the program Esprit 1.9.3.
- Select the picture that was taken with SEM.
- Select mapping, map size: full. Select the element to map, and then acquire.
- Choose the same time to map for the selected element to be able to compare. Export image – add to report.

3.1.3.2 Indication of protein through protein staining

Dyes used:

1. Coomassie Brilliant Blue G (diluted 333 times in deionized water). Coomassie G stains proteins in general (basic amino acids are stained).
2. Rhodamine B (0.1 % in deionized water). Rhodamine B stains silk.

Store at room temperature (Rhodamine B should be kept dark).

PBS buffer:

- Provided is 3×50 mL of 10×PBS (phosphate-buffered saline, pH ~ 6.8), non-sterile.
- Store at room temperature.
- Dilute ten times to 1×PBS in water.
- The pH of 1×PBS should be 7.4 upon dilution, but check the pH to be sure.

Silk staining:

- Add Coomassie G or Rhodamine B on top of the silk.
- Incubate 15-30 minutes at room temperature.
- Rinse the silk thoroughly with 1×PBS until excess/unbound Coomassie G or Rhodamine B is removed.
- The degree of Coomassie G and Rhodamine B staining can be judged by visual inspection of the silk samples by eye (and should be compared to a non-silk control), and documented be light microscopy.

- As Rhodamine B also is fluorescent, the degree of Rhodamine B staining can also be documented by fluorescence microscopy (in water, excitation = 562 nm, emission = 583 nm). This is a good complement to the light microscopy, and is also a suitable option if the silk staining is weak.

3.2 3D print

This chapter describes the manufacturing process to develop a CPLA material for 3D printing. The 3D printer and its setting is also described.

3.2.1 In-House Fabrication of CPLA

CPLA was manufactured in-house to control the manufacturing process and content of the filament. The material was manufactured with 100g per batch and with 70 percent PLA and 30 percent cellulose fibers.

1. Tear up 30 g of cellulose sheets and put in deionized water for one hour.
2. Pour the expanded cellulose in a mixer, fill up with deionized water and mix for 10 000 rounds, takes about five minutes. Pour the mixed cellulose in a large bucket. Clean mixer by adding new deionized water and mix for an additional 10 000 rounds with only deionized water.
3. Mix deionized water and cellulose with 70 g of PLA. Attach a large mixer rod to a tripod. Start with a high gear and a low force, then increase speed until everything moves and commingle for about one hour.
4. Use a funnel and a conical flask with added vacuum to remove water.
5. Add to tray and put in desiccator to dry for about two days.

3.2.2 In-House Extrusion of CPLA Filament

The mix of cellulose fibers and PLA needs to be extruded to be able to 3D print. It takes about four hours to extrude 100 g CPLA.

1. Change temperature to 180 C at initial speed at 20 l/mm.
2. Add a circular nozzle by the outlet; 2.85 mm is the standard diameter for commercial filament to print in the 3D printer Ultimaker 2.
3. Calibrate when the temperature is reached. Start the motor and increase the speed to 50 l/mm.
4. Re extrude the material for decreased cellulose fiber size and increased brittleness.

3.2.3 In-House 3D Printing of CPLA Filament

This material is more brittle than commercial filament and the printer needs to be carefully controlled by the operator for the material not to get stuck in the nozzle, even more with re-extruded material.

1. Choose a CAD file that you want to print. Convert it into a STL-file and open in the software Cura, specified to the 3D printer Ultimaker.
2. In Cura choose settings for nozzle size, temperature, speed, infill, skirt etc.

   Settings for the cubes of CPLA:
   Nozzle size: 0.6 mm
   Temperature, nozzle: 220 C
   Temperature, building plate: 60 C
   Speed: 40 mm/s
   Infill: 100%
   Skirt: 1 layer

   Settings for the mandible of CPLA:
   Nozzle size: 0.4 mm
   Temperature, nozzle: 220 C
   Temperature, building plate: 60 C
   Speed: 20 mm/s
   Infill: 20%
   Skirt: 1 layer

   Settings for the skull of Ultimaker PLA Pearl White filament:
   Nozzle size: 0.25 mm
   Temperature, nozzle: 210 C
   Temperature, building plate: 60 C
   Speed: 40 mm/s
   Infill: 10%
   Skirt: 1 layer

3. Save the Gcode of the model onto a memory card and insert the memory card into the 3D printer.
4. Change material and choose material in the settings on the 3D printer.
5. Find the Gcode of your model on the memory card and print.
6. Be aware if the filament gets stuck in the tube or feeder or somewhere else along the path to the heated nozzle, then pause the print and fix the problem or tune the settings while it print.

3.3 Stakeholders

Interviews and brief literature research were conducted to map the market for a mandible implant of CPLA and silk protein and its stakeholders. The interviews constitute a base for information gathering regarding the value chain for mandible implants. The interviewees are diversified in complementary fields and a mix of experts and professionals to see the whole picture of the product development process. The interviews were qualitative, semi-structured and competence-based with questions related to the research questions of the master thesis project:

What are the most important properties for a biomaterial that will be replaced by bone?
What are the mechanical properties for 3D printed CPLA?
How does the cellulose fibers change during material processing?
Is there a viable adhesion between CPLA and silk protein?

Can a bone-like structure be 3D printed?
Can cellulose be 3D printed?

How does a product development process work for a MedTech product?
Who are the stakeholders for such a product?
What can the customer chain look like?

3.3.1 Interviewees

The interviewees were chosen based on earlier collaborations with Chalmers and Innventia. Complementary interviewees were gathered by researching University Hospitals in Sweden and their collaborations for innovative product development.

Matts Andersson, Chief Executive Officer and Chief Technical Officer at Ortoma

Björn Lovén and Christian Thunborg, Innovation coordinator and Chief of Product Development at SLL Innovation (County Council of Stockholm) and Danderyd University Hospital

My Hedhammar, Founder and R&D director of Spiber Technologies

Linda Forsberg Pettersson, Maxillofacial surgeon and researcher on mesenchyme stem cells for bone regeneration at Umeå University, County Council of Västerbotten and University Hospital of Umeå

Sjoerd Haasl, Act. Dir. CTMH (Center for Technology in Medicine and Health) and Clinical Innovation Fellowship

4 Results and Discussion

The results of this project regarding Material properties, 3D printing and interviews with Stakeholders are presented and discussed.

4.1 Material properties

The material property tests were chosen based on the available mechanical test equipment and recommendations from similar projects. Material properties for a 3D printed mandible implant consisting of cellulose, PLA and silk protein were categorised into:

1. Mechanical properties
2. Cellulose particle properties
3. Protein adhesion

4.1.1 Mechanical properties

3D-printed specimens of in-house manufactured CPLA were subjected to mechanical testing to measure their tensile strength, compression strength and impact strength. Since the specimens were 3D-printed the material itself could not be tested in its full capacity. Instead the adhesion between the printed layers was tested which is important for a specific printed product. The CPLA were compared to a reference of commercial PLA and a commercial filament of cellulose and PLA with additives called Woodfill.

PLA: printed commercial PLA
xPLA: printed commercial PLA with a 45 degrees angle change of the printing pattern, see Figure 9
Woodfill: commercial filament of 70% PLA and 30% cellulose with additives (e.g. softener)
2x CPLA: 2 times extruded material of 70% PLA and 30% cellulose, in-house manufactured
4x CPLA: 4 times extruded material of 70% PLA and 30% cellulose, in-house manufactured

Figure 8 - The different materials for mechanical property testing

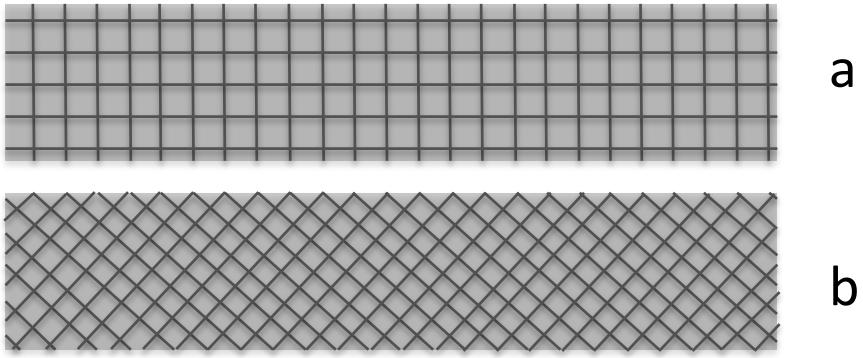

Figure 9 - Grid patterns illustrating the different printing patterns of (a) Woodfill, 2x CPLA, 4x CPLA and (b) xPLA.

4.1.1.1 Tensile strength

The tensile tests were performed according to ISO standards that include the specified dimensions of the specimen, load, equipment etc. The tests gives measures of tensile strength, elongation and, from the stress/strain curve also tensile modulus (stiffness) to compare with existing materials for mandible implants and as indication of the properties possible to obtain for CPLA. In Figure 10 the test instrumentation is shown along with the test specimens after break. There is one specimen of each material due to lack of material and the time consuming manufacturing process for the specimens of CPLA. The xPLA were tested twice to confirm the result since it performed much better than the other materials. The xPLA also broke in different places, seen in Figure 10, specimen b and c. That indicates that there are small differences in the printing process, the Ultimaker 2 is a basic 3D printer without perfect accuracy and the specimens break at their weakest link.

