REVIEWS

Computer-aided tissue engineering: overview, scope and challenges

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Advances in computer-aided technology and its application with biology, engineering and information science to tissue engineering have evolved a new field of computer-aided tissue engineering (CATE). This emerging field encompasses computer-aided design (CAD), image processing, manufacturing and solid freeform fabrication (SFF) for modelling, designing, simulation and manufacturing of biological tissue and organ substitutes. The present Review describes some salient advances in this field, particularly in computer-aided tissue modeling, computer-aided tissue informatics and computer-aided tissue scaffold design and fabrication. Methodologies of development of CATE modelling from high-resolution non-invasive imaging and image-based three-dimensional reconstruction, and various reconstructive techniques for CAD-based tissue modelling generation will be described. The latest development in SFF to tissue engineering and a framework of bio-blueprint modelling for three-dimensional cell and organ printing will also be introduced.

Introduction

A fundamental premise for tissue engineering lies in the fact that sample cells can be cultured ex vivo, introduced with scaffold in the appropriate environment for cell and tissue growth, and the newly grown tissue/organ implanted to restore the tissue function. The technology developed in tissue engineering has been used to create various tissue analogues, including skin, cartilage, bone, liver, nerve and vessels [1–3]. Utilization of computer-aided technologies in tissue engineering has evolved the birth of a new field of computer-aided tissue engineering (CATE), which integrates advances in biology, biomedical engineering and information technology to tissue-engineering application. CATE can be defined as the application of enabling computer-aided technologies, including computer-aided design (CAD), image processing, computer-aided manufacturing (CAM) and rapid prototyping (RP) and/or solid freeform fabrication (SFF) for the modelling, designing, simulation and manufacturing of biological tissue and organ substitutes. Specifically, CATE encompasses the following three major applications in tissue engineering: (1) computer-aided tissue modelling; (2) computer-aided tissue informatics; and (3) computer-aided tissue scaffold design and manufacturing. An overview of CATE is presented in Figure 1.

A review of the development and application of CAD/CAM in biological and tissue engineering was given in an earlier paper by Sun and Lai [4]. Although an overview of the broad spectrum of the CATE will be outlined, the focus of this Review is to report the latest advances and the development of the topics on computer-aided technology to tissue engineering application which have not already been reported in [4]. Particularly, we will focus on the presentation of new topics on computer-aided tissue informatics, new technologies on SFF, new methodology on tissue scaffold design, and new concepts and frameworks for bio-blueprint modelling and application to 3D (three-dimensional) cell and organ printing.

Owing to the length of the material we wish to discuss, we have divided our contribution into two papers. The focus of the first paper, i.e., this Review, is to present an overview of CATE in its three main categories, that is, (1) computer-aided tissue modelling, including 3D anatomic visualization, 3D reconstruction and CAD-based tissue modelling; (2) computer-aided tissue informatics, including computer-aided tissue classification and application for tissue identification and characterization at different tissue hierarchical levels; and (3) computer-aided tissue scaffold...
Computer Aided Tissue Engineering

Anatomic Modeling
* Geometry/Morphology
* Volumetric representation

Biophysics Modeling
* Mechanics/Deformation/Kinematics

CAD-based Modeling
* Contour-based model
* Surface extraction
* Solid model

Tissue classification and Informatics
* Tumor diagnosis
* Morphometric and cytometric study
* Tumor cell detection
* Tissue properties

Cell/Tissue Formation and Cell aggregation
* Cell aggregation
* Cell/tissue growth
* Cell-cell and Cell-Tissue interaction

Figure 1 Overview of CATE

design and manufacturing, including scaffold modelling and design, SFF of tissue scaffolds, bio-blueprint modeling for 3D cell and organ printing. The focus of our research paper in this issue [4a] is to present the use of the CATE approach for biomimetic modelling and design of bone tissue scaffold, including the detailed process of biomimetic modelling, design, tissue and scaffold characterization, analysis and fabrication to a specific case study of femur bone replacement.

Computer-aided tissue modelling

Computer-aided tissue modelling consists of two major processes: non-invasive imaging data acquisition and 3D reconstruction. Construction of a specific tissue model often starts from the acquisition of anatomic data from an appropriate medical imaging modality. This is sometimes referred to as 'image-based tissue modelling'. In image-based tissue modelling, the imaging modality must be capable of producing 3D views of anatomy, differentiating heterogeneous tissue types and displaying the vascular structure, and generating computational tissue models for other downstream applications, such as analysis and simulation.

