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Customized Tissue Engineering For Ear Reconstruction

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Abstract

Tissue engineering (TE) of cartilage for reconstructive surgery has proven to be a promising option for obtaining tissue for 3D structures that results in minimal donor site morbidity. Technological advances in this area are important since many defects can only be treated with customized implants. Most TE strategies rely on the use of resorbable 3D scaffolds to guide the growing tissue, with each tissue requiring a specific scaffold that has precisely defined properties depending on the physiological environment. Rapid prototyping (RP) technologies allow the fabrication of scaffolds of various geometric complexities from a variety of materials and as composites, while even allowing the inner architecture of the object to be varied in a defined manner at any given location. Scaffolds can be manufactured using RP techniques directly from computer aided design (CAD) data sources, e.g. via an STL file. The combination of TE and RP serves as the basis for the production of customized implants, for example the cartilage ear framework, and provides new perspectives for autologous ear reconstruction.

In head and neck surgery, the successful treatment of various defects, including iatrogenic, traumatic or congenital defects, requires augmentation with supportive tissue. The ideal material is autogenous tissue, but such tissue is only available in a limited supply and is often associated with subsequent donor site morbidity. Furthermore, the transplanted tissue is often inadequate with respect to dimension, shape and function. Tissue engineering (TE) provides a very promising method of overcoming these limitations. Most TE strategies rely on the application of resorbable 3D scaffolds to guide the growing tissue.

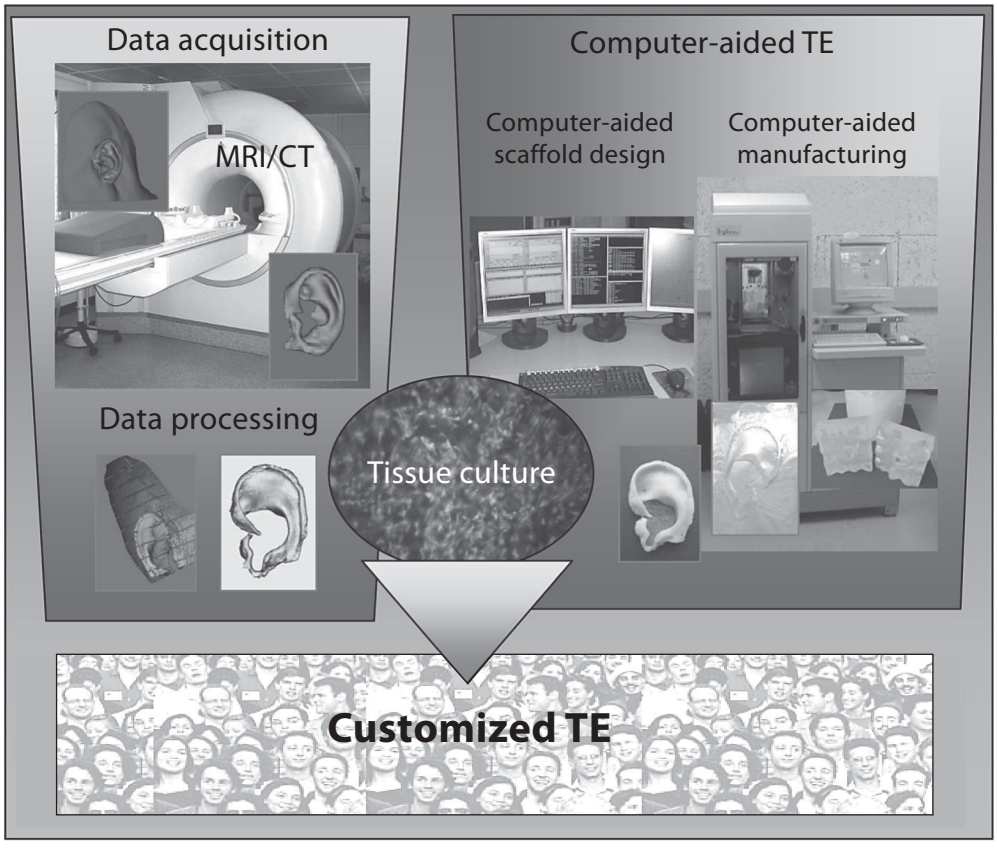

Fig. 1. Overview of the steps involved from data acquisition to a customized autologous implant.