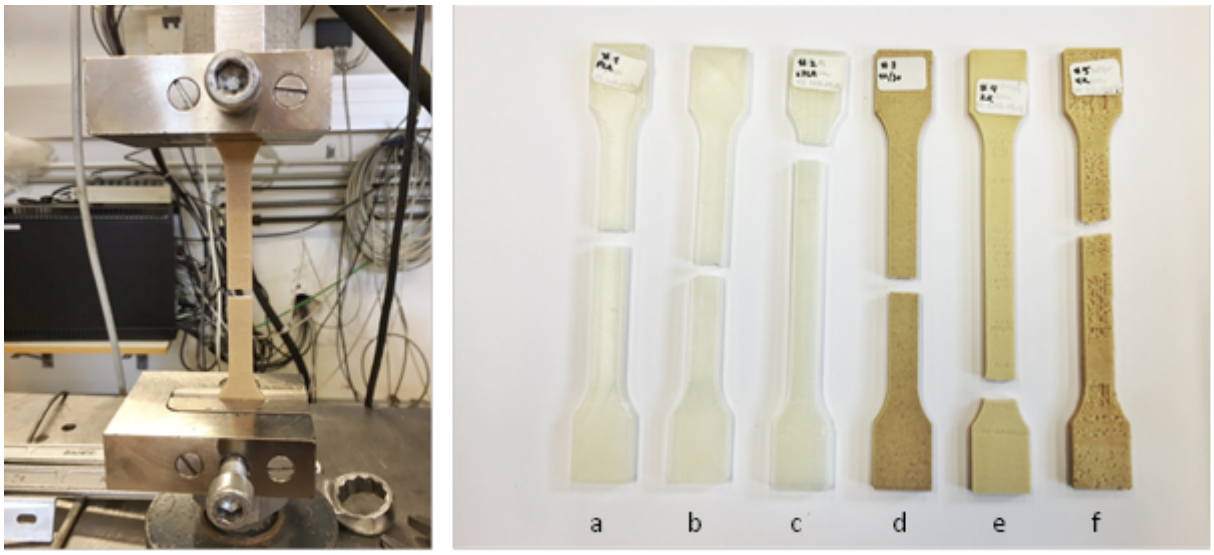

Figure 10 - Tensile test apparatus and test specimen: (a) PLA, (b) xPLA, (c) xPLA, (d) Woodfill, (e) 2x CPLA and (f) 4x CPLA

The stress/strain curve in Figure 11 show the different materials, calculations described in chapter 3 Experimental.

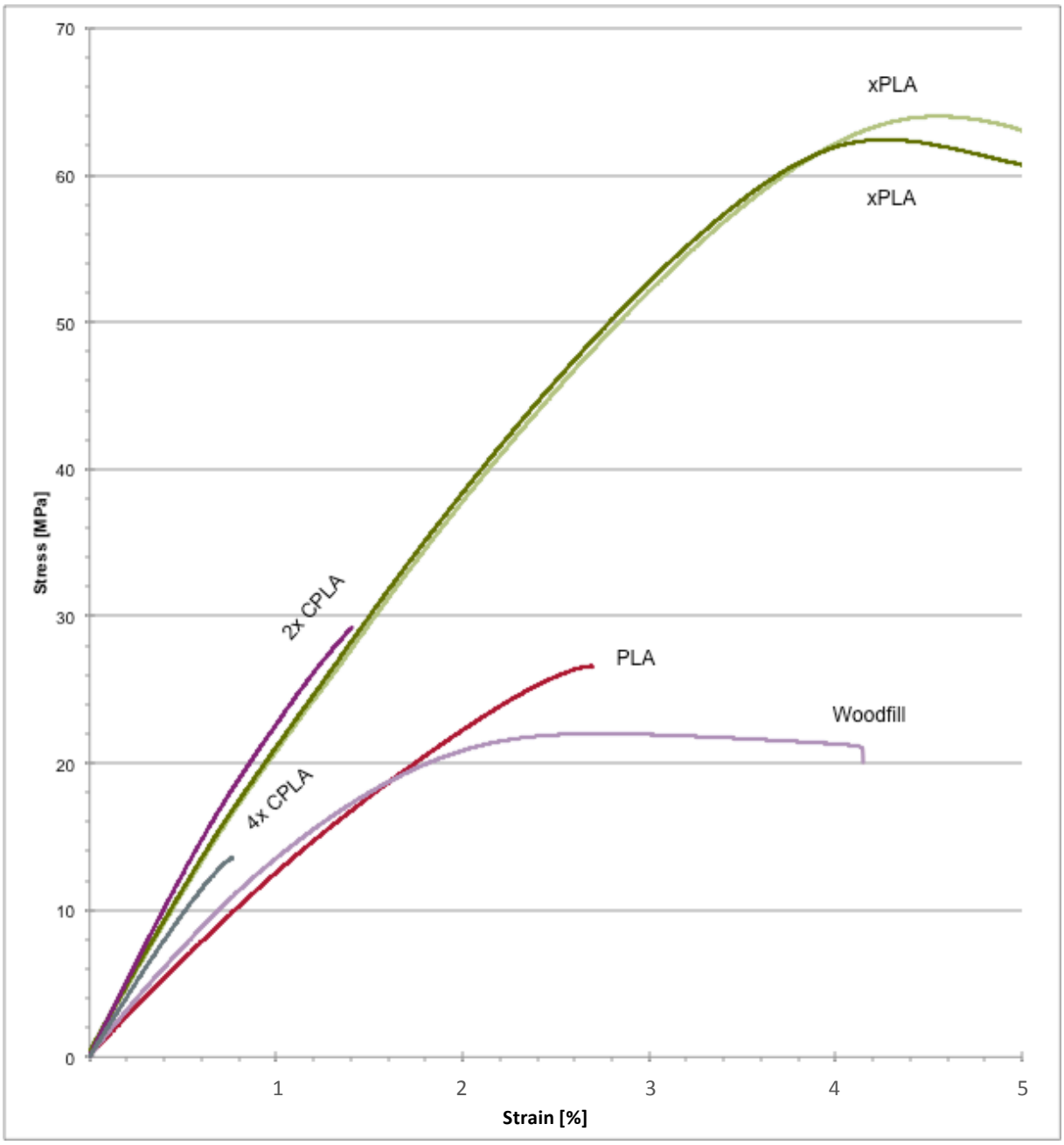

Figure 11 - Stress/strain curve of tensile test result

The test indicates that 3D printed PLA has a higher tensile strain than 3D printed CPLA, when using the same grid pattern. Since PLA is a thermoplastic material (in contrast to cellulose fibers) the printed layers are probably fused together in a stronger bond that makes PLA tougher than CPLA.

xPLA has the best performance, probably since the printing pattern enables the specimen to elongate during high stress without breaking. It shows that the direction of the printed layers is an important factor in how to optimise a 3D printed product.

Woodfill has a low elastic limit compared to PLA and 2X CPLA but the elongation before it breaks is a lot better than PLA and the two CPLA specimens. This might be because of the additives in Woodfill or the production process of the material.

2x CPLA has a higher elastic limit and is not as brittle as 4x CPLA.

4.1.1.2 Compression strength

The compressive strength can be compared to other relevant materials and to the maximum masticatory force for a human mandible of 700N (Scully 2003). Figure 12 show the test rig with a compressed specimen and four types of samples. (a) PLA, (b) xPLA, (c) 2x CPLA and (d) Woodfill.

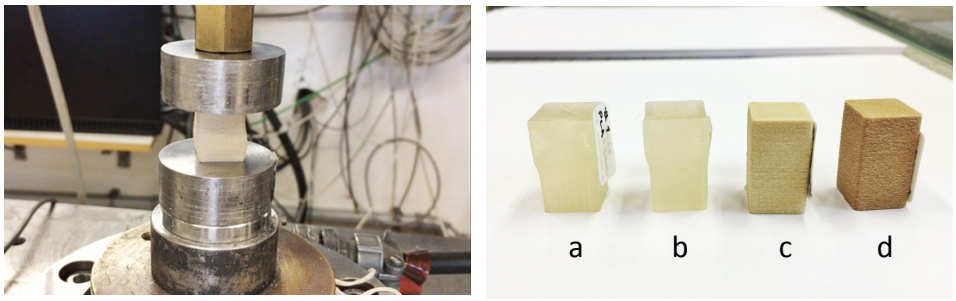

Figure 12 - compression test rig with 2x CPLA during a test to failure and the four test specimen (a) PLA, (b) xPLA, (c) 2x CPLA and (d) Woodfill

The specimen containing cellulose, 2x CPLA and Woodfill, did not buckle while the specimen of PLA did. It indicates that the cellulose fiber material helps the specimen to keep its shape and distribute the load throughout the construction.

2x CPLA were tested until failure and got a different buckling shape than PLA. 2x CPLA buckled at the center of the specimen while PLA buckled on the upper half of the specimen. This also indicates that the cellulose fibers distribute the applied force while PLA yields in an earlier state.

Figure 13 show the stress/strain curve for the compressive strength of the specimens. Even though the specimens of PLA did buckle they could withstand a larger stress compared to the samples with cellulose. A mandible does not need to withstand the usual masticatory force during bone generation. Thereafter the new bone tissue will withstand the load. It is far more important that the implant keeps it shape and do not buckle so that the bone can grow properly and in its intended geometry.