Non-invasive imaging data acquisition

The primary imaging modalities used in the tissue modelling are CT (computed tomography), MRI (magnetic-resonance imaging) and optical microscopy, each with its own advantages and limitations, described as follows.

CT and \( \mu \text{CT} \) (micro-CT)  
CT or \( \mu \text{CT} \) scans require exposure of a sample to small quantities of ionizing radiation, the absorption of which is detected and imaged. This results in a series of 2D (two-dimensional) images displaying a density map of the sample. Stacking these images creates a 3D representation of the scanned area. The main advantage of CT and \( \mu \text{CT} \) as an imaging modality for tissue-engineering purposes is reasonably high resolution. The latest development of \( \mu \text{CT} \) technology has been successfully used to quantify the microstructure–function relationship of tissues and the designed tissue structures [4]. For example, recent reports have shown that a \( \mu \text{CT} \) system was capable of characterizing the micro-architectural and mechanical properties of tissue scaffolds [5], to help the design and fabrication of tailored tissue microstructures [6,7], to quantify the bone-tissue morphologies and internal stress–strain behaviour [8–10], to non-destructively evaluate...
porous biomaterials [11] and to model lung tissue at 10–50 \( \mu \text{m} \) resolution [12].

Differentiation of tissue in CT scans is accomplished through contrast segmentation, the grey-scale value of each voxel (volumetric element, a three-dimensional pixel, the smallest distinguishable three-dimensional element in an object) determined solely by tissue density. As such, CT is inferior to both MRI and optical microscopy in differentiating soft tissues of similar density. It is much more effective in the modelling of hard tissues and sharply defined density changes, such as the interface between bone and soft tissues, as illustrated in the differentiation between the brain and the bone as shown in Figure 2. In addition, \( \mu \text{CT} \) is also commonly used in the study of bone density in research, clinical medicine and palaeontology [5,9,11]. The disadvantage of poor soft-tissue differentiation can be addressed to some degree through the use of contrast agents. Most of the CT contrast agents are short-lived and iodine-based, and primarily of use in imaging the vasculature, as in X-ray angio-

graphy. In addition, there are a number of metals, metal salts and metal particulates that significantly increase contrast in CT. Owing to the toxicity and side-effects of heavy metals in vivo, few of these metallic contrast agents are in widespread clinical use [13,14]. Assembly of an organ blueprint model from a cadaveric sample negates these deleterious side-effects, and metal-bearing contrast agents may be useful. In one specific example, Kriete et al. [12] utilized AgNO\(_3\) to enhance the contrast of \( \mu \text{CT} \) images taken of lung alveoli. The silver, which is not X-ray-transparent, served to outline each alveolar sack.

**MRI**  MRI does not expose the sample to ionizing radiation, hence its increasing use in clinical applications. It images soft tissues as well as bone, and as such is vastly superior in differentiating soft-tissue types and recognizing border regions of tissues of similar density. Much like CT, the output of MRI is a series of 2D images that may be stacked and segmented by signal intensity. Further segmentation may be achieved through region-growing selection of voxels of similar signal value that are contiguous. This can create models of regions of similar signal intensity that are part of an individual structure, e.g. selecting a single ligament rather than all the ligaments in the image.

Although the resolution of MRI is inferior to CT scans, it has been of great use in assembling anatomic atlases of increasingly fine resolution as the technology matures. For instance, Dhainain [15] performed \( \mu \text{MRI} \) scans on mouse embryos in using an 11.7 T (tesla) system. The resolution achieved was 20–80 \( \mu \text{m} \) voxels. The resulting segmentation isolated each of the major developing organs in the embryo. A similar segmentation using publicly available rat MRI data is shown in Figure 3. Using simple region-growing techniques and Mimics software [16], we developed a 3D representation for the central nervous system, heart and kidneys of the subject. Despite the high tissue differentiation capacity of MRI, the resolution is consistently worse than that for either CT or optical microscopy.

