Rapid manufacturing techniques for the tissue engineering of human heart valves

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Abstract

Three-dimensional (3D) printing technologies have reached a level of quality that justifies considering rapid manufacturing for medical applications. Herein, we introduce a new approach using 3D printing to simplify and improve the fabrication of human heart valve scaffolds by tissue engineering (TE). Custom-made human heart valve scaffolds are to be fabricated on a selective laser-sintering 3D printer for subsequent seeding with vascular cells from human umbilical cords. The scaffolds will be produced from resorbable polymers that must feature a number of specific properties: the structure, i.e. particle granularity and shape, and thermic properties must be feasible for the printing process. They must be suitable for the cell-seeding process and at the same time should be resorbable. They must be applicable for implementation in the human body and flexible enough to support the full functionality of the valve. The research focuses mainly on the search for a suitable scaffold material that allows the implementation of both the printing process to produce the scaffolds and the cell-seeding process, while meeting all of the above requirements. Computer tomographic data from patients were transformed into a 3D data model suitable for the 3D printer. Our current activities involve various aspects of the printing process, material research and the implementation of the cell-seeding process. Different resorbable polymeric materials have been examined and used to fabricate heart valve scaffolds by rapid manufacturing. Human vascular cells attached to the scaffold surface should migrate additionally into the inner structure of the polymeric samples. The ultimate intention of our approach is to establish a heart valve fabrication process based on 3D rapid manufacturing and TE. Based on the computer tomographic data of a patient, a custom-made scaffold for a valve will be produced on a 3D printer and populated preferably by autologous cells. The long-term goal is to support the growth of a new valve by a 3D structure resorbed by the human body in the course of the growth process. Our current activities can be characterized as basic research in which the fundamental steps of the technical process and its feasibility are investigated.

Keywords: 3D printing • Rapid manufacturing • Heart valves • Tissue engineering • Polymeric scaffolds

INTRODUCTION

In the last years, tissue engineering (TE) has become an established field for a wide range of medical applications. This is due to the obvious advantages of using autologous cell material from the patient rather than using foreign human or even animal donation material. Well-known examples are applications in skin, bone or cartilage replacement [1–4]. However, the number of activity areas is still growing: current research and development focusses on problems like replacements for bladder [5], cornea [6] and trachea [7], and also cardiovascular structures, in particular blood vessels [8] and heart valves [9–12].

In the past, TE has produced structures that are indeed three-dimensional (3D), but the lack of plasticity is one major limitation. However, the functionality of most components of the human organism is based on a 3D structure. Modern 3D printing technologies provide the technical means to create and support 3D structures but, although success has been achieved in producing 3D structures by TE, it is difficult to achieve an optimal, functional 3D form, especially with regard to heart valves.

The replacement of human heart valves provides an instructive and challenging application for the combination of TE and 3D technologies. The 3D Laboratory at the Technical University of Berlin and the Tissue Engineering Laboratory at the German Heart Institute Berlin (DHZB) are collaborating to explore the possibility of developing a procedure for computer-aided fabrication of valve scaffolds on a 3D printer for subsequent seeding with vascular cells from the patient with the aim of generating a new valve. Prior computer tomography (CT) ensures that the valve fits exactly to the specific anatomy of the patient. This approach is intended to provide a potential alternative to mechanical heart valves or the use of human or animal donation valves.

Medical background

Heart valve failures account for a considerable proportion of cardiovascular disease. From a clinical perspective, repair and preservation of diseased valves is preferable to an extensive replacement. However, since preservation is impossible in 70% of cases, replacement is often mandatory. Currently available heart
valve replacements/substitutes are mechanical prostheses, non-living xenografts of animal origin or homografts of human origin. Each of the currently available solutions function well [13, 14], but all are associated with specific problems, motivating the search for further alternatives.

Tissue engineered cardiovascular substitutes could provide a promising alternative to conventional replacements. Meanwhile, TE, in particular that of heart valves, is a very global term and often serves as a synonym for fabrication of substitutes based on cells and a scaffold, also including application of xenogenic and allogeneic materials. However, one can distinguish between conventional or common TE and ‘classical’ TE, which also includes the engineering of an appropriate scaffold without an animal or human matrix origin to overcome recent limitations.

The TE process for the fabrication of a functional human heart valve substitute consists of several single steps that finally collude for: (i) generation of appropriate cell types, (ii) manufacturing a 3D scaffold and (iii) providing optimal in vitro culture conditions within a bioreactor.