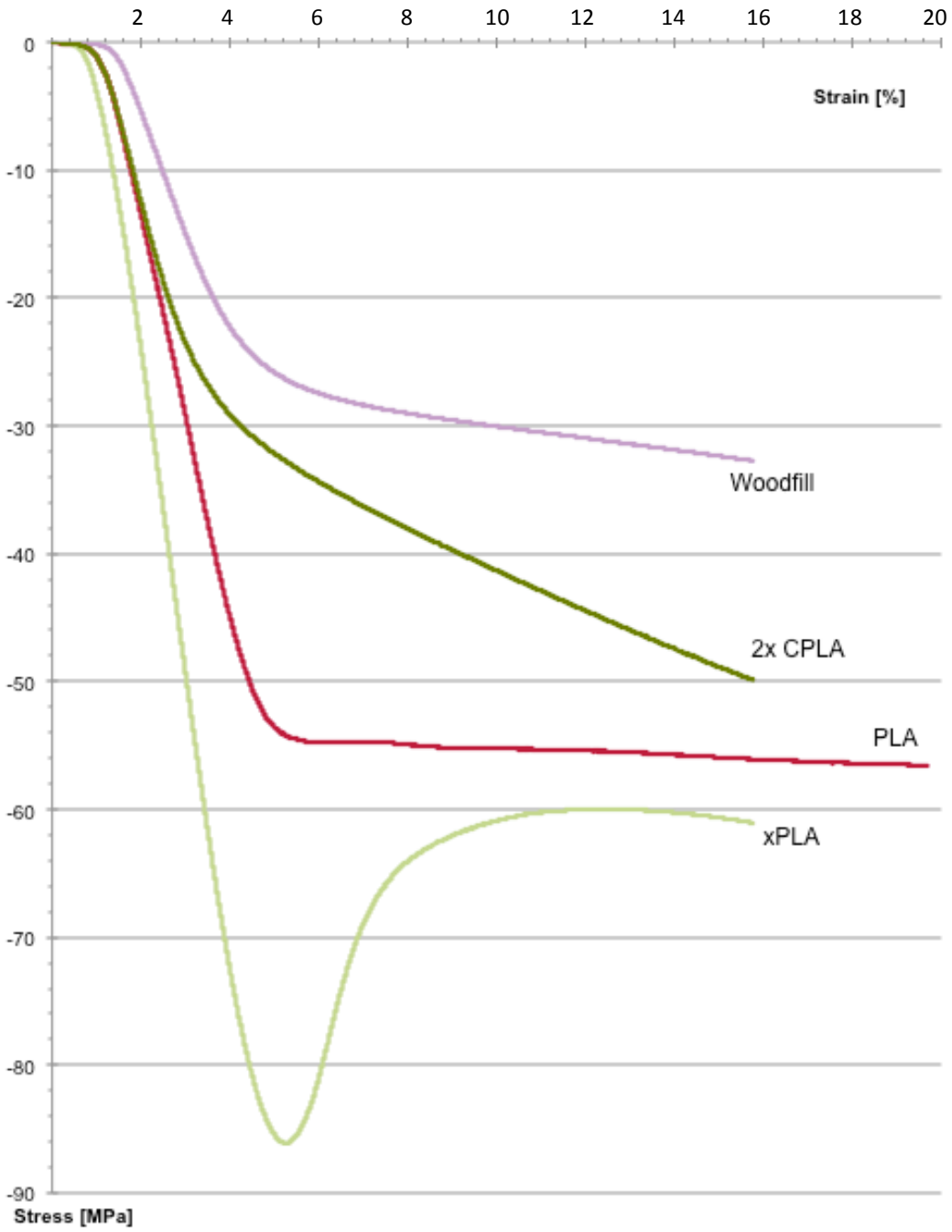

Figure 13 - Stress/strain curve for compression strength

4.1.1.3 Impact strength

Six specimens were tested in the Charpy impact test, of which five were made with the same grid pattern; two were made of PLA (a and b), one Woodfill sample (d), and two CPLA samples, made of material extruded twice (2x CPLA, e) or four times (4x CPLA, f). In addition the xPLA sample (c) made using a different grid pattern was evaluated. The test equipment and specimens before and after testing are shown in Figure 14.

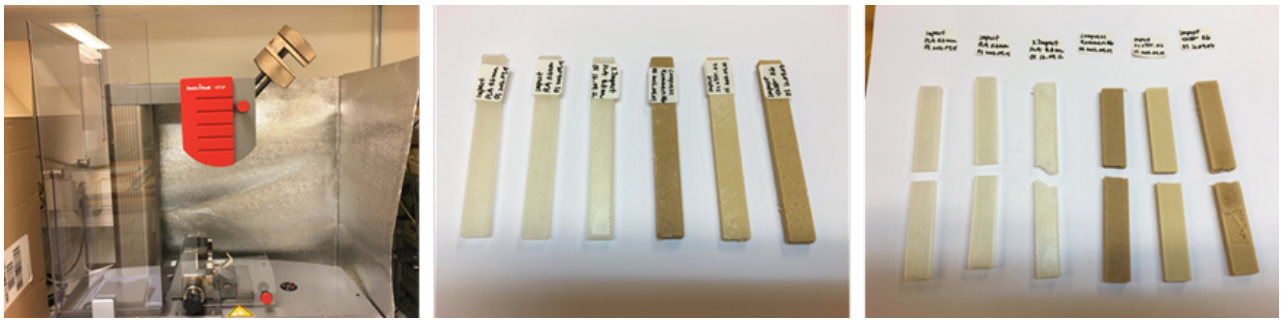

Figure 14 - Charpy impact tester and specimens. (a b) PLA, (c) xPLA, (d) Woodfill, (e) 2x CPLA and (f) 4x CPLA

The absorbed energy was calculated along with the cross section of the specimen to get the mean toughness [J/m^2]. The test shows that 3D printed PLA absorb more energy than 3D printed CPLA, see Figure 15. Since PLA is a thermoplastic material the printed layers is probably fused together in a stronger bond than for cellulose armoured CPLA and has therefore a better performance in an impact test.

That is the same reasoning as for the tensile strength. The cellulose fibers need to enhance their 3D printability for the CPLA to match PLA in these tests. On the other hand, for a mandible implant that will regenerate bone, there is no need for the scaffold material to be able to withstand the normal tensile- or impact forces. The scaffold will be fixated while bone generates and then the bone itself will be the loadbearing material.

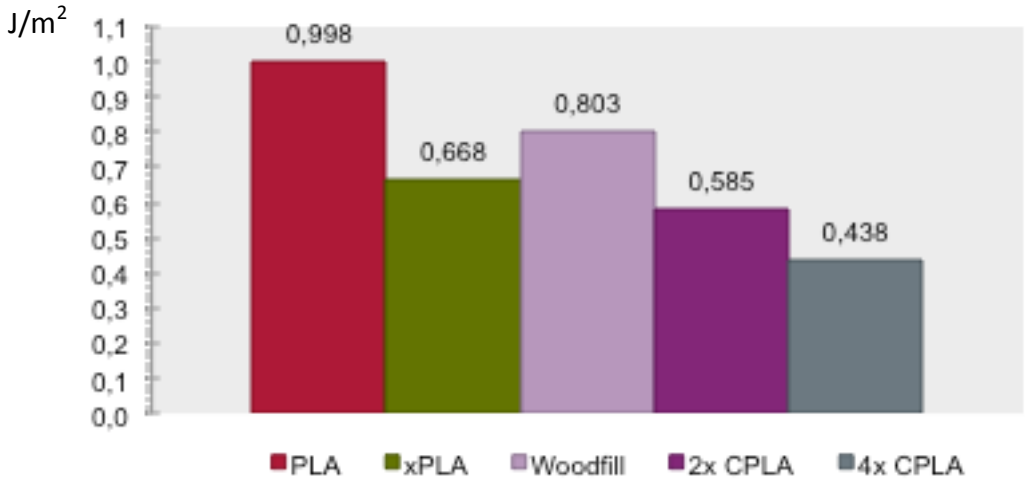

Figure 15 - Absorbed energy for Charpy impact test

4.1.1.4 Summary Mechanical properties

The mechanical tests were primarily made to compare PLA to CPLA, printed with the same grid pattern. PLA had better strength than CPLA but buckled more during the compression test. Since the mandible implant will grow bone, high strength in the scaffold material is not required. The implant will not be exposed to any high loads while the bone regenerates, the scaffold will hold the soft tissue in place and make it possible for the patient to talk and eat during the regeneration of bone. Table 1 show a comparison between the tested materials

and the material properties for PLA found in the Cambridge Engineering Selector (CES) material database.

Material	Tensile strength	Compressive strength	Impact strength
	MPa	MPa	kJ/m^2
PLA	26	56	0.998
xPLA	63	86	0.668
Woodfill	22	34	0.803
2x CPLA	29	50	0.585
4x CPLA	13	-	0.438
Default PLA	47-70	66-86.4	1.3-2.8

Table 1 - Material strengths of 3D printed materials in comparison to default PLA

4.1.2 PARTICLE PROPERTIES

CPLA was made by melt-extrusion, to obtain a coarse fibre for 3D printing. To improve 3D printing of CPLA, the influence of reextrusion was studied. While doing so, it was found that the re-extruded filament becomes denser and more brittle. 3D-printed CPLA were extracted with chloroform to remove PLA. The size distribution of the remaining cellulose particles was measured to see how cellulose is affected by being re-extruded. In addition the infrared absorption spectrum revealing functional groups of the material were evaluated.

4.1.2.1 Particle measurement of cellulose fibers

The particle size of the cellulose fibers were measured and Figure 16 display the distribution of fiber length. The black curves correspond to non-extruded material, the blue curves correspond to two times extrude material and the red curves correspond to four times extrude material. The distribution show a significant difference between the materials since the untreated material has a somewhat even distribution of fiber length between 0.5-4.5 mm while the treated materials fiber lengths are primarily under 1 mm. It also shows that the material extruded four times have even shorter fiber lengths than the material extruded two times. That implies that the number of extrusions correlates with the shortening of fiber lengths.

When 3D printing CPLA it is preferable with short fibers. Large fibers clog the nozzle of the printer and interrupt the print. Large fibers also give an uneven print surface that is hard to control. With shorter fibers the material gets more brittle, which makes the printing process more complicated since the filament breaks while fed into the printer. It is a balance between precision and brittle material and the most successful print seem to be at about 3x extruded CPLA. With longer fibers the scaffold can naturally get a more porous characteristic that is preferable if the detail of the porosity is secondary.

