Soft and hard implant fabrication using 3D-Bioplotting™

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Abstract

At the Freiburger Materialforschungszentrum we have developed a new process (3D-Bioplotting™) that permits most kind of polymers and biopolymers to be used in 3D scaffold design, including hydrogels (e.g. collagen, agar), polymer melts (e.g. PLLA, PGA, PCl) and two-component systems (e.g. chitosan, fibrin). Cells can be incorporated within the construction process, making this an ideal Rapid Prototyping technique for Organ Printing. Tailor-made biodegradable soft or hard scaffolds can so be fabricated in a short time using individual computer-tomography data from the patient. In-vitro tests showed promising results and in-vivo experiments are now under observation.

Introduction

With an increase of computer power during the last years, new ways of creating prototypes have increased in importance, leaving behind the times where prototypes had to be created by hand. 3D construction software, denominated Computer Assisted Design (CAD), can shape an idea as a virtual model. The main application of Rapid Prototyping is to aid the visualization of a 3D structure (Concept Modeling[1, 2]). With increasing demand for customized parts, the construction of 3D objects to aid the fabrication of machine parts, for example in the creation of molds, has gained in importance (Rapid Tooling[3, 4]).Today, with improving mechanical properties and decreasing production time, “ready for use” parts can be made using Rapid Prototyping techniques to create working models (Functional Modeling[5]).A more recent application for Rapid Prototyping techniques lies in the medical field (Tissue Engineering). Rapid Prototyping has started to be tested for the construction of titanium plates[6], drug releasing systems[7] and support structures for bone and nerve defects[8].

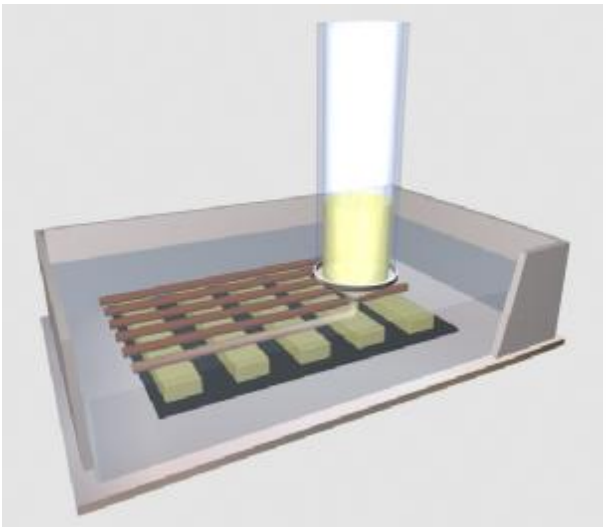
A great number of different Rapid Prototyping techniques have emerged for solid free-form fabrication during the last years[9-12]. These techniques can be distinguished by the materials used to build the layers of the object as well as the method of hardening. The material used to build each layer can be in the form of a foil, a powder, a slurry or a liquid film. The hardening of the object can be achieved by means of temperature changes, solvents, light emission or chemical reactions.

The main techniques are 3D Dispensing, Selective Laser Sintering (SLS), Laminated Object Manufactering (LOM), Stereolithography (SLA), 3D Printing and Direct Write/Direct Printing. All these techniques are essentially restricted to the type of monomer or polymer it can use, and usually have only one or two methods of hardening. Most conventional techniques do not permit handling of a wide range of bioactive components which are temperature sensisitive and require exclusively non-toxic chemicals.

At the Freiburger Materials Research Center we have developed a new technique of 3D dispensing as a new tool for bioactive polymer processing (3D-Bioplotting™ that allows the use of a large variety of materials, including a wide range of biopolymers and bioactive components, as well as a wide range of solidification methods for Rapid Prototyping purposes[13].

3D-Bioplotting™

This new technique, 3D-Bioplotting™, uses a pressure-controlled dispenser movable in all three dimensions. This way, 3D objects can be built, as typical of Rapid Prototyping techniques, layer by layer. Each layer is composed of single strands, which orientation can be varied for each layer. By setting a certain distance between the strands, porous structures can be built.