Despite great progress in the field of cardiovascular TE over the past number of years, there are no ‘classical’ TE heart valve substitutes available with the potential to integrate into the surrounding tissue, to regenerate and to grow. Unfortunately, biopsies from living donors cannot be used for the TE of heart valves. Therefore, cells with a similar physiology have to be evaluated. In the literature, tissues from the great saphenous vein [15, 16], the ascending aorta [15] and the bone marrow [10, 17] are discussed as potential cell sources. Autologous cells have been preferably used to avoid immunological problems later in clinical application [9–11]. In a recent study, we used vascular cells from human umbilical cords. Compared with other tissues, the umbilical cord has the advantage that there are no ethical objections attached to its use as it is removed after delivery and the patient does not have to undergo further invasive procedures to isolate autologous cells. In the last few years, our studies have successfully demonstrated that vascular cells from human umbilical cords are feasible for the TE of heart valves [9]. These cells do not belong to the group of stem cells, but to already differentiated cells that have been found in a similar manner in native heart valves.

Currently, in the field of ‘classical’ TE several natural, i.e. fibrin and collagen [12, 18], and synthetic materials have been used and the applicability of resorbable and solid polymers has been analysed [8, 9, 19–26]. Figure 1 shows the first results of engineering heart valve scaffolds based on resorbable materials.

A variety of synthetic biomaterials have been used for regenerative medicine, in particular for TE applications. Resorbable polymers certainly belong to the most important materials, in particular polymers based on polyglycolid acid (PGA) and polylactic acid (PLA) and their co-polymers. These materials combine appropriate properties with good biocompatibility and degradation into non-toxic metabolites. PGA and PLA have been well known for several decades [27–31], notably since the 1960s when they were used as components of degradable suture materials [32–40]. Owing to this properties, PGA and PLA have also been used for wound coverage, regeneration of tendon defects, controllable agent release and in cardiovascular TE [1–3, 9, 19, 41–51]. Our present study focuses on PGA- and PLA- based co-polymers, as previous studies have demonstrated that the latter exhibit suitable properties for the fabrication of heart valve scaffolds [21, 45].

International research groups concur that dynamic in vitro conditioning after the cell-seeding process is essential for TE constructs. This involves exposing the developing tissue constructs to biomechanical and physical impulses in a bioreactor that imitates the subsequent in vivo conditions at the place of implantation. It is

Figure 1: Fabrication of TE heart valve scaffolds consisting of different resorbable natural and synthetic materials. From top left to bottom right: homograft; Fibrin scaffold; PGA scaffold; PGA/PLA scaffold; P4HB scaffold.
widely acknowledged that dynamic conditions promote the development of the extracellular matrix responsible for increased stability of cell layers [46]. If conditioned, endothelial cells change their morphology by aligning with the flow direction [47]. Only certain, specially equipped bioreactors can offer the right environmental conditions in vitro by suspending cell-seeded constructs in permanent strain to flow and mechanical pressure [46, 48]. Setting and handling of the bioreactors used so far varies from research group to research group, but the basic principle is similar. So far, a fully developed model for in vitro standardized fabrication of a functional TE heart valve is not available. The optimal in vitro conditions for the development of tissues are still unidentified, but it is widely confirmed that a uniform distribution of the cells on the scaffold, a high sterilization potential, an efficient cell culture medium and the influence of physical strain have positive effects on the generation of tissue [49]. Therefore, there is an urgent need for bioreactor enhancements, keeping in mind the use of good manufacturing practice (GMP) materials, easy handling, suitability for sterilization and accompanying analytics.

3D Printing and rapid manufacturing

The term 3D printing is an example of a so-called additive production method, denoting a process of making a 3D solid object of virtually any shape from a digital model [52–54]. 3D printing is achieved using an additive process yielding successive layers of material in different shapes. Therefore, the term printing is rather misleading in this context. Qualified 3D data models are created from computer-aided design (CAD) constructions, 3D object scans or—as in the present context—from CT or magnetic resonance imaging (MRI) data [55].

A number of technologies have been developed recently, each suitable for just one or a small number of specific materials [52, 56]. The most widespread technologies use special binders to ‘glue’ modified gypsum powder layer-by-layer. Other techniques use heating procedures to weld plastic filaments into 3D structures or lasers to produce—also layer-by-layer—3D structures using polymer [57–59], metal [60, 61] or ceramic-oxide [62] powder. Typically, layers of <100 µm can be obtained today.