Various rapid prototyping (RP) technologies – such as stereolithography, solid free-form fabrication, fused deposition modeling, 3D printing and negative molding – can be utilized in the fabrication of 3D models [1]. Using slice data acquisition through CT or MRI, a defect can be detected and defined according to an exact mathematical model. Then, in combination with data-processing technologies such as computer-aided design (CAD) and computer-aided manufacturing, this procedure can be clinically applied using alloplastic materials, for example as calvarium titanium implants [2]; thus, allowing the generation of the ideal implant for any particular individual defect (fig. 1).

However, taking advantage of such state-of-the-art techniques in generating scaffolds for TE using RP is very challenging [3]. The necessary structural features of scaffolds include high porosity, adequate pore size, interconnectivity for cell seeding and nutrient diffusion capabilities. The material must also be non-cytotoxic, and should enable cell adhesion and proliferation. Furthermore, it must provide specific biomechanical stability and elasticity, as well as controlled degradation and resorption rates to match tissue replacement.

Such customized scaffolds can be seeded with cells of various sources, e.g. with differentiated cells like chondrocytes or periosteal cells for cartilage tissue; preadipocytes for fat tissue; or mesenchymal stem cells for bone, cartilage or tendon TE. Every different type of cell requires optimal material characteristics and seeding strategies in order to generate the desired tissue. For optimal tissue development, specific supplements and growth factors are also essential. In addition, a variety of bioreactors are currently in use to support tissue development and tissue architecture for clinical application. This article provides a overview of the combination of RP and TE in generating individual autologous ear implants.

Imaging Data Acquisition

In order to produce a scaffold with the aid of RP techniques, the basis of the manufacturing process (i.e. the imaging data acquisition) has to be appropriate. This requires that the resolution of the slice images be high enough to adequately represent all the necessary details. However, it must be taken into account that even with today's most powerful computers such large datasets are still very difficult to process. In principle, setting resolutions on image acquisition scanners like the CT and MRI scanners so they do not natively support image capture, but are instead computed by interpolation, is counter-productive since it impairs data transmission and does not lead to better image quality. Today, very advanced cubical interpolation and filtering algorithms are available, but details that are not contained in the primary image cannot be retrieved or enhanced during subsequent data processing.

With respect to the type of image acquisition technology used, the superiority of CT scanners in image resolution and quality is so great that their application is adequate in most cases. This includes the representation of cartilage structures that will be discussed later. Experience shows that slice distances in the range of 1–2 mm, combined with a planar pixel size of 0.5–0.7 mm, are optimal for later reconstruction. Lower values of these parameters do not necessarily lead to significantly better results, but only serve to complicate data processing. This also applies to complex cartilage structures like the concha. However, in this particular case, a high number of tonal values (at least 3,000 unique ones) are additionally needed to ensure proper segmentation later on.

The image processing is performed on personal computers, and thus requires that an appropriate data interchange format be chosen. Nowadays, this can be done using DICOM format slice images on CD-R media in most cases. In order to ensure an unobstructed process, images should not be compressed. When using WORM (write once, read many) and MO (magneto-optical) media it must also be considered whether the devices and their associated formats are mostly proprietary to the manufacturer of the scanner. Thus, the compatibility of such devices and media combinations with the data processor should be checked in advance.

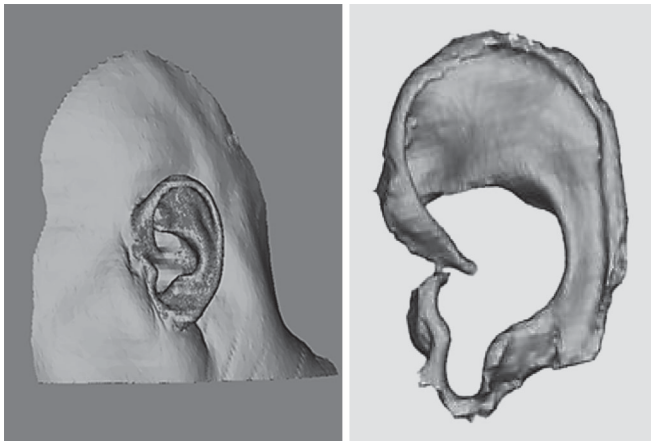

Fig. 2. Data acquisition and processing of a virtual model of the cartilaginous part of the missing contralateral ear.