**Optical microscopy**  Without modelling software capable of reassembling dissected histology slices, optical-microscopic methods of developing 3D tissue modelling would not be possible. To examine a sample to high resolution using optical microscopy, it must be physically sectioned to a thickness of between 5 and 80 \( \mu \text{m} \) and placed on to slides, providing a square sample perhaps 1 cm \( \times \) 1 cm for fine resolution. The division into these slides is a labour-intensive process, and the resulting images of the target organ would be thousands of 2D images that must be both digitally stacked into 3D columns as in CT and MRI and arranged in correct X and Y positions. This is computationally and memory-intensive, but within the capabilities of many computer-modelling programs.
With the understanding that the technique would be labour-intensive and require a great deal of computer time, optical microscopy also brings the benefit of a century of refinement. Pathologists have become skilled at identifying any individual cell in the body visually, even in cases of diseased tissue, the origin of their name. Stains may be applied to individual slides or all slides. These stains may be as simple as dyes or as complex as fluorescing antibodies that bind only to a single type of cell. Differentiating every tissue type down to the level of the individual cell is possible for optical microscopy in ways far beyond either CT or MRI, not by density or signal intensity, but by the features of the cells themselves. One particular example of optical microscopy used in tissue modelling was reported in [12] with regard to the imaging of lung tissue, detecting fluorescence from the lung parenchyma above 545 nm. The individual images taken were then stacked, and carefully re-aligned, for a 3D representation. While the alveoli were modelled at extremely high resolution, the principle drawbacks of this method were made readily apparent. Specifically, both the sectioning and imaging are time-consuming processes, and the sectioning can result in distortion of the individual slides. Also, from a practicality point of view, pathologists cannot be expected to examine thousands of individual slides of an entire organ and identify each and every cell in the image. Therefore it will be a significant challenge to train computers to identify individual cells by their visual characteristics, even with the aid of complex staining.

**Modality hybrids**

Another method for determining a more precise 3D model is to combine multiple modalities used on the same specimen in order to correct for deficiencies in any single modality. For instance, 3D models derived from MRI and CT could be combined to display heterogeneous soft tissue, for which MRI is excellent, within a high-resolution bone structure such as the skull, for which CT is better suited. A combination of CT and PET (positron emission tomography) has been studied as a means to provide both structural and metabolic information for clinical applications such as precise localization of cancer in the body [17].

A CT/optical-microscopy combination might be of use in correcting the histological distortion from the physical sectioning required for optical microscopy, otherwise an ideal modality for high-resolution high-tissue-differentiation imaging. The CT angiography-derived vascular tree may be a means to help correct this histological distortion in the final model. The optical-microscopic method would image the vasculature just as the CT scan would, but the individual vessels might be moved, owing to cutting distortion in any given slide. By comparing the optical vascular model to the CT-derived vascular model which does not require significant cutting, the histological distortion might be correctable. At the very least, the comparison could determine whether the final model derived from optical microscopy was grossly distorted. An illustration of the multi-modality μ-CT/optical-microscopy hybrid model, which uses the vascular tree from a μ-CT angiograph to correct distortion in images from optical-microscopic sections, is presented in Figure 4.

**Imaging-based 3D reconstruction**

The basic 3D interpretation format of consecutive slice images is a volumetric model consisting of voxels. The volumetric model consists of brick-like components, each representing a set height, width, and depth. Like pixels, each voxel represents a yes/no value indicating its existence in the model and a grey-scale or colour value. As such, volumetric models tend to have rough surface textures with many right angles at the scale of the individual voxel, as shown in Figure 5.

Although volumetric data from medical-image data can be directly altered to STL (stereolithography) format and printed on a rapid prototyping system, there are many advantages to further convert the images model into a CAD-based solid model. CAD-based solid modelling relies upon ‘boundary representation’ by means of which a solid model is defined by the boundaries that enclose it. These bounding surfaces are mathematically described by polynomial functions such as non-uniform rational B-spline (NURBS) functions. This method facilitates the construction of the model by minimizing the size of the files and ensuring the closure of bounding surfaces.
Figure 4  An illustration of the μCT/optical-microscopy hybrid model

Figure 5  Image volumetric representation

a) 2D pixel representation   b) 3D voxel representation   C) 3D NURBS representation