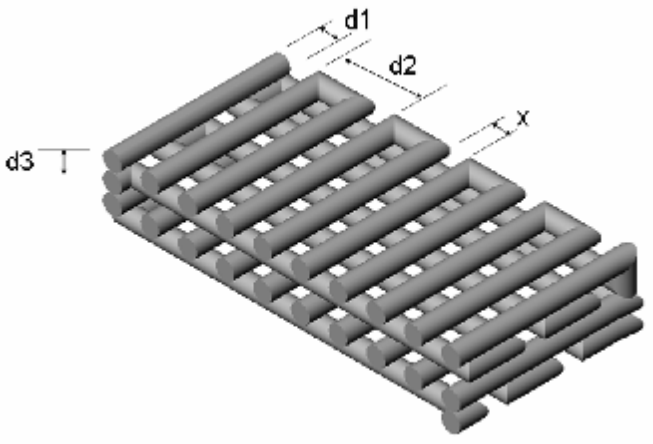


Figure 1 (left): Schematics of the 3D-Bioplotting™ process, building of the first layer
Figure 2 (right): Design of a porous 3D scaffold made through 3D-Bioplotting™

The surface and surroundings onto which the object is built (plot medium) defines the solidification method used. Whereas high-temperature polymer melts can be plotted on a glass plate using air or inert gases, many other materials will need certain substances to increase the solidification speed. A certain liquid plot medium, matching densities with those of the plotting materials, can also be chosen to provide a buoyancy to the plotted layers, so that soft strands will remain in the correct position instead of sacking due to gravitational force. This makes this technique the only, that can produce 3D hydrogel scaffolds in a direct plotting process, without the use of moulds.

Figure 3: Vertical cut of plotted polyurethane scaffolds. The left scaffold was plotted in air, while the right one was plotted in a plott medium (water).

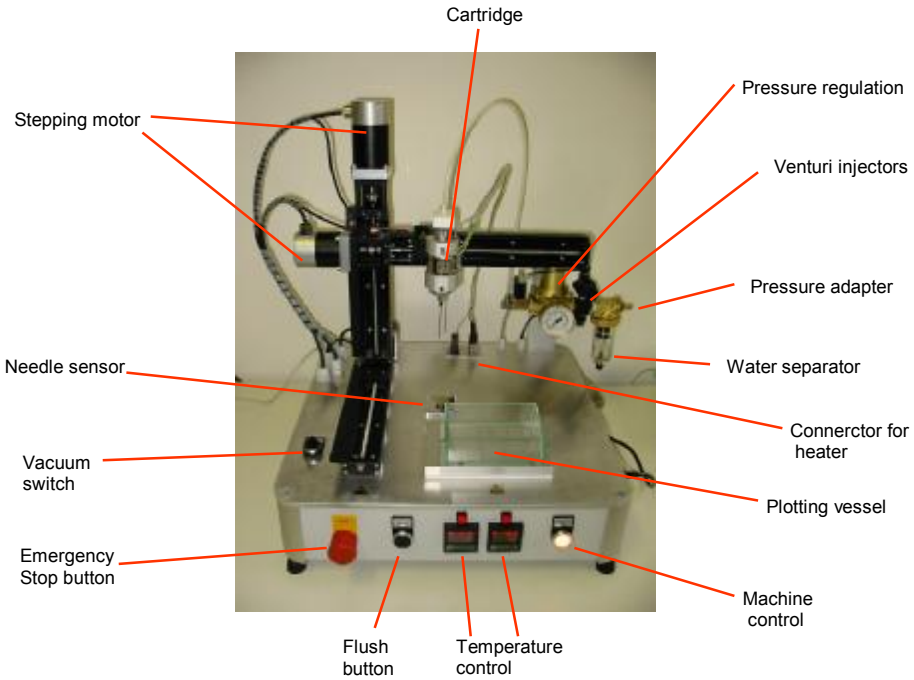


Figure 4: The 3D-Bioplotter at the Freiburg Materials Research Center, constructed by Envisiontec GmbH

We have used 3D-Bioplotting™ to create scaffolds made out of polymer melts, ceramics, monomers and even hydrogels[14]. The hardening methods available to this technique are temperature changes, evaporation of solvents and chemical bonding of monomers and polymers.