Initially, 3D printers could only be used for rapid prototyping, i.e. for the production of models with prototyping character for scientific applications or to support product development in many branches of industry, usually resulting in dramatic reductions of product development time and costs: while human model builders often need weeks or months to build prototype models, 3D printers achieve this task in a few hours or days, usually with significantly better quality. In addition, new or modified prototypes can be easily conceived and produced. Finally, the prototyping process simultaneously provides digital construction data for further use in development and production [52, 63, 64].

More recently, the application range of 3D printing has been extended to rapid manufacturing: both the printing quality and the range of admissible materials have been expanded such that it is possible not only to produce models of good quality, but also objects offering real product quality. At first sight, 3D printing is time-consuming and costly if considered per item, i.e. this technology is not appropriate for industrial mass production. On the other hand, the prerequisite of qualified 3D data models opens the way for individual, custom-made solutions. Moreover, in terms of the often extremely costly production of moulds and other tools, 3D printing proves efficient when it comes to producing industrial goods in small quantities or when the part to be produced changes frequently. Typical examples are the production by 3D printers of parts for some 3D printers themselves, due to the fact that the number of the respective 3D printers produced per year does not exceed some hundreds, or the production of parts for the automotive industry such as hinges, motor parts, parts of the interior panelling etc. [52, 64].

The suitability for custom-made solutions or the flexible production of small series also provides a prerequisite for a large number of applications. Examples of potential activity areas that have not yet been mentioned above are various implants (cranium, hip joint, knee, shoulder etc.), prosthetics including prosthetic dentistry, orthopaedic shoes and hearing aids, support of operations (drilling and positioning templates, for example), production of medical devices and direct 3D tissue printing of organic materials [65, 66], neural tissues [67], bones [68–70], blood vessels [71], etc.

WORK FLOW

The basic work flow of our approach can be described by a rather simple algorithmic strategy whose steps will be specified in more detail below:

1. CT yields the necessary sufficiently exact individual data of the geometry of a human heart valve to be replaced for a specific patient.
2. Using a specific software, the layer-based CT data are transformed into a digital 3D data model. Unlike standard 3D applications on the computer, 3D television or 3D cinema, we not only need surface data, but also an exact representation of all internal structures.
3. Using machine-specific software and often after an additional application-specific manual intervention, the 3D computer model is transformed into a digital 3D printing model that considers both machine- and material-dependent aspects and, in particular, the special requirements for data completeness and the thickness of all model components: structural parts that are too ‘thick’ are probably optically inadequate and use too much of the cost-intensive material; parts that are ‘too’ thin risk not being sufficiently solid. This aspect is particularly important in rapid manufacturing, which yields objects of product quality to be used according to their specification.
4. On the 3D printer a custom-made scaffold is fabricated. Instead of simply producing an object representing sufficiently exactly the geometry of the valve, a so-called growth matrix is needed having a geometrically regular ‘porous’ structure in order to allow controlled subsequent growth of the cell material on the scaffold and into the inner structure. As mentioned above, the search for a suitable material is a crucial part of the research.
5. The scaffold is introduced into a bioreactor for cell-seeding with vascular cells of the respective patient.
6. The long-term vision is that following a sufficiently long growth process in the bioreactor, growth to a fully functional valve will be concluded in the body of the patient while the scaffold is finally resorbed by the body.
DATA COLLECTION AND DATA PROCESSING

A preferably high-resolution CT of the individual patient yields the basic data for the shape of the defective valve. In simplified terms, we ‘stick together’ the layers of the CT to obtain a 3D data model. The result of a CT looks like a collection of coloured X-ray images, but in reality it is already the result of image processing on digital data. Basic data are usually available as a huge set of discrete points in space which are first transformed into a grid of triangles or polygons in space as a raw frame for the further representation as a surface or volume corresponding to the geometry in question. Further steps are a mathematical smoothing process of the surface or volume to approach the final geometry as precisely as necessary. In most image processing applications, the final rendering adds colours, textures, lights etc.

In our context, we use a volumetric digital data set provided by the CT produced at the DHZB in the DICOM data format. In a segmentation step, a standard procedure in medical and industrial CT applications, the data for the (aortic) valve geometry are isolated for new data processing under the conditions of the intended 3D print. A polygon mesh is created and the generated data are exported as an STL data set. Digital data resulting from CTs or scans are often incomplete or ambiguous. While the respective requirements for visual 3D representations can be less strict, 3D printing requires very exact data without any data holes and with a clear geometrical orientation. Data completion and repair are important steps which are assisted by specific software, but which require manual intervention based on long experience. The intended fabrication of a real 3D object also requires the definition of wall thickness in accordance with the object geometry, the material and the specific 3D printing technology [52, 75]. The above steps are part of a well-established procedure. The final specifications result from parameters prescribed by the subsequent cell-seeding and requirements imposed by the cardiac surgeon with respect to the final implantation and will be the subject of continuous improvement.