Figure 5 illustrates the difference between volumetric and surface representations. Figure 5, left panel, represents a series of slice images, with a single pixel protuberance from a vertical flat surface. The volumetric model displays this single pixel as a voxel in Figure 5, middle panel. The surface interpretation of these 2D pixels is different, however, and when expressed as a NURBS surface, as in Figure 5, right panel, the single offshoot pixel is expressed as the apex of a curve. In addition to being a closer approximation to most 3D structures, the boundary represented CAD model is capable of being altered through Boolean operations and analysed by enabling CAD and analysis software packages. Application of the volumetric and surface representation to develop a new 3D model based upon the Visible Human database.
dataset has been summarized by Hohne [18]. By first segmenting the pixels on the basis of the RGB (red, green and blue) optical data from the sections of the Visible Human male with significant knowledge-based assistance, they were able to differentiate the individual organs. Surface inclinations and surface textures were determined at these segmented border lines. The resulting surface-represented model is one of great realism, which achieves subvoxel resolution. Digital sectioning of the model is possible, displaying desired cross-sections of selected organs. A surface-rendered model from the Visible Human male torso data is displayed in Figure 6 (taken from [18]).

Some biological elements may defy the resolution of the preferred imaging modality for a given use, but occur in patterns that may be synthesized, the artificial layout then being added to the final model. Examples might include nephrons, peripheral nerves or spinal-column neurons in series, and vascular trees. For instance, there has been work in synthesizing vascular-tree patterns computationally with an emphasis on fully perfusing a given volume of tissue, simulating natural branching patterns and arterial radii. Karch et al. [17] used a modified computational method of constrained constructive optimization to create vascular-tree patterns for simulated regions of tissue, taking an initial vessel and basing branching patterns upon aspects of flow and local arterial characteristics. The patterns are strikingly biomimetic, despite being solely generated by a computer without input from biological data, as shown in Figure 7. These models could be combined with another model through a Boolean subtraction to create channels in a tissue model or scaffold – channels that may be of smaller scale than may be detected by the imaging method.

A number of reverse-engineering approaches exist to enhance the 3D model reconstruction from a 2D-image-based modality. Boolean operations upon the surface model can allow further information to be added to a model, some biological units too small for imaging such as small vessel networks may be synthesized rather than imaged, and information may be combined from multiple modalities. Boolean operations allow multiple models to be combined, either through addition or subtraction, intersection or union. Figure 4 of [19] represents one such operation, removing a series of 3D channels in the cube at the left from a biomimetic bone structure derived from CT. The resultant model, suitable for construction as a bone scaffold, is
a biomimetic shape possessing the desired porosity. An approach to reconstruct a CAD-based model from medical-imaging data after 3D region-growing can be found in our previous papers [4,20,21].

Computer-aided tissue informatics

It is a matter of inevitability that we will have maps of hundreds of genomes, thousands of protein structures, tens of thousands of genes and databases of hundreds of thousands of single nucleotide polymorphisms (differences in a single nucleotide that cause changes in gene function). With a wide array of imaging modalities (CT, MRI, optical microscopy, ultrasound and electrical-impedance monitoring) and an even wider array of cell-expression analyses (systematic sequencing, proteomics, DNA microarrays, expression arrays, yeast two-hybrids and high-throughput screenings), bioinformatics will provide the key connections and correlations within the vast amount of data [22]. The understanding of cellular constituents and metabolism at the smallest scale can be directly applied to the characterization and analysis of features on the tissue scale (tissue informatics). Finer detection and recognition of tissues will enhance biological modelling at the anatomic and system level.

This section will discuss informatics techniques at multiple scales: (1) those aspects of cellular bioinformatics with potential application to tissue identification and characterization, (2) currently used methods for identifying features at the tissue level, (3) methods for automated recognition of organs and anatomical systems, and (4) specialized techniques in maintaining continuity of complex branching structures such as neuron processes and vascular trees at all scales of analysis.

Computer-aided cell analysis

Automated methods exist for cell counting, determination of cell geometry, chromosomal counting, correlation of DNA expression determined through micro-arrays, interpreting fluorescence data, determining a cell’s lineage and cross-correlating gene expression with predicted in vivo pathology. All of these features have predictive value for determination of tissue viability and the differential fate of cells seeded with the goal of tissue culture. DNA expression, as determined by microarrays, offers a highly detailed description of cell function, identifying hundreds of expressed genes at any one time. DNA expression may be further enhanced by immunofluorescence data, whereby the presence of a single chromosome, gene or protein may be measured. Once expression data have been accumulated, large-scale computational cross-correlation may be performed, comparing this expression with expressions common in pathology. The data may further be used to extrapolate a cell’s lineage and eventual differentiative identity or pathological condition.