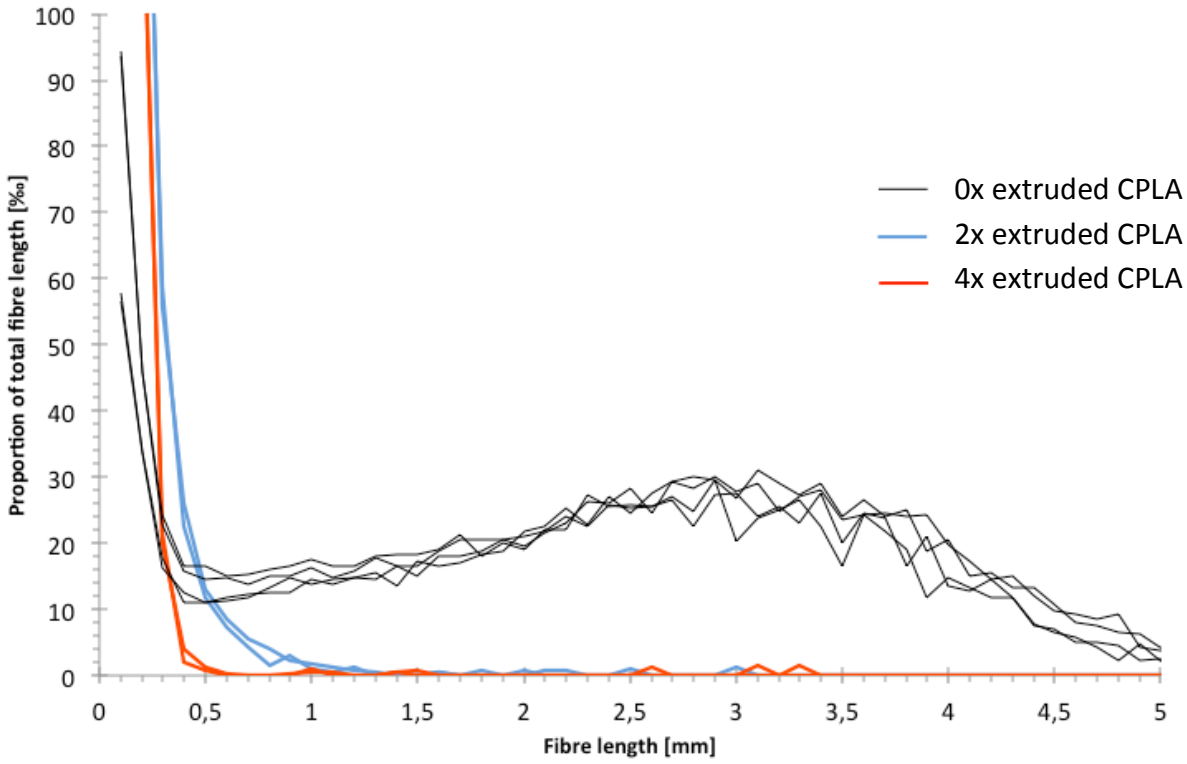

Figure 16 - Figure length distribution. The fiber length shortens with the number of extrusions.

4.1.2.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was used to reveal the functional groups of the cellulose fiber material after removing the PLA. The fiber fraction was analysed to see if it was affected by the extrusions, either with respect to particle size or the composition of functional groups. Figure 17 shows the fundamental structure of (a) PLA and (b) cellulose.

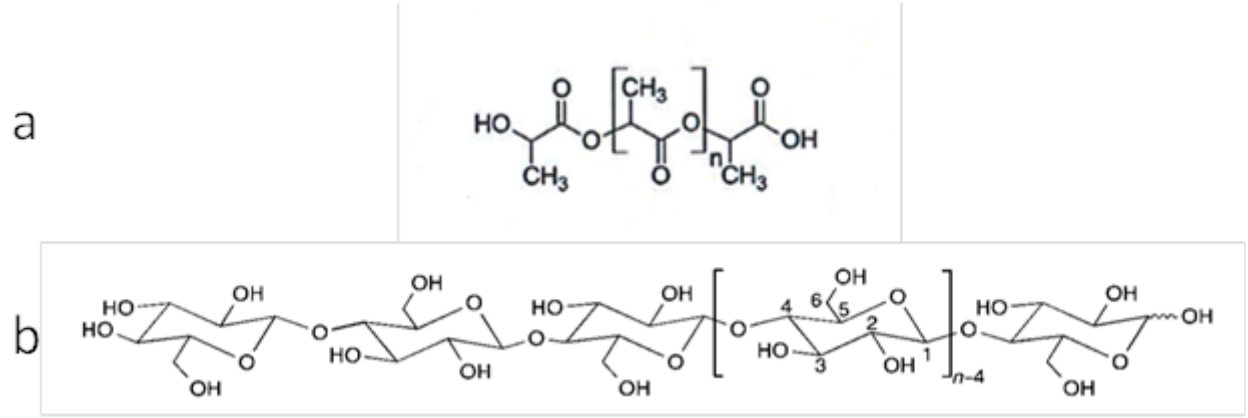

Figure 17 - The structure of (a) PLA and (b) cellulose

The Fourier transform converts the data to the spectrum in Figure 18. The red curves correspond to four times extrude material, blue curves correspond to two times extrude material and black curves correspond to non-extruded material.

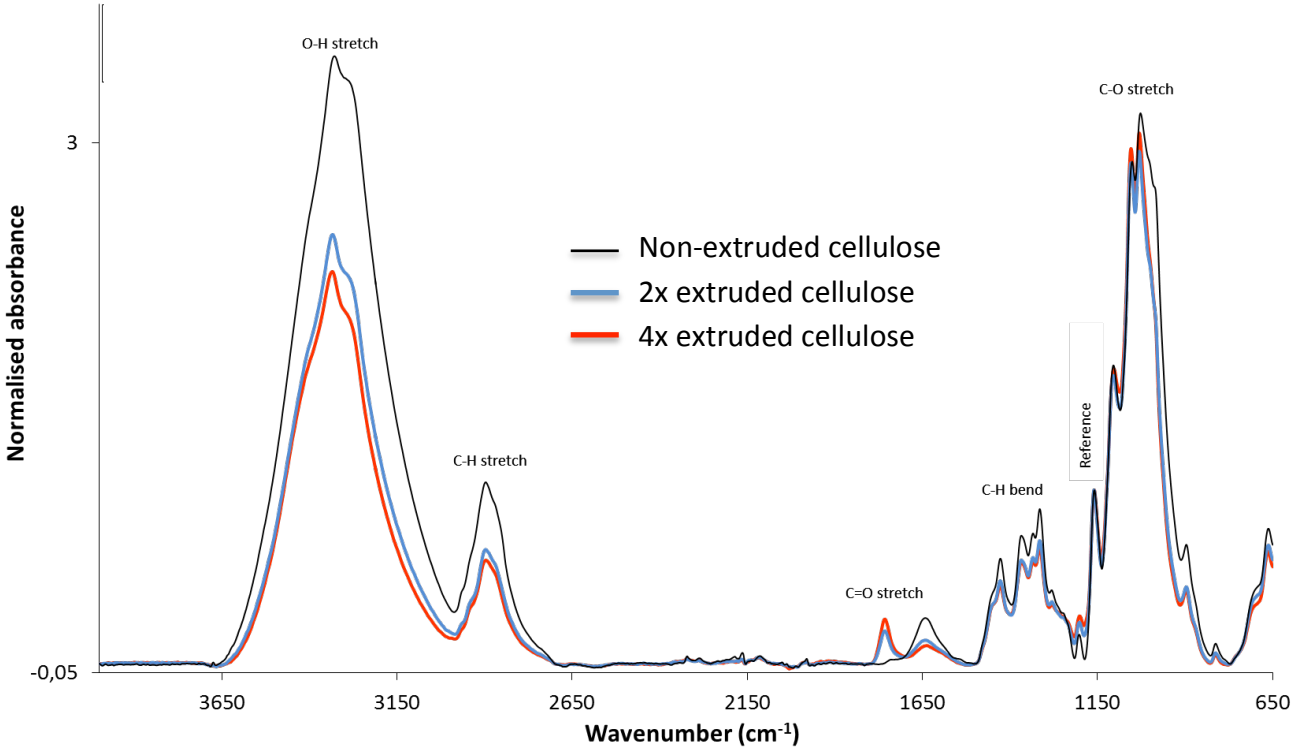

Figure 18 - Fourier Transform Infrared Spectroscopy

For the increased number of re-extruded material CO groups increase while OH groups decrease since the spectrum is normalised against 1160 cm^{-1}, which is the band corresponding to the glycosidic bond in cellulose. The analysis shows that re-extruded material has reduced particle size and the bond between glucose units break. It results in a higher order of the cellulose as the lateral order index (LOI) in Figure 19 show. 2x extruded and 4x extruder has a higher order of the fibers, a more compact material that correspond with the results from the particle measurement. That indicates that the amorphous

structure is removed with the chloroform that removes PLA and the ordered structure is enriched. To test that properly, a reference of a non-extruded cellulose sample should have been extracted in the same way as the extruded CPLA.