In our current study, we used three different software packages to facilitate the above procedure, generating appropriate data models in <5 h. The size and the shape of the resulting valve models represent the original valve within a technically induced tolerance of <0.5 mm, which has not been evaluated yet.

3D PRINTING AND RAPID MANUFACTURING PROCESS

Among the available 3D printing technologies only selective laser sintering (SLS) has shown to be adequate in this context: a suitable material in powder form is applied layer-by-layer to a working platform. Guided by a computer, a laser heats up the powder on the basis of the 3D data for the current layer to produce a solid structure in that layer. Depending on the applied temperature, the laser induces a sintering (melting) process below (above) the melting point of the material. This process is repeated multiple times until a complete, solid 3D object formed by a few hundred up to a few thousand layers of <100 μm has been created. The final object must be cleaned. In most cases, a refinement of the surface is necessary. Figure 2 shows the SLS machine used in the 3D Laboratory of the TU Berlin.
The fact that none of the few materials certified by the machine manufacturer (currently, some synthetics, and for printers in the industrial range also some metals and ceramics) is adequate for our purposes has motivated the search for a suitable material and adaptation of the machine and the printing process. As each material is usable for 3D printing only in a specific temperature range defined by the glass transition and the melting temperature, the laser output has to be adapted by software and hardware measures for non-certified materials. In addition, the polyglycolic acid/polyactic acid (PGA/PLA) co-polymers we intend to use are extremely costly, making it necessary to avoid any loss of material in the production process. As the volume of 1,800,000 cm³ of the production space in our SLS printer is by far too big for the fabrication of a heart valve scaffold, a new, swappable unit of <150 cm³ with a separate material feeding unit, appropriately adapted mechanics and a modified laser unit had to be constructed at the TU Berlin. The continuous testing and improvement of the printing process in the given context constitutes a major part of our research.

The intended TE process requires establishing the necessary prerequisites for cell-seeding on the fabricated valve scaffolds. Figure 3 shows a 3D valve model with the exact geometry of the valve printed on the SLS machine at the TU Berlin. Our ultimate goal goes beyond this rather easily attainable result: we have to fabricate a growth matrix, i.e. a porous 3D object with this geometry and, additionally, a 3D mesh structure where size, shape and spatial reparation of the pores are material-dependent and have to be adapted for cell-seeding. The geometrical construction process has been successfully established and is subject to a continuous improvement process in cooperation with the TE process.

MATERIALS

The key to the potential success of our approach lies in the choice of a suitable material that meets the complex and partly contradictory requirements of medicine, TE and 3D printing. The certified standard synthetic materials, namely polyamides, are dedicated to the fabrication of robust technical structures and by no means suitable for our application.

From the TE and the clinical point of view, the optimal material should (i) not cause any inflammatory or toxic immune responses; (ii) exhibit controllable stability and degradation; (iii) metabolize in the body after fulfilling their function; (iv) have thermoplastic properties and the feasibility to be molded into any 3D shape; (v) possess a porous structure, preferably in a network structure for the migration of cells [76]; (vi) be suitable for sterilization; and (vii) exhibit the appropriate mechanical properties such as elasticity and stability. The 3D printing, i.e. the SLS fabrication process, leads to several additional necessary properties [77–79].

Powder-like and granular structures of PGA and PLA co-polymers are available from leading manufacturers. We tested several variants that differed mainly with respect to the percentage composition of both components and in terms of shape and particle granularity. All available materials had to undergo a detailed analysis of various aspects. All resorbable polymers have been analysed for their individual temperature features such as melting point and glass transition point in order to determine the working temperature range. Microscopic and macroscopic analyses have been performed to determine the material structure, namely particle size and shape.

Figure 4: Three examples for a PGA/PLA co-polymer with consistent scale.

To achieve fabrication precision with sufficiently accurate surfaces, a powder structure with a particle size in the range of 30–50 μm and a regular, preferably spherical, shape are required. However, all tested resorbable polymers have large particle sizes of >150 μm and a strong granular or fibrous shape (Fig. 4). Therefore, the original polymeric material had
to be processed by grinding. In view of the observed glass transition points ranging from 44 to 50°C uncooled materials would melt in a conventional mill during the grinding process.