Imaging-Based 3D Data Processing

After importing the datasets into a PC workstation, they can be processed with special software products that are commercially available. The most common are Mimics from Materialise (Leuven, Belgium) and Amira from Mercury Computer Systems (Chelmsford, Mass., USA). The task of the software is to generate a 3D computer model based on the 2D slice images. Furthermore, the software also provide services such as editing, measuring and direct interfacing to RP machines. It should be noted that in most cases multiple sub-datasets can no longer be combined at this stage. Therefore, the desired regions have to be contained in 1 continuous record.

Different tissue types are represented in the scanner images by varying grey values, whereby the segmentation of the target type has to be made by choosing a certain bandwidth. This decision tends to be somewhat intuitive since it depends on the calibration of the imaging equipment and the reconstruction method. Scanning is then automatically performed on all slices present with the same values. On the basis of the resulting mask, a cubical interpolation with subsequent smoothing is carried out to achieve a continuous virtual 3D model. This can be, for instance, either the entire bone structure of the head or only the cartilage portions. In subsequent steps, subsections can also be separated by setting distraction layers.

Using this procedure, a model of the existing structures is produced. In order to obtain a scaffold for further processing, the mockup can be used in different ways. In the case of the ear, it can simply be mirrored because of its symmetry and small area of support (fig. 2).

If no guide information from the respective patient is available, extrinsic information can be used as a basis for constructing the implant piece-by-piece by means of standard CAD systems like those used in engineering. After completion, the computer model can be exported to one of many data formats used in RP. Common types

include STL as a 3D exchange format with limited possible resolution, and CLI which is already adapted to the RP machines and thus cannot be subsequently edited.

Customized Scaffold Design and Manufacturing

Scaffolds are 3D biomaterial structures that mimic the function of the natural extracellular matrix within the tissue they should replace [4]. Each tissue requires a specific scaffold with precisely defined properties. The scaffold design depends on the physiological environment which is needed by the donor cells to build a proper tissue.

Scaffolds serve as:

- an adhesion substrate for the cell, facilitating the localization and delivery of cells when they are implanted;
- temporary mechanical support of the newly grown tissue by defining and maintaining a 3D structure;
- a guide for the development of new tissue with the appropriate function, e.g. by release of agents such as growth factors.

To be successful in TE, scaffolds must fulfill a specific set of requirements, such as biocompatibility, and possess adapted surface and physiochemical properties that promote or inhibit the initial cell attachment. Pore size, interconnectivity and pore morphology are responsible for the homogenous distribution of the cells into the scaffold, and the trouble-free transport of nutrients, metabolites and degradation products which are the basis for tissue formation and vasculature. The mechanical properties have to be in line with the host environment, e.g. load-bearing tissue substitutes for cartilage. Sufficient mechanical strength and stiffness are also required to deal with wound contraction forces and remodeling processes during tissue building and simultaneous scaffold degradation. Finally, an adjustable rate of degradation or a smooth transition between tissue build-up and scaffold degradation, as well as a moderate immunological response to the scaffold material itself and the degradation products, are indispensable in successfully building an effective tissue substitute.

A number of different scaffold materials of both natural and synthetic origin and/or degradable and non-degradable have been investigated, for example:

- collagen, hyaluronic acid, fibrin, alginate and Pluronics for cartilage development;
- demineralized bone matrix, hydroxylapatite, ceramics, coral and various types of different glass for bone;
- polylactic acid, polyglycolic acid, polycaprolactone for bone and cartilage;
- poly(ethylene glycol)-terphtalate for human nasal cartilage;
- poly(L-lactide-co-glycolide) for nerve TE;
- fibrin glue for cartilage and urethral reconstruction.

None of the known biomaterials used in TE are ideal for the production of individual cell carriers through RP technologies. Having determined the appropriate material for the desired application, it must be checked whether the material can be processed