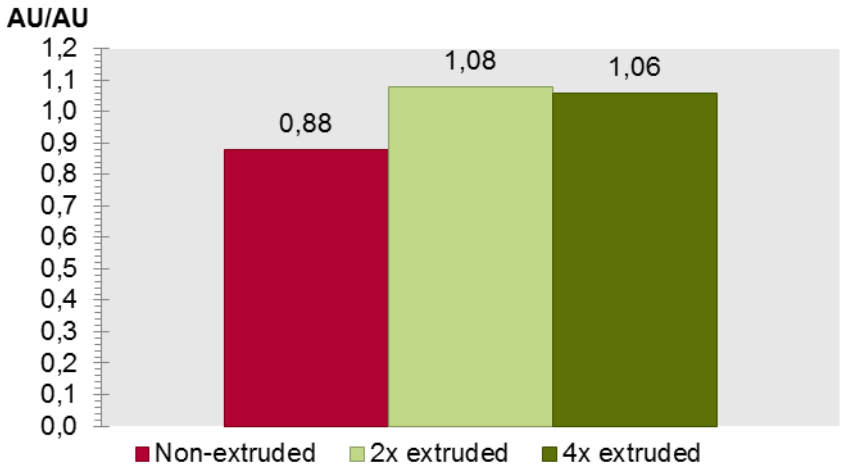

Figure 19 - Lateral Order Intex

4.1.3 PROTEIN ADHESION

The mandible implant should regenerate bone through silk protein that induces bone cell growth and the adhesion between the scaffold material and protein is therefore crucial. The tested proteins are the commonly used laboratory protein Bovine Serum Albumin (BSA) and two different silk proteins from the silk worms B. mori and A. assama. The intention was to also include spider silk protein but the availability of the material made that difficult within the scope of the master thesis project. The methods used were element mapping with Scanning Electron Microscope (SEM) and staining of protein with Coomassie G and Rhodamine B followed by visible indication of protein.

4.1.3.1 Indication of protein through mapping of nitrogen and sulphur

Nitrogen and sulphur are elements of protein. Proteins consist of long chains of amino acids with connecting peptide bonds. Nitrogen is present in all amino acids while sulphur is present in the amino acids methionin and cysteine.

BSA protein and proteins from B. Mori and A. Assama were compared and the initial test was to detect the proteins on a piece of glass with mapping of nitrogen. Nitrogen is not present in glass and was detected and visualised as the bright red areas within the blue circles shown in Figure 20. The protein accumulated at the edge of the solution for BSA and B. Mori. That could depend on the surface tension of the solution or the solubility of the protein in

deionized water in this case. For A. Assama the protein distributes more evenly over the surface and that is preferable when a scaffold of CPLA should absorb the protein solution.

BSA (laboratory protein) B. Mori (silk protein) A. Assama (silk protein)

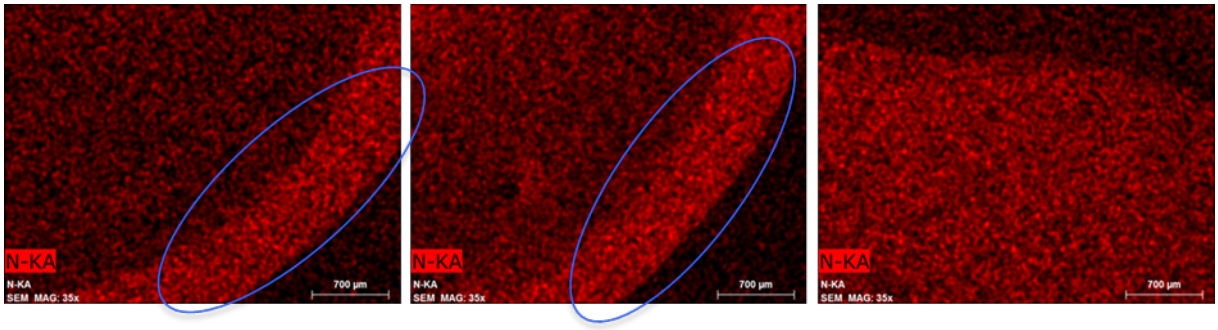

Figure 20 - Mapping of nitrogen on the edge of dried protein solution with 30x magnification, the measurement line shows 700 µm. The blue circles indicate the accumulation of the protein.

The test series in Figure 21 show protein solutions adsorbed on cubes of CPLA. Similar to the drops on glass were that a slight accumulation of the protein BSA and B. Mori were indicated while A. Assama seems to be absorbed by the cube of CPLA. It is preferable for the protein to be absorbed into the structure in order to attach inside the CPLA scaffold. In that way the regenerated bone might have a better chance to grow in an even 3D structure that can give better load bearing properties.

BSA (laboratory protein) B. Mori (silk protein) A. Assama (silk protein)

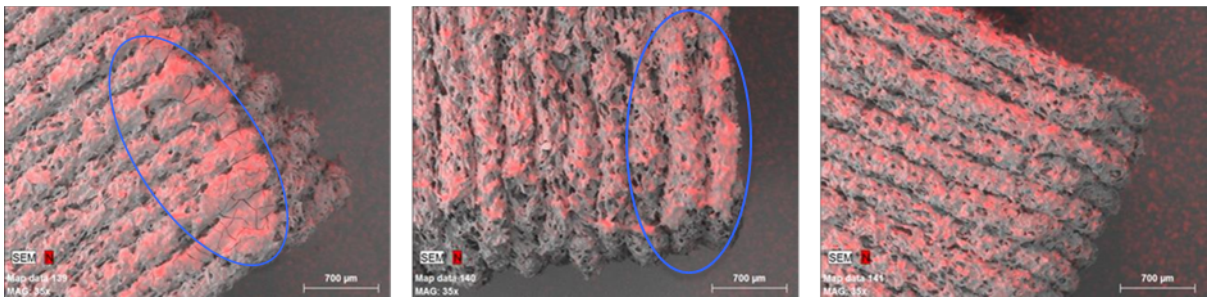

Figure 21 - Mapping of nitrogen on the edge of dried protein solution on a cube of CPLA with 30x magnification, the measurement line shows 700 µm. The blue circles indicate the accumulation of the protein.

3D printed cubes of CPLA were soaked in protein solution from B. Mori. They were compared with specimens soaked in protein-free solutions. The presence of protein was visualised using dye solutions of Coomassie G and Rhodamine B. The cubes and a piece of pure silk were put in the dye solution and then rinsed thoroughly with buffer to remove unbound dye.

Coomassie G is specialised in detecting general protein while Rhodamine B binds specific to silk protein. There was no noticeable difference with Coomassie G staining if a cube of CPLA contained protein or not. When the excess dye of Rhodamine B was rinsed there was a difference in colour between the cubes with or without protein. Rhodamine B is also

fluorescent and fluorescent microscopy would be a good complement to what is seen with the naked eye. The microscope uses fluorescence detection to study properties of organic compounds.

4.1.3.2 Indication of protein through protein staining

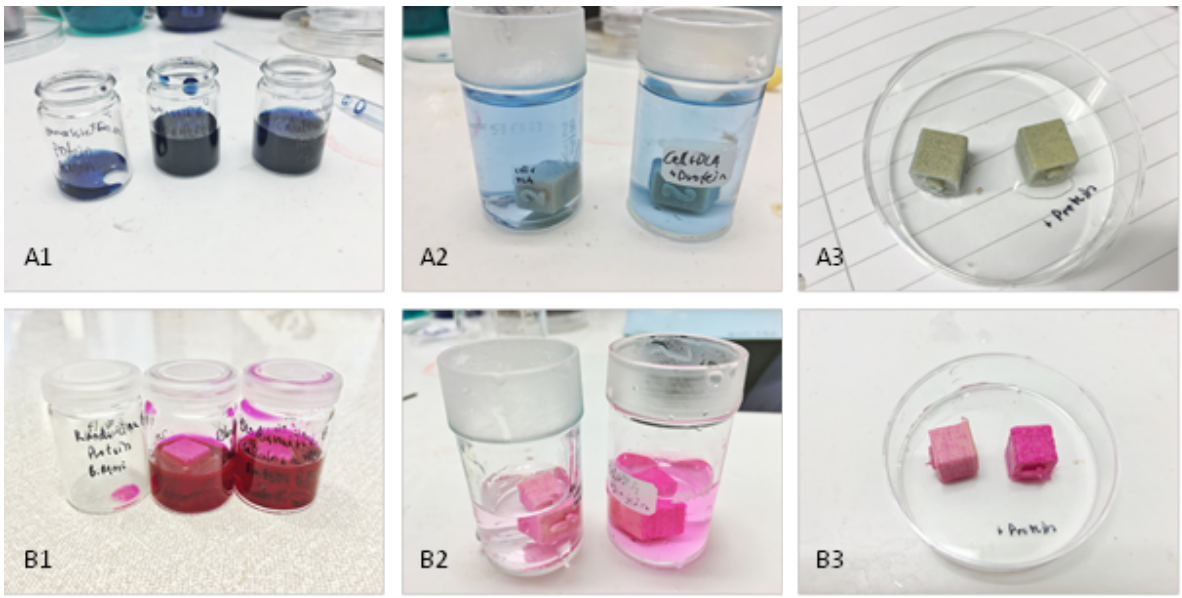

Figure 22 - Test adhesion of silk protein from B. mori on 3D printed cubes of CPLA with protein staining. The A series is dyed with Coomassie G while the B series is dyed with Rhodamine B. (A1) and (B1) show soaking of a small piece of silk protein, one cube coated with silk protein and one cube without protein. (A2) and (B2) show the rinse process of dye solution with PBs buffer. (A3) and (B3) show the rinsed cubes and the visual detection of protein with Rhodamine B.

4.2 3D print

The development phases from material manufacturing to 3D printed final demonstrator are presented as:

1. Initial 3D printing
2. Test specimens
3. Mandible
4. Cranium

Commercial PLA was used for the initial 3D prints, the references of test specimen and for the cranium. The commercial PLA and CPLA have softening agents and other additives to ease the 3D printing. Commercial CPLA were used as a reference to all 3D prints of the in-house fabricated CPLA.

Figure 23 - 3D printed cubes with two, four and six times extruded material.