Materials	Selective Laser Sintering	Stereo-lithography	3D Printing	Direct Printing	3D Dispensing	Laminated Object Manufacturing
Metals	+	-	+	-	(+)	+
Ceramics	+	+	+	-	+	+
Polymers	+	+	+	+	+	+
Glas	(+)	-	-	-	(+)	-
Paper	-	-	-	-	-	+
Wax	(+)	-	-	+	+	-
Hydrogels	-	(+)	-	-	+	-
Hydrogels + proteins/cells	-	-	-	-	(+)	-

Table 1: Materials used with different Rapid Prototyping techniques (+ = known application, - = not known, (+) = theoreticaly possible, but no application is known)

Our main interest lies presently in the field of Tissue Engineering. Using computer tomograhy data from a patient, a support structure for a defect can be modelled by means of CAD software and then a tailor-made 3D object fabricated using biocompatible or biodegradable materials[15-17]. A decrease in the duration of self-regeneration is beeing tested by cultivating cells from the patient onto the scaffold in-vitro. This will also reduce the risc of rejection.

Drugs can be added to the material, which will be released in-vivo to promote the self-regeneration process or reduce the risc of infection[18].

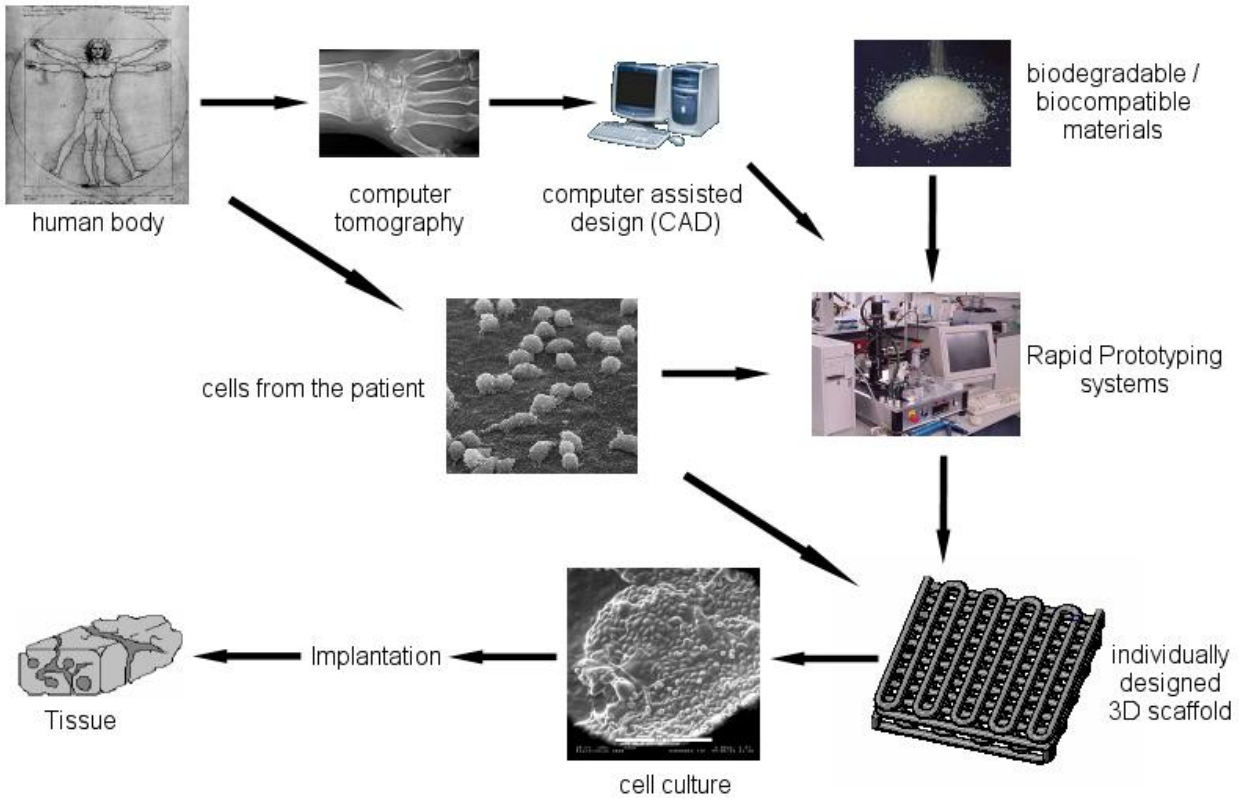


Figure 5: Schematics to the process of creating a scaffold out of data from a patient, cultivating cells onto the scaffold and the growing of new tissue after implantation.

Scaffold design

The design of the scaffold is chosen accordingly to the needs of the medical application, being an important factor in the mechanical properties of the object. Blocks of material have several disadvantages, namely more material on the site which can, during degradation, provoque an infection and lead to slower regeneration time. A porous structure has much more advantages, the surface is much larger, allowing a better controlled degradation of the polymer; cells have more place to grow, allowing a larger concentration of cells per volume. Additionally, the flow of nutrient media is permitted through the gaps between the strands.

While a large variety of designs are allowed for the interior layout of the scaffold[19, 20], we have found that a non-woven cross design has an adequate porosity while having an excellent structural stability. Using this non-woven design, changes in the strand thickness and distance between strands can be adjusted to each particular applicaton. For example, thicker strands with medium sized gaps are best for bone replacement, while thin strands with small gaps between them are needed for nerve regeneration.

We have tested the compressive strength of round PLGA scaffolds (5 mm high, 14 mm diameter) with different gaps between strands. Surprisingly, only few of the scaffolds actually break. After

a certain pressure is applied, the layers start to collapse, the sooner the higher the distance between strands. Most scaffolds end up more flat and wide, but retaining the porous scaffold structure.