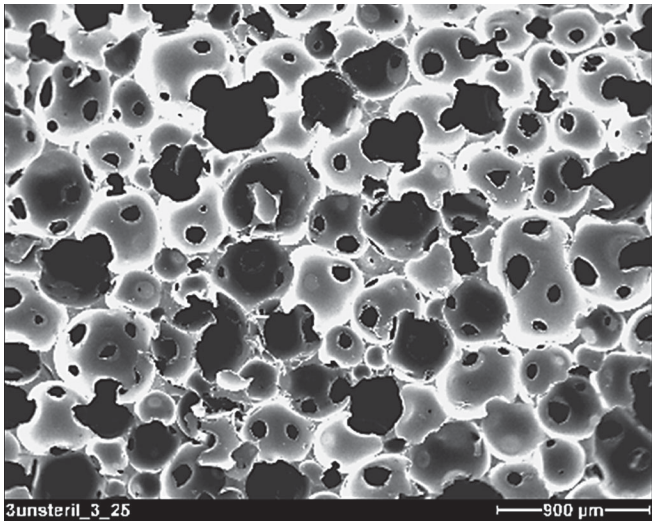

Fig. 3. Newly developed polyurethane material based on polycaprolactone, with 80% porosity and high interconnectivity under electron microscopy.

with the desired macro- and micro-architecture, and whether the material is compatible with the associated technical equipment. To meet the scaffold requirements mentioned previously, TE uses RP techniques. In an attempt to optimize our material properties, we developed a biomaterial based on polycaprolactone as a degradable polyurethane. This foam has 80% porosity, high interconnectivity and open surfaces with excellent biocompatibility for chondrocytes (fig. 3).

Rapid Prototyping

RP technologies utilize very specialized processes commonly used in the industrial production of technical equipment, which allow the fabrication of physical objects directly from CAD data sources via STL files. Based on such computer-aided techniques, objects of any geometric complexity can be formed without the need for final assembly. They can be fabricated from a variety of materials and as composites, and the process even allows the inner architecture of the object to be varied in a defined manner at any given location. RP techniques can be easily automated and integrated with imaging techniques to produce scaffolds that are customized in size and shape. In combination with TE, the basis for the production of quality customized implants is assured.

There are currently more than 20 different RP techniques being applied in various fields. Solid free-form fabrication is an umbrella term for a variety of different techniques such as stereolithography, selective laser sintering, 3D printing, 3D plotting and fused deposition modeling. These solid free-form fabrication systems permit the fabrication of complex objects via a manageable, straightforward and relatively fast process.

Table 1. Comparison of different RP techniques used in TE scaffold fabrication for head and neck surgery

	Resolution µm	Materials	Advantages	Disadvantages
Stereo lithography	70–250	reactive resins, PEG acrylate, PEG methacrylate, polyvinyl acetate, HA, dextran methacrylate, polypropylene fumarate	good mechanical strength, easy to use, easy to achieve small features	limited to reactive resins (mostly toxic), must be photosensitive, extremely dense, low void volume
Selective laser sintering	400–500	bulk polymers, polyethylene, ceramics, metals, compounds, PEEK, polyvinyl alcohol, PCL, polylactic acid, HA	high accuracy, high porosity, good mechanical strength, broad range of bulk materials, no support structure needed, fast processing	material must be in powder form, elevated temperatures from local high-energy input, uncontrolled porosity, material shrinkage when sheet-like structure is made, resolution depends on the laser beam diameter, powder may be trapped
3D Printing	100–500	ink and powder of bulk, polymers, PLGA ceramics, starch, dextran, gelatine	no inherently toxic components, fast processing, low costs, high porosity, can be performed in an ambient environment	weak bonding between powder particles, diminished accuracy, rough surface, component resolution and efficiency of removal of trapped materials are concerns
3D Plotting	100–250	swollen polymers (hydrogels), thermoplastic polymers, reactive resins, ceramics, PBT, PEOT	broad range of materials, broad range of conditions, incorporation of cells, protein fillers	slow processing, low accuracy, limited resolution, low mechanical strength, no standard condition, time-consuming adjustment to new materials
Fused deposition modelling	160–700	thermoplastic with good melt viscosities polymers/ceramics, PCL, PP-TCP, PCL-HA, PCL-TCP, PEGT-PBT	low costs, no trapped particles or solvents, highly reproducible, fully interconnected pore network, variation of pore morphology across scaffold realizable, input material in pellet form, preparation time is reduced	elevated temperatures, range of bulk materials limited by melting point and processing conditions, no natural materials, medium accuracy, positive value for pore channels is applied, high temperature, rigid filament, pore heights are determined by size of polymer fiber, no incorporation of biomolecules

Table 1. Continued

	Resolution µm	Materials	Advantages	Disadvantages
Injection molding (high pressure at high temperature; low pressure at room temperature)	135–500	hydrogel, PCL, polyester, collagen, ceramics, PLGA	broad range of materials, low pressure/room temperature, high accuracy, complex shapes and defined wall thickness can be fabricated reproducibly, can be automated	concerning compression molding: thermal degradation of polymers, no defined porosity and wall thickness, skin formation on polymer surface

PEG = Poly(ethylene glycol); PEEK = polyaryletheretherketone; PCL = polycaprolactone; PLGA = poly(lactic-co-glycolic acid); PBT = polybutylene terephthalate; PEOT = polyethylen oxid terephthalate; HA = hyaluronic acid; TCP = tricalcium phosphate.