4.2.1 Initial 3D print

Initial testing with the 3D printer was done during the Fuzzy Front End of the project. That

included learning the Cura 3D printing slicing software and how to tune the Ultimaker for optimal resolution, see chapter 3 Experimental. When the print of commercial CPLA was satisfactory and the in-house CPLA was ready the tuning of the 3D printer for re-extruded CPLA began. The more re-extrusions the filament was subjected to the more brittle it became. Figure 23 show 1 cm^3 sized cubes and the difference of 2x, 4x and 6x extruded CPLA. As the filament became more brittle it also became more homogeneous. With a finer filament the prints got sharper and easier to control but also denser and the natural porosity of the material was reduced.

4.2.2 Test specimens

Test specimens were made to test the mechanical properties of the 3D-printed materials PLA, CPLA and Woodfill (commercial CPLA). The commercial filaments PLA and Woodfill needed no extra tuning of the 3D printer while the in-house manufactured CPLA were more difficult to print due to the filament being brittle and broke during 3D printing. The stiffness broke the filament into small pieces that resulted in an uneven flow and therefore an uneven print. Figure 24 shows six specimens for tensile test, the first three are printed in transparent PLA with different printing angle, the later three is printed with Woodfill, 2 times extruded CPLA and 4 times extruded CPLA. The specimens for compression test and impact test were manufactured in the same way and with the same obstacles.

Figure 24 - 3D printed specimens for tensile tests

4.2.3 Mandible

A 3D printed mandible was developed to make a realistic demonstrator and the beginning for a proof of concept. This phase started with an open source CAD model of a mandible in miniature with a basic material, proceeded with Woodfill and finally with a full size 3D scanned mandible of in-house manufactured CPLA.

Figure 25 - 3D printed mandibles in PLA

The first print was with an open source model with commercial white coloured PLA and nozzle size 0.4. Figure 25 show the same CAD model before and after the 3D printer was tuned with the right settings

The next print was with the same model but with the material to Woodfill, commercial CPLA. Woodfill consists of 30% wood fibers that easily get stuck in the small nozzle of the 3D printer. The mandible was printed with nozzle size 0.6 and the surface finish is not as good as for the mandible in PLA.

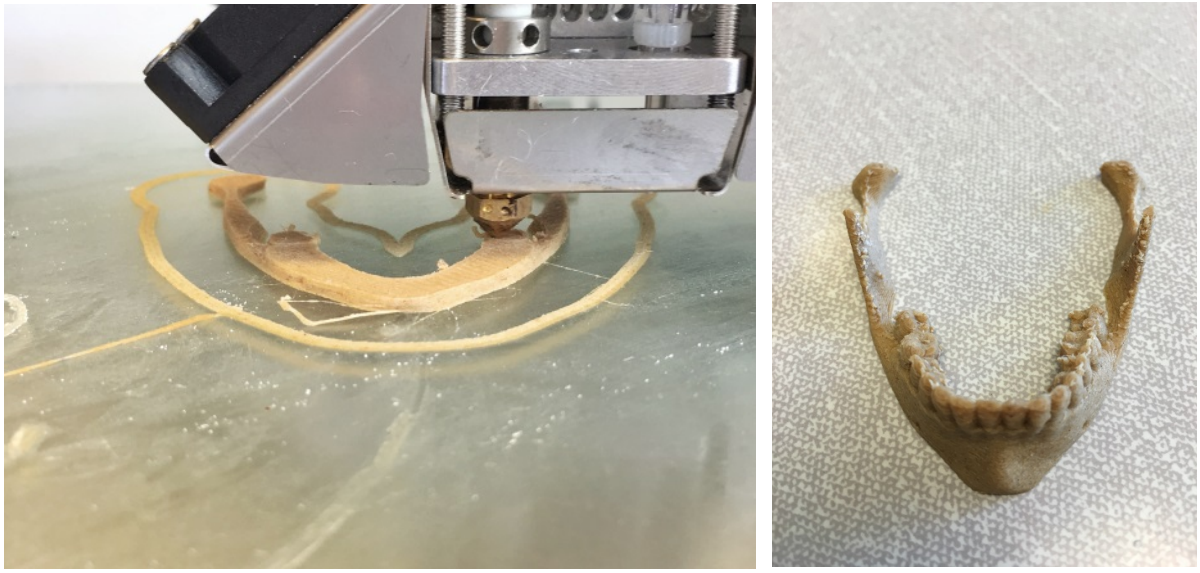

Figure 26 - 3D printed mandible in Woodfill

The third round was a scanned 3D model given by Ortoma. The scanning of a human mandible is not risk free due to the radiation. The soft tissue in the temporomandibular joint is extra sensitive and therefore never scanned if not necessary. The models in Figure 27 are printed in Woodfill with nozzle sizes 0.6 (miniature) and 0.4 (full size).

Figure 27 - 3D printed real mandible of Woodfill, miniature and full size

4.2.4 Cranium

An open-source CAD model of a cranium was used for the final demonstrator to look authentic. The model was divided into three parts for optimal finish because of the complex bone structure inside the cranium. The cranium was printed in Pearl White PLA to mimic bone as a part of proof of concept, to show that the 3D scanned mandible fit in a human skull, see Figure 28.

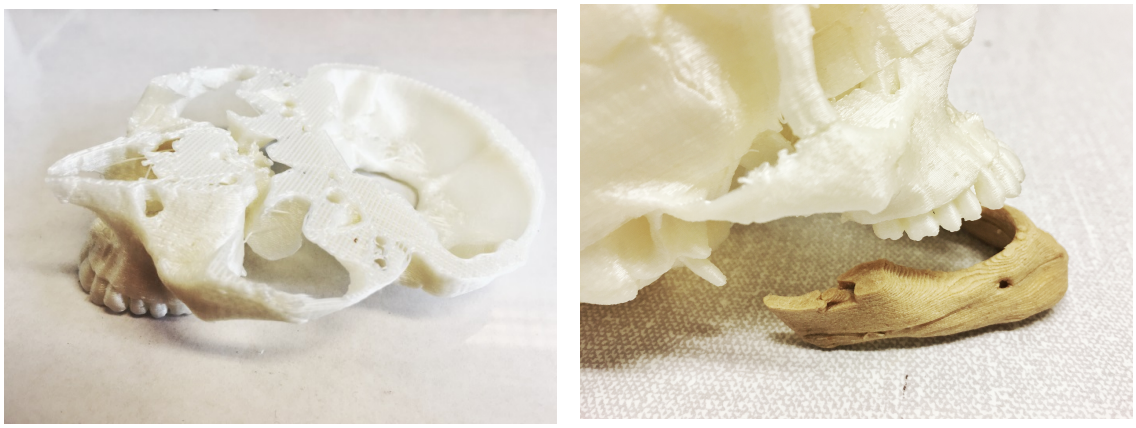

Figure 28 - Details of cranium in PLA and mandible in 4x CPLA

4.3 Stakeholders

Stakeholders are mapped to analyse the future for a mandible implant of CPLA and silk protein for bone regeneration. Five interviews were conducted and their common knowledge on the subject clarifies the market for such a product. The interviews together with literature map stakeholders and potential contacts to pursue the product development.

4.3.1 Interviews

The interviewees are professionally connected to implants, orthognathic surgery, bone regeneration research and MedTech innovations. They gave guidance to what to explore

further within given subject and here follows relevant information from the interviews to this project. For interview structure see Theory.

4.3.1.1 Matts Andersson

Dr. Matts Andersson is the innovator and founder of Ortoma, Chief Executive Officer and Chief Technical Officer in the company (Ortoma 2016). Ortoma provide controllable and individualized treatment solutions to help surgeons with pre-operative planning and navigated surgery through 3D scanning technology. Andersson is well known and acknowledged for his achievements in dentistry as innovator of the Procera system, a world leading trade mark and business at Nobel Biocare. Andersson is also Adj. Professor at Chalmers University of Technology and has formulated product development projects together with Olivia Sjöblom and other students.

- The market for 3D printing and 3D scanning is exploding, especially in dental care with Computer Aided Design (CAD). Half of all reconstructions go through a computer. Also a large market share within cancer related reconstruction. Ortoma has a 3D printer in-house.
- Bone growth is more common when you grow the bone in a more aerated location in the body than on site.
- A difficulty with mandibular cancer and bone regeneration is that you want to remove the tumour as early as possible but have nothing to repair with while the new bone grow in another part of the body. The idea of this thesis is therefore excellent for that purpose; to manage the added stress while building up the new bone.
- All manufacturers of implants in Sweden are foreign; Streiker, Biomet, Johnson&Johnson, Link etc. Sweden has to improve its ability to take market shares. You can compare with Switzerland, they are not as good as Sweden on R&D but more capable to get market shares.
- The most used material for mandibular reconstruction is with bone from the hip. The largest obstacle is to get good circulation. Other material used is Titanium.
- Difficulties are rejection and to maintain viable circulation, especially for bone regeneration.

4.3.1.2 Björn Lovén and Christian Thunborg

Björn Lovén is Innovation Coordinator and Christian Thunborg is Chief of Product Development at SLL Innovation (Stockholms läns landsting, County Council of Stockholm), Danderyd University Hospital.

- SLL innovation has two different 3D printers; one ordinary for prototypes and demonstrators and one new Stratasys with poly jet technology. The Stratasys can print several materials at a time and biocompatible material.

- Incentive to buy a 3D printer:
  1) In house manufacturing, co-workers at SLL or Danderyd University Hospital. Need in house manufacturing to test a product for CE certification.
  2) External customers, mostly start-ups, to print prototypes/demonstrators to communicate a potential product to customers, rapid prototyping.
  3) Direct use, customize existing products.
- A surgeon in the Netherlands has printed a transparent cranium and a surgeon in China has printed a vertebra.
- The next challenge for 3D-printed medical equipment is to be able to print a combination of materials with silicon. Prostheses for children need to be updated as the children grow – a perfect alternative with 3D printing.
- Their printer has only one biocompatible material, which is MED610 and can be in contact with skin for up to 30 days. There are difficulties with the sterilisation of 3D printed products, more so with the support material. Water-based support material is developed to ease the sterilisation.
- SLL Innovation assists in a start-up phase and before CE certification. Scientists are not needed in the same extent as they used to, more important with product developers that can mix existing solutions and knowledge into new products. SLL Innovation work with medical clinics, clinical trials, doctors, surgeons, nurses, customers etc. to do a risk analysis.
- Develop mostly products, sometimes processes, and tries to see the entire cycle.
- Maybe 3D printing grows in Sweden's dental business because of the larger percentage of privatisations compared to other businesses, which result in a more innovative climate.