In first tests with a cryomill providing permanent cooling with liquid nitrogen (Fig. 5), we obtained satisfactory particle sizes with a mean value of almost 36 μm after up to 12 grinding cycles (Fig. 6). Light microscopic examination and the analysis of flow properties approved the results from the successful grinding process.

The test materials revealed an amorphous melting temperature ranging from 180 to 220°C. The SLS printer allows for temperatures up to roughly 200°C. With an appropriate adaption of the fabrication process this material class proved to be feasible for fabrication [80].

In summary, we were able to determine a material class that in principle ensures the technical feasibility of the printing process and makes a preselection of suitable materials.

TISSUE ENGINEERING PROCESS

For subsequent cell-seeding of the manufactured heart valve scaffolds vascular cells, i.e. endothelial cells from the human umbilical cord vein, and myofibroblasts from the arteries were obtained. These cell types have been characterized in detail in previous studies from our lab [9, 24, 50, 51]. The human umbilical cord as a potential source of vascular cells has several advantages: after delivery, redundant cord tissue can be used for direct isolation of the cells from cord vessels without any ethical objections and the patient does not have to undergo any additional surgical intervention. Further, sufficient amounts of cells can be obtained due to the length of an ordinary human umbilical cord providing enough tissue. Cells can be cultured in a new bioreactor which is used exclusively for the proliferation of cells [51]. Additionally, isolated cells can be cryopreserved in liquid nitrogen as needed for further applications. Additionally, isolated and expanded cells can be
cryopreserved in liquid nitrogen as needed for further applications. After cryopreservation cells can be successfully thawed, recultured and expanded for subsequent fabrication of TE heart valves.

Cell-seeded heart valve scaffolds were cultured in a specially designed pulsatile bioreactor providing dynamic flow conditions. The recent bioreactor used in our studies combines the cell-seeding process and the conditioning process in the same device to minimize the risk of contamination. The bioreactor consists of two cylindrical perfusion chambers which rotate in two directions, as demonstrated in Fig. 7. Cells were seeded onto the scaffold under dynamic conditions and afterwards were conditioned in the same chambers. The development of new bioreactors and the improvement of ambient culture conditions in these systems is an ongoing process. With accompanying analyses and documentation one can draw conclusions from the development of new tissue and tissue components, such as composition of the extracellular matrix needed to successfully generate living and functional heart valve tissue. A concluding detailed analysis consisting of microscopic, mechanical, histological, immunohistochemical and molecular biological examinations presents the results of each step in the entire TE process.

CONCLUSION AND PERSPECTIVE

Heart valve replacement based on a combination of 3D rapid manufacturing and TE represents a challenging and highly innovative approach and a potential alternative to established techniques by avoiding some of their disadvantages, mainly by opening up opportunities to use autologous cell and data material. Several fundamental steps that substantiate future feasibility and applicability have been accomplished: we have defined the key procedural steps of the fabrication process; data generation and data processing can be described; an appropriate 3D printing technology as well as suitable rapid prototyping and TE processes are available; a qualified class of resorbable materials for the scaffolds has been identified and is currently undergoing intensive studies and tests; and adequate TE techniques are rather well established. Current work concentrates on the perfection of the adaptation of the 3D printer to our application, a continuation of the material research, the specification and the fabrication of the growth matrix and its operation in a real TE process. Further significant progress with respect to the treatment of open questions concerning these aspects can be expected in the near future. However, the current work is still being conducted on a basic research level and does not yet allow us to predict exactly when the technology will be ready for clinical application. Our approach is situated somewhere between classical clinical methods and the still hypothetical vision of fabricating organs or parts of them directly from cell material.

Our method provides an instructive example for the possible future use of 3D techniques, namely for rapid manufacturing in the field of medical applications. It also illustrates the need for interdisciplinary cooperation, given that our work relates to such diverse disciplines as medical, biological, mathematical, information technological, machine technical and material sciences. The potential can certainly be considered to be huge. Although numerous research groups in various fields of medicine are working on this topic, we still have a rather long way to go before clinical application can be realized. To our knowledge, none of the efforts to develop solutions for direct use in the human organism—with the possible exception of dental prosthetics—have yet reached the status of practical applicability.

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Figure 7: The bioreactor used at the Tissue Engineering Laboratory of the DHZB. Left: the bioreactor including the power unit; right: perfusion chamber with a non-woven PLG scaffold.
Conflict of interest: none declared.

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