DNA microarrays are glass slides upon which thousands of discrete DNA sequences are printed in a known pattern. A cell sample, when placed upon the slide, will interact visibly with the DNA spots with which it shares expression. Visual automated detection and intensity measurements of each spot are performed and the type of genes that are expressed for any given cell sample are catalogued [23]. Although the advancement of DNA microarray technology has revolutionized genetic research, there is a flaw in statistical analysis. With thousands of points of data for every sample, the statistical value of correlations requires a large number of samples to be performed, and the analysis is computationally complex [24]. Immunoassays offer even greater specificity than DNA microarrays, as they may be used to identify any desired cellular component or element of expression, but large sample sizes are required for this method as well. As such, the automation of spot analysis is essential to expedite results for both modalities. This is accomplished through generation of a grid to isolate each spot and quantify the grey-scale value of each point of expression [25]. The clustering of this data by correlation is performed largely without supervision, owing to the quantity of data. Work is underway to allow the software to make use of prior knowledge of gene function in clustering the microarray data [26] and to use pattern-recognition and multiple-image resolutions to filter out non-significant points [27].

Correlational data from these different methods of cell expression may be used to determine both a cell’s lineage and its fate, and into what cell type it is destined to terminally differentiate. ALES (A Lineage Evaluation System) is one such statistical utility. Through input of a cell’s expression data, and the expression data of its daughter cells, ALES assembles a lineage tree—a hierarchy of gene-expression or cell-characteristic data akin to a family tree. Once these data are accumulated, a cell may be analysed for its gene expression to determine exactly where on the tree it will fall, to the level of the cell division which produced it [28]. Alternatively, gene-expression data may be correlated not with cell identity but with patient outcome, in the case of pathology. By following the outcomes of these patients, a gene index may be developed to determine associations between gene expression and patient prognosis [29].

Computer-aided tissue identification and analysis

There are a number of tools specific for tissue analysis. Through various optical-microscopy stains, nuclei, cell boundaries and elements of the extracellular matrix (ECM) may be viewed selectively, with differences in cell size, cell density, nuclear size and matrix composition as visual parameters. Knowing the context of what tissues are expected in...
a given region, and the parametric values possessed by such
tissues, histological slides may theoretically be differentiated
in this manner.

Once the images have been acquired, an automated
method must exist to separate and identify the individual cell
or tissue types, depending upon the scale of the intended
reconstruction. In the case of MRI and CT, this tissue dif-
ferentiation is determined by contrast segmentation, which
separates the tissues by grey-scale value of signal intensity.
Optical methods operating in RGB colour provides triple
the discriminatory ability of grey-scale. Further methods
such as region growing, which provide a mask of all con-
tiguous voxels of a certain threshold, may be applied. While
tissue separation may be performed to some degree through
contrast segmentation, tissue identification inherently re-
quires a knowledge-based approach. In the literature regard-
ing automated identification of cells and tissue, it is fre-
quently repeated that the discriminatory ability of software
is far below that of human counterparts. However, an auto-
mated method for tissue characterization is essential for the
construction of a high-resolution biomimetic model, as
the workload involved in such characterization is greater
than would be economical for a pathologist to perform.

Tissues can be very heterogeneous, with cells expres-
sing complex transitional phenotypes that cannot be
accounted for in even the most complex knowledge-based
system. Such outlier cells in a tissue sample would not be
recognized by a pattern-recognition system and might in-
clude mesenchymal stem cells, immune cells migrating
through the tissue, cells whose nucleus or other distinguishing
feature for some reason did not stain properly, or
cancerous cells. In the case where one or two cells appear
different from the rest of the surrounding tissue, it may
be safest to augment the data with K-nearest neighbour
classification [30]. This method of pattern recognition will
classify the anonymous cell as identical with its majority of
neighbour cells, provided there are no distinguishing char-
acteristics in the anonymous cell negating this classification.