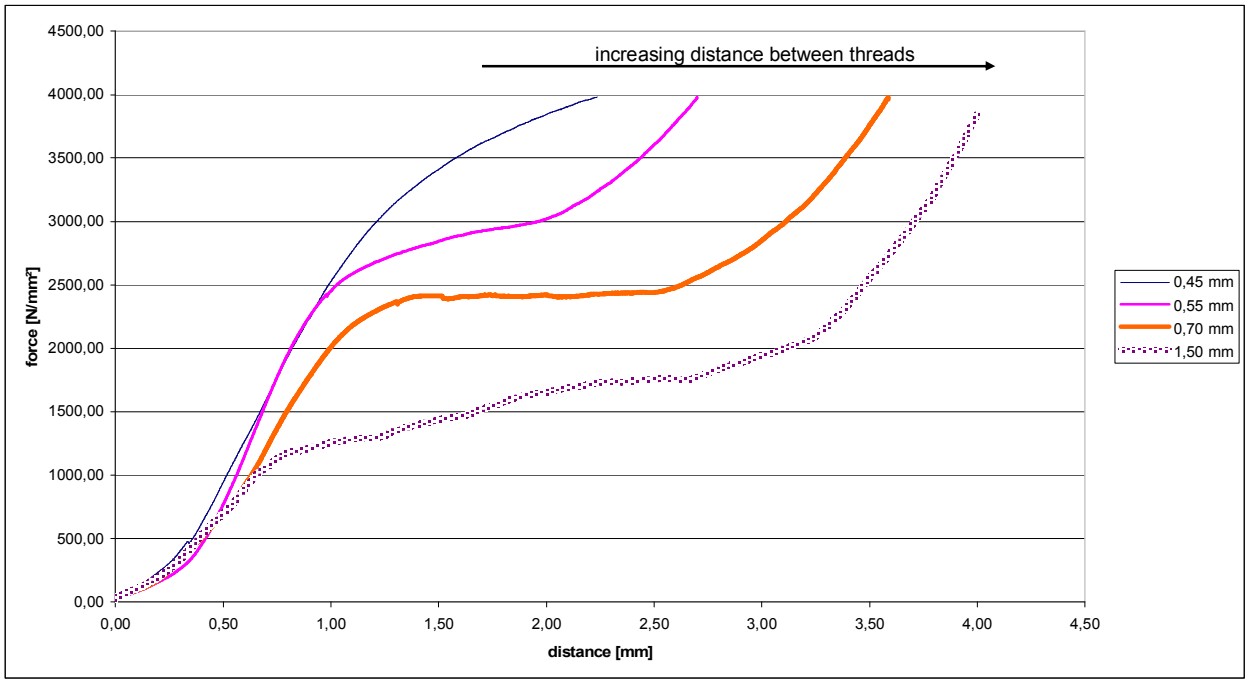


Figure 6: Compressive strength of PLGA scaffolds with different distance between strands

Cell cultivation on 3D scaffolds

On some RP applications, like nerve regeneration or bone defect support, cells can be cultivated on the scaffold to decrease the duration of self-regeneration. Because we use biocompatible materials and biopolymers, the question of the compatibility of the materials used and the cells does not arrise. More important is the question of cell adhesion. Many biocompatible polymers (eg. polycaprolactone) are hydrophobic, meaning cells will not adhere to the scaffold.

If a hydophobic polymer has to be used, a plasma sterilisation will create hydrophilic groups on the surface of the polymer. The polymer surface is modified to have a better adhesion having a positive effect on growth (Figure 7). Another method to have cell growth on hydrophobic 3D scaffolds is obtained by coating of the scaffold with other materials. We have found collagen to be an excellent coating candidate.

The design of the scaffold has also a great impact on cell growth. Very narrow gaps between strands will not permit the flow of nutrient media to the interior of the scaffold, the structure behaves similarly to a compact 3D block of material. A compromise between biological and mechanical properties has to be found for each application (Figure 8).

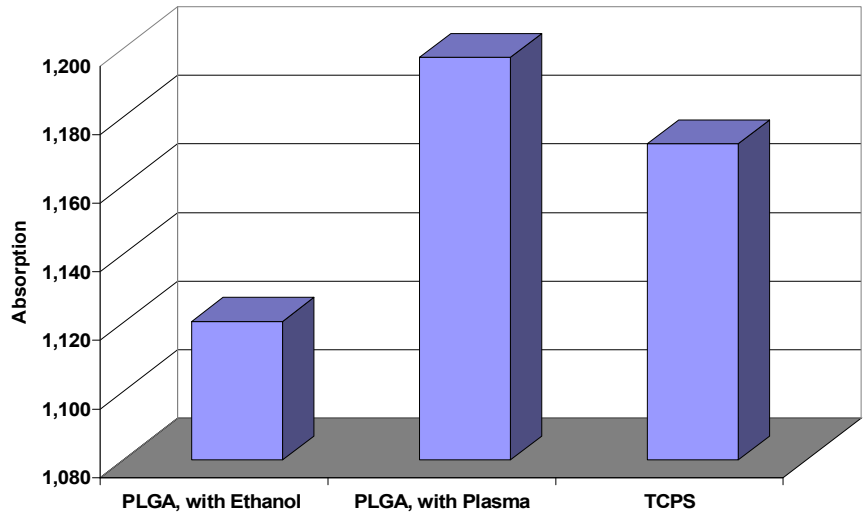


Figure 7: Cell growth on two similar scaffols using different sterilitazion methods. TCPS = no scaffold