4.3.1.3 My Hedhammar

My Hedhammar is the founder and R&D director of Spiber Technologies. Spiber research and develop artificial spider silk and its areas of application.

- Spiber supplied this project with silk protein, methods for testing and provided information about the properties for silk protein.
- Spiber has conducted equal experiments and discussed the SEM pictures from this project that have similar results.
- There are different opinions concerning additives, some scientists claim that a minimum of additives is preferable since the body can reject the implant if it is too complex.
- Spiber have tested their silk protein subcutaneous on rats and it takes about 3 months for the protein to be absorbed.

4.3.1.4 Linda Forsberg Pettersson

Linda Forsberg Pettersson is a maxillofacial surgeon and researcher on mesenchyme stem cells for bone regeneration at Umeå University, County Council of Västerbotten and University Hospital of Umeå.

- Forsberg Pettersson described her research on mesenchyme stem cells and reconstructive maxillofacial surgery.
- The golden standard of today is free vascularised flaps – bone with soft tissue and blood vessels. Titanium rails are also used for reconstructions.
- There are trials in Finland where they grow bone in a muscle first, then insert the grown bone where it is needed.
- Their research with Peyman Kelk is now in a phase where they need a scaffold material for the stem cells. This opens up for collaboration since Innventia specialises in material science and development.

4.3.1.5 Sjoerd Haasl

Sjoerd Haasl is Act. Dir. CTMH (Center for Technology in Medicine and Health) and Clinical Innovation Fellowship.

- The process for a new medical technical product divides into two possible paths:
  1. The official way through research, laboratory testing, clinical trials and CE-certification
  2. Enthusiastic entrepreneur/researcher/surgeon that show a proof of concept and form a multidisciplinary team to develop the product
- Haasls recommendation: Compose a proof of concept, start clinical trials and patent quick. To publish invention can kill innovation force due to no earnings; it is better with licences or patent. Important to gain/keep market shares.
- A reversed process is beneficial, to find a need then look for a solution.

4.3.2 MedTech Development Process

When putting a medical technical product on the market it needs to be taken through certain steps in order to ensure patient safety. The Swedish Medical Products Agency (Läkemedelsverket) is responsible for supervision of existing laws and regulations and approval of medical technical products. The vast majority of medical products are purchased by the Swedish county councils and therefore regulated through the law (2007:1091) regarding public procurements. There are different standards from the International Organization for Standardisation, ISO, on how to collect the right information and documentation to be able to get approved for clinical trials. Läkemedelsverket inspect the collected information and method for the clinical trial before giving clearance. When the product is new or includes a new material the clinical trials are crucial to get the final certification, Conformité Européenne, CE mark.

A mandible implant counts as a class III product: "Examine, amend or replace anatomy or physiological process". A medical technical product needs to fulfil the essential demands from Läkemedelsverket and EU. They contain general demands, for example demands that the product will not risk the patients' clinical state or safety. There are also demands regarding manufacturing, production, identification and instruction manuals to eliminate risks in all areas. An authorized third party, Notified Body, is thereafter involved in the conformity assessment procedure. For products like mandible implants that are developed by a company without medical expertise, the Notified Body is involved during the process and consults the project from clinical trials to CE marking. Notified Bodies for implants in Sweden are SP Sveriges Tekniska Forskningsinstitut AB or Intertek Semko AB. When these have verified that the product follows all laws and regulations, the product is ready to market within the European Economic Area (EEA).

4.3.3 Competitors

Manufacturers of implants for the Swedish market are Streiker, Biomet, Johnson&Johnson and Link. They are all large foreign companies with large market shares. Cooperation with (university) hospitals, surgeons, researchers within the field and suppliers is needed to be able to market and develop a mandible implant. It needs to be a multidisciplinary team to drive the product development further.

4.3.4 Stakeholders for A Mandible Implant

Composition and examples of stakeholders to create a multidisciplinary partnership for development of mandible implants:

Supplier	Spiber, Ortoma
Customer	Hospitals
Regulatory & CE certification	Medical Products Agency & Notified Body
Clinical studies	Hospitals, Swedish MedTech
Research & Proof of concept	Innventia, Spiber, University Hospital, SLL Innovation
Multidiciplinary development	Scientist, entrepreneur, PD, county, surgeon

5 Conclusion

A bone regenerative 3D printed mandible implant of CPLA and silk protein may be a suitable material composition in the future for the market of mandible implants.

5.1 Biomaterial

- A test specimen made of cellulose and PLA can withstand well beyond the maximum masticatory force for a human mandible on 700N. It indicates that an optimised scaffold of a mandible implant also can withstand the forces while bone is regenerated.
- Pure PLA has better tensile and impact properties than CPLA due to the 3D printed layers are better fused together with a pure thermoplastic polymer. CPLA has better compression properties than PLA due to the increased stability with a fiber strengthen composite. Since compression is the most important mechanical property for a mandible implant a scaffold of CPLA is preferable.

5.2 3D printing

- During the material manufacturing process and re-extrusion of the material the cellulose fibers gets broken down into shorter chains and can thereby more easily get through the nozzle of a 3D printer. The precision of a print can thereby increase and possibilities as controlled 3D printed porosity can be investigated. With every re-extrusion the material gets more brittle which is undesirable.

5.3 Stakeholders

- The Swedish market is open for a new mandible implant since the existing solutions are not satisfying enough. Regenerated bone is easier for the body to accept than a foreign object and this concept can work on demand with no extra lead-time for bone to regenerate elsewhere.
- Collaboration between SLL Innovation, Spiber and/or researchers at the University Hospital of Umeå can lead to a continuation of the research and development for a bone regenerative 3D printed mandible implant of CPLA and silk protein.

6 Future work

	PLA and its copolymers
	Simulated body fluid
Next step	Topology optimization
	Biomimic, porosity
	Proof of concept

PLA and its copolymers need further investigation to see which material is most suited for in vivo trials. Test CPLA for other mechanical properties such as shear force and fatigue tests is also important to explore to what extent cellulose is relevant in a scaffold material.

One step closer for a proof of concept is to evaluate the material in Simulated Body Fluid to see how the material behaves inside the body. See procedure in Appendix 9.3.

To optimise the strength of a mandible implant one can use topology optimisation on a CAD model of a mandible. It will by Finite Element Method (FEM) calculate how to redesign the material layout for a given set of loads. Software programs to explore are Ansys, Solid Works and Autodesk within medical. There is research for the bridging of topology optimization and additive research that needs further investigation.

Topology optimization with biomimicry could lead to a product better than the real thing. For example a mandible could get a perfect porosity to regenerate bone in an optimal pattern. Porosity is also important to further investigate since the bone needs oxygen and circulation. The porosity can then help to gain a maximum surface for the bone cells to grow on in a minimal space.

The multidisciplinary project TechMark Arena has lead to the identification of common fields of interest between students. During this master thesis project the importance of porosity within different disciplines was identified and would be fruitful to pursue.