The visible data available to the pattern-recognition
programs include density of cell outlines and locations of
nuclei, where a bulge would occur in the cell in the direction
of the axis of the camera angle. These sharp contrasts may
be interpreted in terms of texture, essentially as bumps
defined by cell membrane perimeters and nuclei peaks. The
result is a 3–4D matrix (depending on technique), defining
the surface in a manner similar to human perception of
touch. This form of texture interpretation is currently under
clinical experimentation to discriminate between cervical
texture patterns indicative of different stages of cervical les-
sions [31]. Texture analysis on a single chip with cellular
neural network architecture is being developed, capable of
measuring texture, which includes histogram equalization
of the textured images, filtering with the trained kernel
matrices, and decision-making based on average grey-scale
or texture energy of the filtered image [32]. Such rapid
analysis would enable construction of a texture library for
tissue types, perhaps enabling faster identification than cell-
by-cell visual classification.

Anatomic registration
In addition to recognizing the individual cells in a selected
region of tissue, there is also value in automated recognition
of organs and body systems. The data for such operations
would come from non-invasive imaging modalities such as
CT and MRI. The automated recognition of organs from
this data could quickly and robustly separate out organs and
defects on the anatomic level, despite individual patient
variation. Such methods operate on either landmark/feature
detection, such as identifying the major vessels of an organ
such as the kidney [33] or by a shape-based method by
which imaged data is compared with a known shape with
some margin for error.

The simplest feature detection uses contrast segmenta-
tion of an imaging modality and some foreknowledge of
the thresholds expected for a given data set. For instance,
Anderson et al. [34] established an automated segmentation
program by which the grey matter, white matter and
cerebrospinal-fluid spaces are segmented from MRI data.
This is actually more difficult than it may seem, for while
MRI does register soft-tissue differences through different
grey scales, the signal-to-noise ratio is fairly low. As such,
the Anderson team had to use noise filters and compensate
for field inhomogeneity through a recursive method that
adapts to the intrinsic local tissue contrast. This method was
determined to work for several kinds of MRI input, and may
find use directly in clinical application. More complex uses
of landmarks in medical imagery are finding use in anatomic
registration. One such example of feature recognition can
be seen in the work of Hellier and Barril [35], who used the
sulcal patterns of the brain to register the brain structure
reliably for 18 patients, minimizing inter-patient fun-
ctional variability when evaluated on magnetoencephalo-
graphy data.

The fundamental difference between shape-based and
feature-based registration is that shape-based registration
software, while it still considers Euclidean point-to-point
information about features, also measures structural corres-
pondence in terms of normals and curvature. One study
offering direct comparison of these methods showed 2–33 %
accuracy improvement of shape-based over feature-based
registration methods [36]. Shape-based approaches com-
pare the curve-represented model of a known structure
and compare it with the same structure as medically imaged.
One such example was reported in [37] for the 2D segmenta-
tion of the left cardiac ventricle. In this example, the algo-

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teaching it the general shape of the ventricle and the degree of variability to be expected between a number of different subjects. Using this knowledge base, the shape-based algorithm adjusted the topology of its expected ventricle model to match the imaged ventricle, thus identifying the region in each 2D section. In the same study, a similar 3D match was performed with the prostate [37].

More so than informatics technology at the cellular and tissue level, anatomic registration presents potential for immediate clinical applications. Clinical applications being investigated include automated recognition of colonic polyps using computed tomographic colonography, increasing the accuracy of radiation treatment around the spinal cord by accurately determining the cords location and shape prior to gamma surgery, and identifying the patient-vascular pattern prior to liver surgery [38–40].

**Continuity analysis**

Another aspect of tissue informatics at all scales of analysis is determination of connectivity between slices of a given imaging modality. Whether using confocal microscopy, CT or MRI, the output format of the modality is ordered 2D images with some amount of non-imaged distance in between. While 3D reconstruction can be performed on these images, some elements, especially cable-like elements such as neuronal dendrites and blood vessels, are not always obvious as to their connectedness between layers. For instance, in assembling 3D surface-represented reconstruction of the visible human optical data, it was necessary to manually inspect each section, mark it for significant vessels, and then connect the vessels with their own anatomic knowledge [41]. Creating an automated application capable of connecting cable-like elements in 3D would be a valuable endeavor.