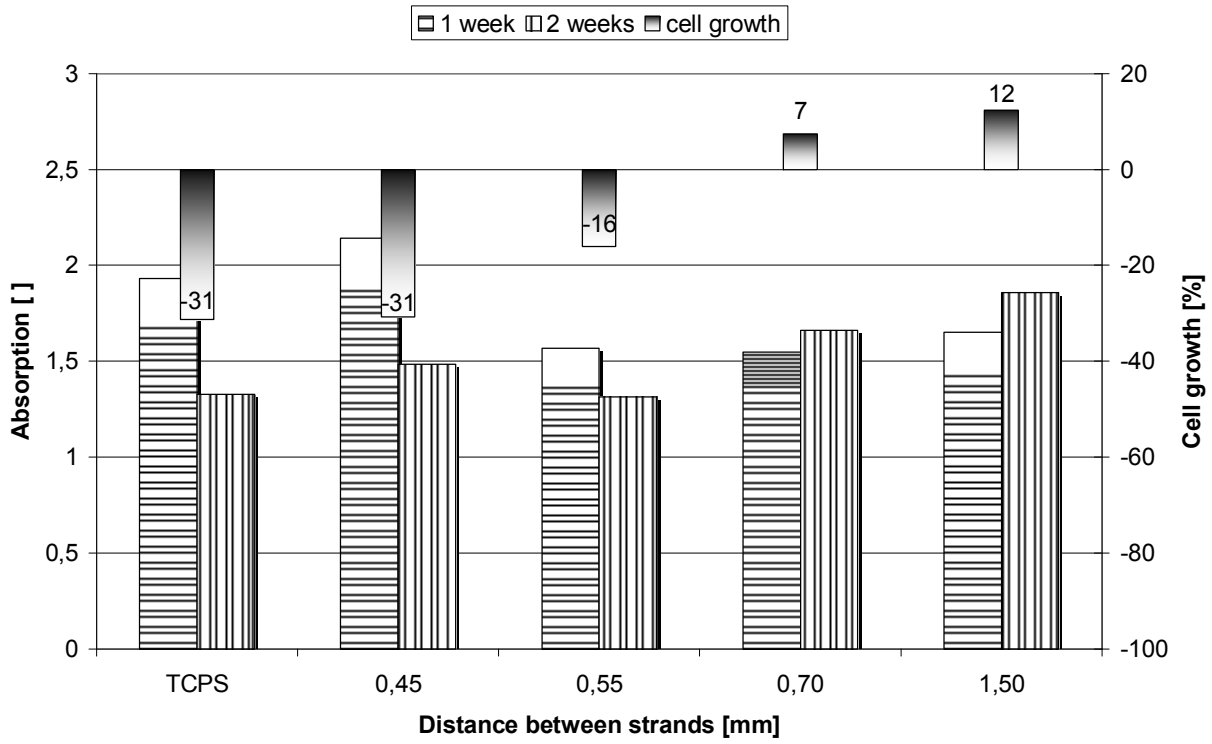


Figure 8: Cell growth of 3D PLGA scaffolds using different distances between strands. TCPS = no scaffold (the numbers indicate cell growth during the second week with respect to that of the first week)

Plotting Hydrogels

One of the challenges of Rapid Pototyping is the fabrication of 3D constructs made out of hydrogels. Few results have been achieved in this area, most use moulds or support structures to create collagen and other hydrogel scaffolds[21].
Hydrogels have a great potencial in biomedical applications using Rapid Prototyping, soft tissue implants could be tailor made to each patient. 3D objects like ears and noses, as well as nerves and arteries could be fabricated with similar mechanical properties to the original parts. The addition of cells to the material previous to the plotting process would allow creating scaffolds with certain types of cells placed in predeterminated positions.

With our technique, 3D Bioplotting™, plotting of hydrogels is possible. Scaffolds of collagen, chitosan, alginic acid, fibrin and agar-agar have already been successfully plotted. The structural design of the scaffolds can be varied like for the scaffolds using harder materials.