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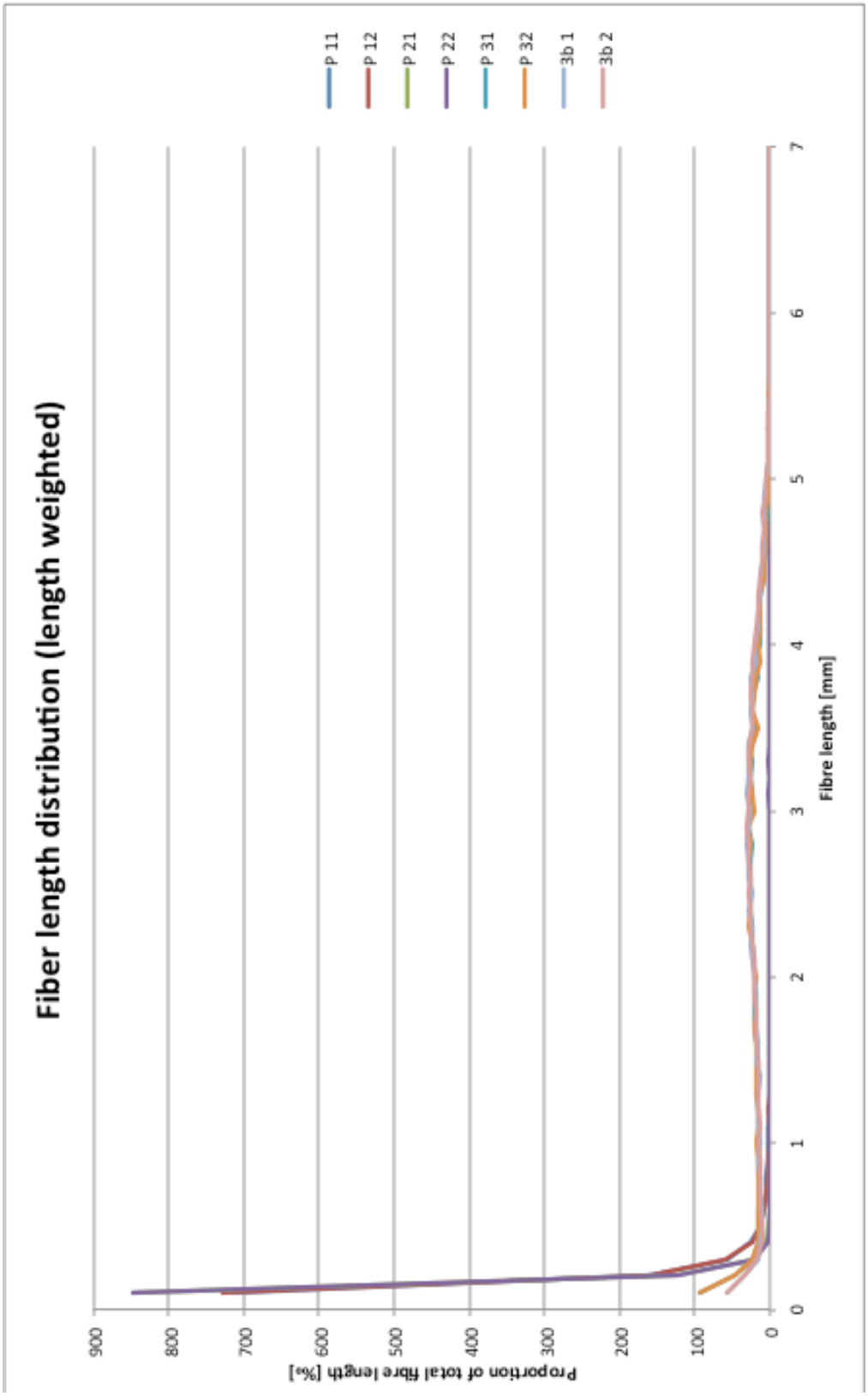
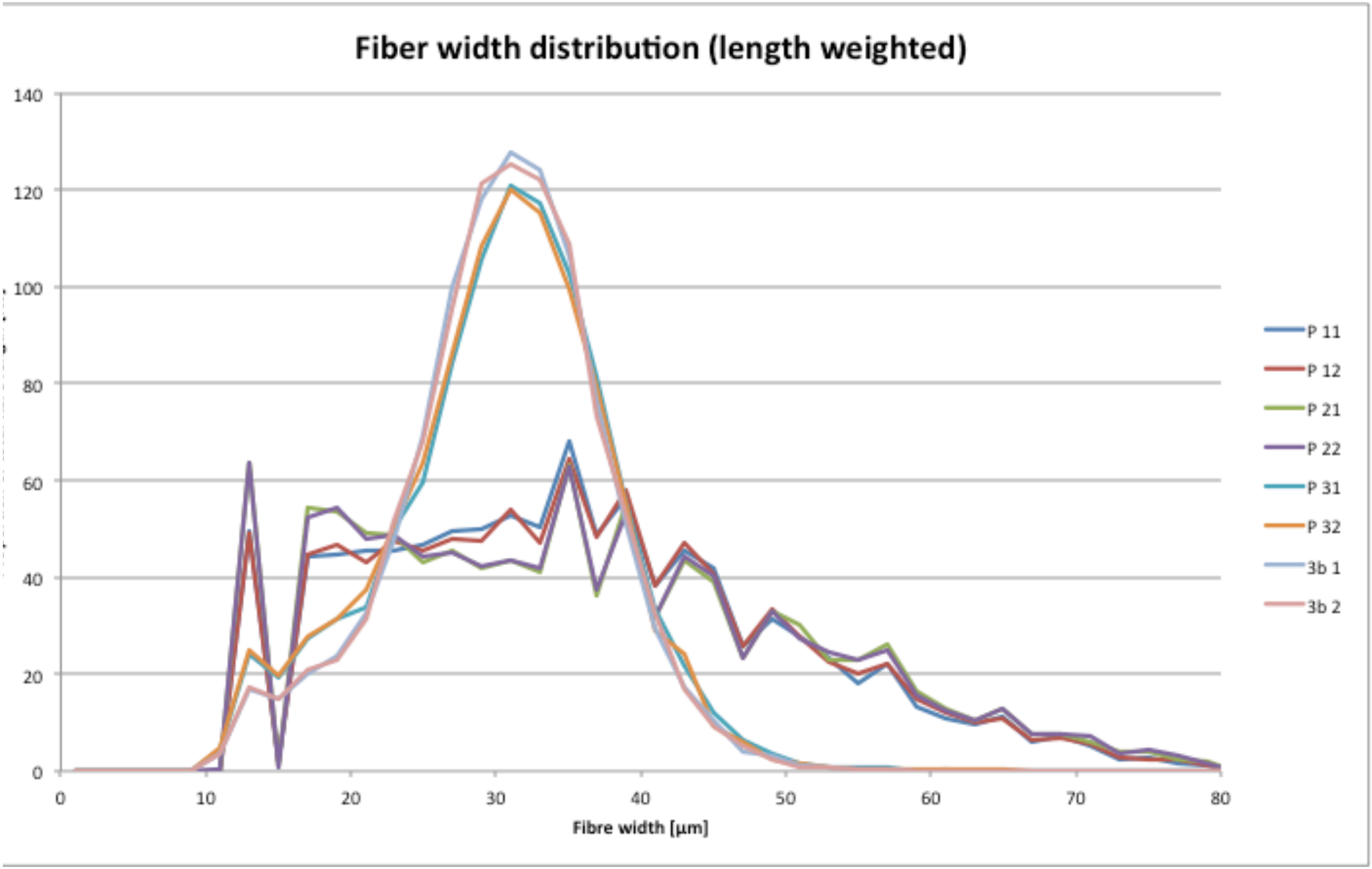
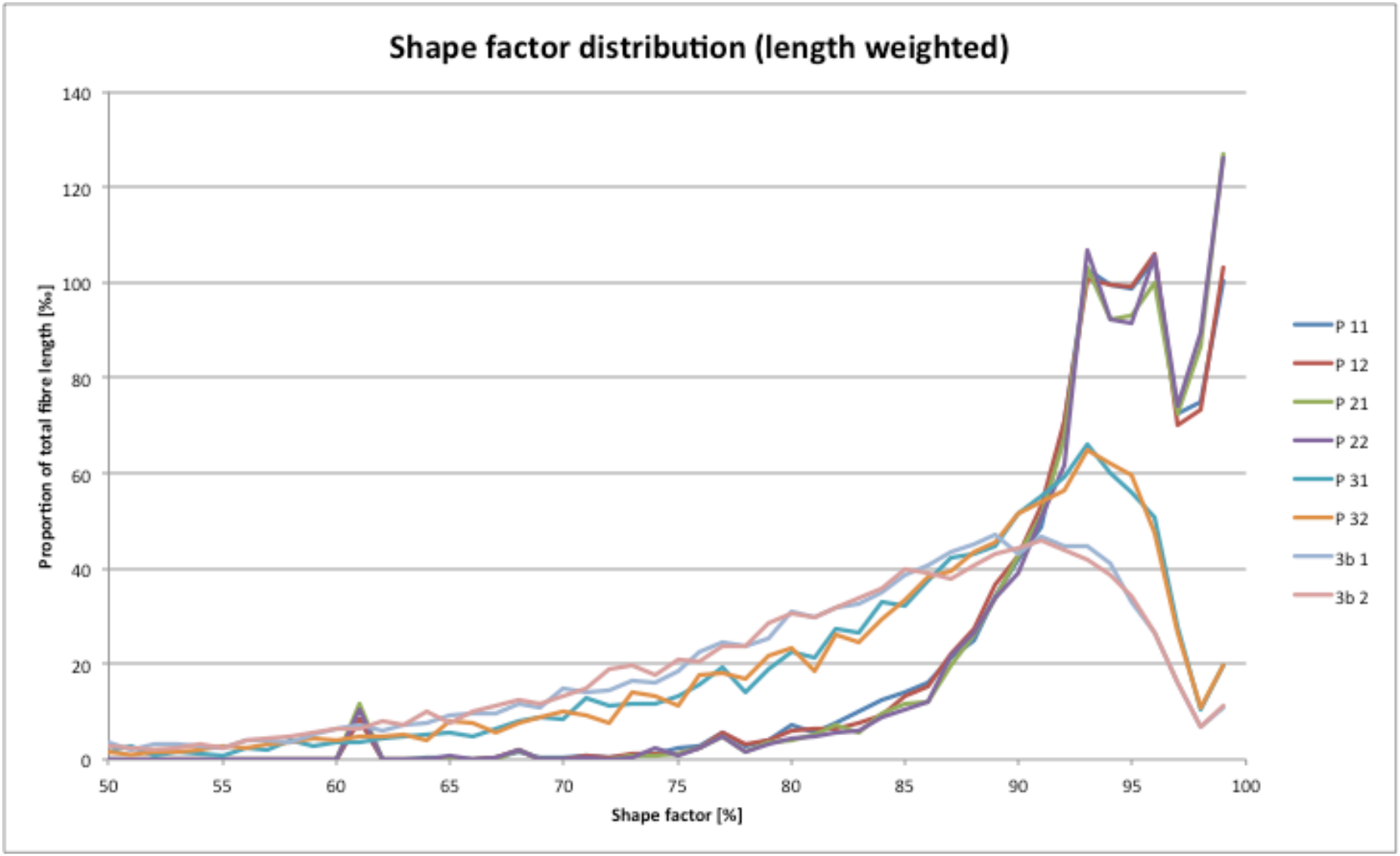
8 Appendix

8.1 Cellulose measurement from FiberTester




Fiber width distribution (length weighted)
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
Fibre width [µm]
P 11
P 12
P 21
P 22
P 31
P 32
3b 1
3b 2


Shape factor distribution (length weighted)
Proportion of total fibre length [‰]
140
120
100
80
60
40
20
0
50
55
60
65
70
75
80
85
90
95
100
Shape factor [%]
P 11
P 12
P 21
P 22
P 31
P 32
3b 1
3b 2

iii

8.2 Cellulose fiber functionality with FTIR