At the smallest scale, the cable-like elements for which tracing is valuable include cellular processes like neuronal axons and dendrites. Traditional methods of tracing neurons three-dimensionally have consisted of skeletonization methods by which neuronal connections are inferred by stick-like connections between 2D images found with confocal microscopy. New methods, based on shape-recognition applications, treat each process as a 3D cylinder model and apply a set of four directional kernels based on imagery to follow the topology of the neuronal process. The end product is a robust, noise-resistant application capable of labelling all somas present in a confocal sample with dendrites and axons appropriately mapped [42]. A similar approach is of value on the larger scale when mapping the vasculature of a complex organ such as the liver. Selle et al. [40] took liver angiographic data and segmented them into the vascular elements of the liver. At that point, the application analysed the structure of the hepatic vessels, divided the vessels into arteries and veins, determined the territories fed and drained by each vessel, and then interpolated small vessel primitives to fully perfuse the regions of tissue in between. The territorial distribution of the liver from that study is shown in Figure 8. Connectivity in other organs is being explored through a combination of imagery and computation, such as a map of brain connectivity through use of functional MRI and fast marching methods [43].

**Computer-aided tissue scaffold design and manufacturing**

Design of 3D tissue scaffolds or tissue substitutes for tissue-engineering application should consider the complex hierarchy and structural heterogeneity of the host tissue and/or scaffold environment. Other than important factors of porosity, pore size, interconnectivity and transport property for nutrients that would enable the ingrowth of new cells and cell-tissue formation, the designed scaffolds should also be
able to have compliant mechanical properties with the host environment as well as required mechanical strength after implantation in the design of load-bearing tissue scaffolds or substitutes such as bone and cartilage [44,45].

Biomimetic modelling and design for tissue scaffold
The load-bearing tissue scaffolds have certain characteristics of their own in order to function as a true bone substitute that satisfies the biological, mechanical and geometrical constraints. Such characteristics include: (1) biological requirement – the designed scaffold must facilitate cell attachment and distribution, growth of regenerative tissue and facilitate the transport of nutrients and signals. This requirement can be achieved by controlling the porosity of the structure, by providing appropriate interconnectivity inside the structure, and by selecting appropriate biocompatible materials; (2) mechanical requirement – the designed scaffold must provide structural support at the site of replacement while the tissue regenerates to occupy the space defined by the scaffold structure. Scaffold structures need to be defined to possess the required mechanical stiffness and strength of the replaced structure; and (3) anatomical requirement – it must be of an appropriate geometric size that fits in at the site of replacement [46,47].

A CATE-based approach for biomimetic modelling and design of load-bearing tissue scaffolds and replacement has been introduced [46–48]. This approach starts with the acquisition of non-invasive image and the image processing of appropriate tissue region of interest, followed by a 3D re-construction of anatomical structure using enabling image-constructive and reverse-engineering techniques, CAD of scaffold unit cells to represent various tissue anatomical and morphological features, characterization of the structural heterogeneity and mechanical properties for both tissue and designed unit cell through either analytical, numerical or quantitative CT methods in order to select candidate unit cells for final scaffold, and a final design of scaffold with specified internal architecture and anatomically compatible external geometry. Detail of the process is reported in our research paper in this issue [4a].

SFF in tissue engineering
Tissue scaffolds can be used to either promote natural regeneration, in vivo, or be used to create a bioartificial organ, in vitro. The make-up of the ECM, pore size, biodegradability, cell-adhesion factors, growth factors, immunological response, cytotoxicity and the required vasculature of the tissue and scaffold all play an important role in the design of the scaffold [50]. Two types of strategy are utilized in developing scaffolds. In the first strategy, the scaffold has to provide support in vivo. In the second strategy, the scaffold only provides support in vitro until the cells are strong enough to support themselves in vivo [44]. Some of the above concerns can be addressed by biomimetic modelling and design, as described above. Application of SFF technology in tissue engineering constitutes another important component in CATE.

There are a number of different materials, techniques and processes that are being explored and combined [51–56]. For example, porous biodegradable scaffold matrices have been fabricated by many researchers using leaching and gas-foaming techniques [54], extrusion combined with salt leaching [52] or other techniques such as combining leaching with freeze-drying [56] or PLGA [poly(DL-lactic-co-glycolic acid)] foams with leaching and embedded hydroxyapatite (HA) short fibres for fibre reinforcement [57]. Although many applications of using rapid prototyping techniques in biomedicine and tissue engineering have been reported [57–59,67], SFF technology for manufacturing tissue-engineered constructs still appears to be in a relatively early stage. Among available techniques, 3D printing (3DP) and fused deposition modelling (FDM) seem to be the most promising processes in tissue engineering because of the versatility of using scaffolding materials, the possibility of making the scaffold in a cell-friendly environment and the feasibility of controlled deposition with high precision and at rates much higher than comparable methods such as indirect mould fabrication [6,7,57]. Because of these advantages, new developments on SFF to tissue-engineering fabrication are mostly the variations of these two processes, for example, 3DP-based TheriForm™ fabrication and Ink-Jet 3D-printing, and FDM-based precision extruding and micro-nozzle/micro-syringe deposition. The following section focuses solely on some new developments using the 3DP and FDM processes, as well as their variations, in tissue scaffold fabrication.