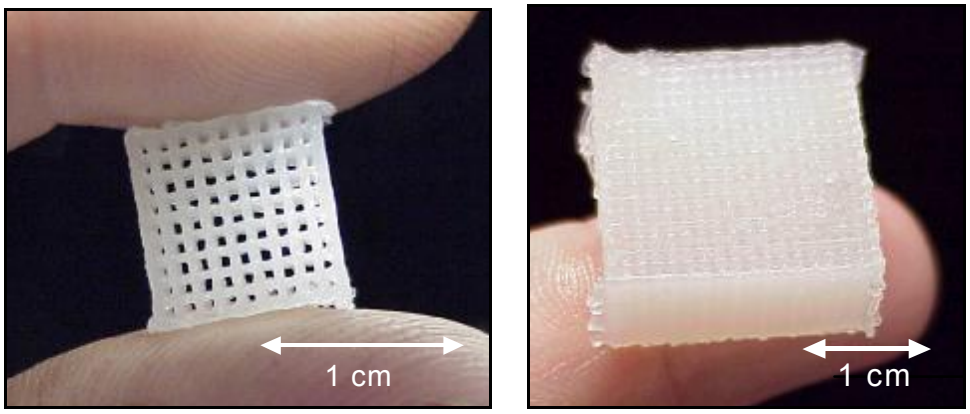


Figure 9: 3D scaffolds made of collagen

Conclusion

We have developed a new technique of biofunctional Rapid Prototyping, which we refer to as 3D-Bioplotting™ and apply for Tissue Engineering. Using this method, 3D scaffolds can be made out of a large variety of materials, incuding polymers, ceramics and hydrogels. The 3D scaffolds, which can be tailor-made from CT data from the patient, have a porous interior design, allowing the flow of nutrient media and the cultivations of cells in the scaffold. Different materials have been sucessfully plotted and many of these allow the growth of cells on the surface. The design of the scaffolds is being optimized to increase the cell growth on them and the mechanical properties of the different designs are beeing investigated. These advantages give 3D-Bioplotting™ a great potential in the field of desktop manufactering for medical applications.

Outlook

Our main goal is the possibility of plotting a scaffold with cells inside of the material. This would allow the plotting of one scaffold with several cell types on it, an important step to Organ Printing. Our first tests have shown that plotting with cells is possible using our technique.Presently we are investigating the bone regeneration on sheep using hydroxy apatite,

PLGA and hydrogel scaffolds. Bone and bone precursor cells have been cultivated on the scaffolds and results of this experiment should be available towards the end of the year.

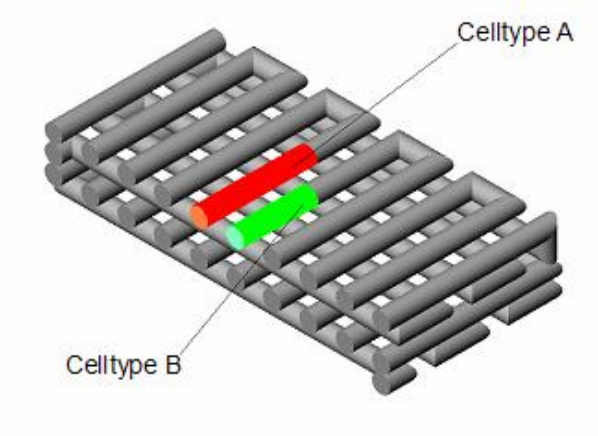


Figure 10: Our main goal: Organ Printing

Aknowledgements

We would like to thank the Stifterverband fuer die deutsche Wissenschaft, the Fonds der Chemischen Industrie and the Deutsche Forschungsgemeinschaft for financial support as well as Professor Schmelzeisen and Ute Hübner from the Universitätsklinikum Freiburg for the biological tests.