By applying the expertise of RP techniques to medical scaffold production, most of the macro- and micro-architectural requirements for TE applications can be satisfied. In particular, the proper micro-architectural design of the scaffold is critical in order to ensure the required discharge of nutrients and oxygen not only on the surface, but also throughout the inner areas of the scaffold.

However, each of the RP technologies currently available has its individual strengths and weaknesses (table 1), and all are highly specific with respect to the processability of particular materials. Not all scaffold materials can be processed with all of the types of RP equipment, which results in a significant reduction in the availability of suitable biomaterials. This means that the planning of any scaffold production must first take into account whether or not the desired biomaterial is available in the required form, such as solid pellet, powder, filament or solution. The next question is whether the characteristic material properties, e.g. biocompatibility, are affected by such production process as solidification, heating, etc. It must also be ensured that the choice of scaffold materials is compatible with the selected RP technique, and that it is possible to fabricate the scaffold in the required way.

Injection Molding

Injection molding is a very widely used technique for the manufacturing of a variety of parts, from the smallest component to entire body panels of automobiles. TE scaffolds are fabricated by using molds, which can be fabricated from a CAD data file by STL or 3D printing, e.g. from metal, ceramic or silicone [5]. Liquid material is injected into a mold that is the inverse of the desired shape [6].

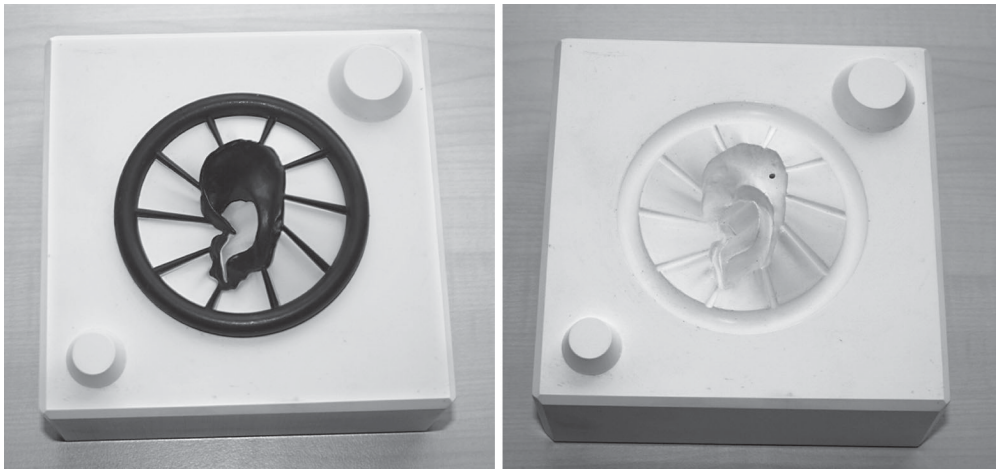

Fig. 4. The mold for producing scaffolds with 2-component foaming of the polyurethane.

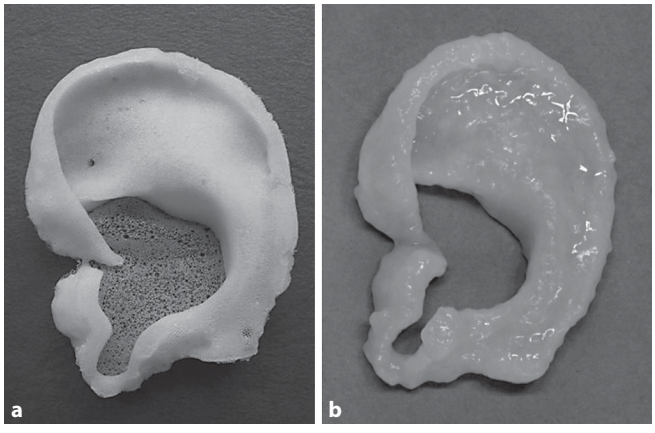

Fig. 5. Individual scaffold (**a**) after seeding with chondrocytes and a cultivation period of 4 weeks (**b**).

In principle, there are 2 different types of injection molding:

– molding of liquefied materials with high compression and high temperature;
– molding at low pressure and room temperature.

Molding at low pressure and low temperature is characterized by high reproducibility, automatization and production efficiency. It enables the use of a number of biomaterials that are not compatible with the fabrication processing conditions of many of the previously mentioned techniques. Complex-shaped porous scaffolds, as well as tubular scaffolds with thin walls and small diameters, can be produced at a resolution of up to 135–500 μm [7].

We developed a specific mold for generating complex polyurethane scaffolds (fig. 4). After data acquisition, data processing and the production of the desired scaffold

using RP technology, the mold must be vitalized by cell seeding, which represents the cellular part of TE.

Tissue Engineering

According to Langer and Vacanti [8], TE is defined as: 'an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain or improve tissue function'.

To produce viable tissues, TE uses strategies [9] such as the:

- implantation of the isolated cells directly into the defect (cell-based therapy);
- implantation of a bioactive scaffold material that implements the ingrowth of the desired tissue (e.g. joint repair, implantation of scaffold material, or stem cells that migrate into the scaffold and rebuild bone or cartilage);
- implantation of a cell/scaffold combination.

These attempts should improve or even replace standard biological functions.

Differentiated autologous cells are highly specialized cells that are characteristic of each single tissue with a clearly defined function, e.g. chondrocytes for cartilage. However, using these cells for building substitute tissues results not only in the reduction of the limited mass of the donor tissue, but also in undesirable collateral tissue damage. A further serious problem is that the differentiated cells, when taken out of the normal 3D environment, tend to dedifferentiate in the 2D cell culture environment within a few days. Dedifferentiation means that they are not producing a tissue-specific matrix, but that they change their morphology and, after a longer period of time, even their genotype. To achieve and maintain their typical cell qualities, cells have to be cultured in an appropriate 3D environment or scaffold.

Cells such as cartilage cells, keratinocytes and muscle cells proliferate rapidly. Others cells, such as hepatocytes and cardiomyocytes, proliferate slowly or not at all. Therefore, alternative sources of cells, like stem cells, are often needed.

Cell Seeding

Single cells can be dropped, injected or sucked as a highly concentrated cell suspension onto and into a scaffold, assuming that the force of gravity will subsequently disperse the cells throughout the entire thickness of the scaffold (fig. 5). However, this strategy is often associated with cell loss. Cells can fall through the pores into the culture dish or they may not necessarily penetrate into the scaffold due to insufficient interconnectivity or hydrophobic surfaces.

Another possibility for cell seeding is the encapsulation of cells, known as macrocapsules, mircocapsules and 3D multicellular masses, into hydrogels e.g. fibrin glue alginate, type I collagen, methylcellulose or pluronic F127. These particles can then

either be subsequently seeded onto the material scaffold directly or the hydrogel/cell particles are shaped by a 3D plotter.

Cell culture additives, like growth factors, are also a major concern in TE. Growth factors are defined as proteins that act as signaling molecules between cells, attach to specific receptors on the surface of a target cell, and promote differentiation and maturation of these cells. All kinds of growth factors are used in TE, such as insulin-like growth factor, transforming growth factor β, or interleukin for cartilage TE [10].

Future goals of TE are the enhanced development of customized TE products to avoid the problems of reducing limited donor tissue, creating collateral tissue damage and unwanted immune responses. To this end, the in vitro culture of cells must be improved so that cell dedifferentiation can be inhibited. In the attempt to establish co-cultures of different cells, the development of bioreactors that enable the co-culture of specifically sized implants has to be ensured. Computer-aided TE will become increasingly important in the future to enable the development of customized scaffolds using the appropriate techniques, materials, and micro- and macro-architectures, without the loss of geometrical resolution, accuracy in detail or material properties such as biocompatibility.

Conclusion

The combination of TE and RP enables us to take the next step in regenerative medicine, i.e. the fabrication of customized implants. Customized implants are designed by the defect data gained from medical imaging technologies, transferred into the language of RP machines, and then realized as a biomaterial scaffold. These individualized scaffolds (such as those used in ear reconstruction) can be seeded with autologous cells, and, after just a short period of time, implantation of the 'individually customized' implant can take place.

Acknowledgments

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