Chemicals Used

- PLGA, Boehringer Ingelheim, Resomer LG 824
- Hydroxy apatite, Typ SF mikrofeines Pulver C13-09, Chemische Fabrik Budenheim, Mainz, Germany
- Glutardialdehyde, M = 100,12 h/ml, Fluka 49629, 50% in water
- Collagen, extracted from bovine tendons
- Polyvinylalcohol, M = 145 000, Clariant GmbH, Frankfurt, Germany, Mowiol 28-99
- Acetic acid, Merck, Germany
- EZ4U, Biomedical, Austria
- PBS, PAA, Austria

Plotting of Materials

3D-Plotting of PLGA

The Resomer LG 824 was purchased as a white powder from Boehringer Ingelheim. This powder was filled into a glass syringe and heated to melting point. A slow degradation of the polymer during the plotting process could be seen in the gradual change of the color of the polymer from white to yellow.

During the plotting process, a reduction of the viscosity also takes place due to degradation, for this reason only small amounts of the polymer were filled into the syringe each time. Used syringes were cleaned with dichloromethane before being refilled, to remove all rests of degradated PLA, or new syringes were used.

3D-Plotting of hydroxy apatite with Polyvinylalcohol

A very fine hydroxy apatite with particle sizes ranging between 1 and 10 µm was sieved through a 50 µm sieve on a sieving machine (Retsch, Haan, Germany - Model AS 200 control) producing a powder without larger agregates. A 10 % solution of polyvinylalcohol was made by dissolving the polymere in boiling water. 37,5 g of hydroxy apatite powder were mixed in 62,5 g of the polyvinylalcohol solution and stired firmly for 20 minutes. A white paste with large aggregates is created, which can be pressed through a 100 µm sieve. The sieved paste was filled in a PE syringe for the plotting process.

After plotting, the scaffolds were kept on the plotting surface in a freezer at -15 °C for 2 hours. The frozen scaffolds were freeze-dried (B. Braun Biotech, Melsung, Germany – Christ, Model Alpha 2) for 2 days using 0.05 mbar and -20 °C. Afterwards, the dried scaffolds were inserted in an oven (Nabertherm, Lilienthal, Germany – Model HT04/17). The oven was slowly heated to 350 °C using a heating rate of 25 K/h. After 5 hours at 350 °C, the temperature was raised to 1150 °C using a heating rate of 100 K/h, at which the scaffolds were sintered for 12 hours. The oven was cooled slowly at a rate of 50 K/h to room temperature, to avoid cracking of the ceramic scaffolds.

3D-Plotting of Collagen

Collagen was extracted from bovine tendons. The collagen was dissolved in 0.5 M acetic acid, the inhomogenous solution was grinded. The hydrogel was precipitated in a 5% NaCl solution, filtered and dissolved once more. The solution was filtered through a 1 mm sieve to remove unsoluble collagen and then filled into dialysis tubes, which were kept in destilled water. The water was exchanged every 6 hours for two days. After dialysis the collagen/water mixture was frozen in a -70 °C freezer for 3 hours, after which they were freeze-dried for 1 day at 0.001 mbar. This fine collagen was grinded and then solved in 0.5 M acetic acid, to produce a 3% mixture. The collagen was plotted into ethanol, the ethanol was discarded after plotting and glutaraldehyde added to crosslink collagen. The scaffolds were afterwards washed three times with destilled water.

Seeding of the Scaffolds

The plotted scaffolds were sterilized using plasma sterilization (STERRAD 100S), after which they were washed 5 times using PBS (once per hour) and 2 times with nutrient media (once every 24 hours). $1*10^6$ cells and nutrient media were added and cultivated for 7 days at 37 °C under a 5% CO_2 atmosphere. The nutrient media was exchanged every two days. Before measurement, the scaffolds were moved to another well plate, so cells on the plate were not measured.

EZ4U-test

The substrate is first dissolved in 2,5 ml activator solution at 37 °C, coloring the solution yellow. Per well, 25 µl of the substrate is added to 200 µl cell culture, after which these solutions are incubated for 5 hours at 37 °C. After incubation, the plate is shaken on the reader (Anthos Reader 2001) and the absorpion at 450 nm (492 nm) against 620 nm is measured. The absorpion of a blank value is deducted from the measured values.

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