3DP process
The use of 3DP with starch polymers for scaffold fabrication was reported by Lam et al. [58]. A mixture of cornstarch, dextran and gelatin was used to create cylindrical scaffolds using a Zcorp 3D printer (Z402). The powder blend consisted of 50% (w/w) cornstarch, 30% (w/w) dextran and 20% (w/w) gelatin. Distilled water mixed with blue dye was used as the binder with the intention of not using organic solvents. Scaffolds were designed on CAD software, then sliced using a slicing algorithm. Post-processing was done to increase the strength of the scaffolds by drying at 100°C for 1 h. Afterwards, tests were conducted to examine their material properties, such as shrinkage, water absorption, porosity and mechanical properties. Porous scaffolds exhibited compression stiffness of 0.059 to 0.102 MPa for the various different designs that were reported.

FDM process
FDM technology has been extensively studied by Hutmacher’s group [59] with PHIL (poly(6-hexanolactone
Filaments of [poly(ε-caprolactone)] were used to create bioresorbable scaffolds. Testing was done on its material properties related to anisotropy and porosity. Channel sizes ranged from 160-700 μm. Compressive stiffness ranged from 4 to 77 MPa. Different layer patterns were used such as square and 'honeycombed'. The FDM scaffolds were also seeded with fibroblast cells and were successful in creating cell growth and proliferation. Cells initially started at the junction points of the scaffold, grew along the rods, began filling up the pores, and then finally, by week 4, the entire scaffold was filled with cells. Seeding with osteoblast-like cells also showed a similar migration pattern [60].

Theriform™ process Licensing the technology developed at MIT, Therics Incorporated has applied the 3DP process to tissue engineering and developed the Theriform™ process. Various experiments have been reported using the process: for example, Zeltinger et al. [61] created PLLA (poly(lactic acid)) scaffolds for testing the effects of porosity on cellular growth in vitro. Fibroblasts, smooth-muscle cells and epithelial cells were seeded on to the scaffolds. Their process involved salt leaching to create the pores in the scaffold. Kim et al. [62] created a scaffold from PLGA that contained interconnected scaffolds to improve circulation. A Cellmax Quad flow perfusion system used to provide a flow rate of 1.4 ml/min through the scaffold. Hepatocytes were grown on the scaffold successfully and showed benefits under flow conditions, owing to the channels. This has the potential to improve liver implant experiments where poor vascular flow has led to transplant-cell death. However, the procedure also combines salt-leaching techniques in combination with rapid prototyping to determine the porosity of the scaffold. A study on the use of the Theriform™ process to fabricate pills with highly controlled release rates as they dissolve was reported in [63]. This could also be applied to controlled amounts of drugs and growth factor. Experiments by Wu et al. [63] reported the feasibility of computer-aided drug design using solid freeform fabrication. Concentrations of the drug can be minutely deposited while creating the pill, so that the amount of drug released over time can be controlled. Different materials and drugs could also be used within the same pill. The similar concept can be extended to tissue scaffolds to control the degradation and release of biological chemicals and factors.

Microsyringe/micronozzle-based biopolymer deposition

A micronozzle-based layered manufacturing technique, also referred to as 'precise extrusion manufacturing' (PEM), was developed for tissue scaffold fabrication [64-67]. Using PEM, thermoplastic material (PLLA) was deposited through a heated, compressed-air sprayer. The nozzle diameter of the sprayer was about 0.3 mm. The liquefi er was heated and kept at 160°C during the process. Yan et al. [65] were able to create controlled pore sizes of 200-500 μm. Their machine worked off of a CAD model and used NC (numerical control) technology for manufacturing the scaffold. We [66,67], too, reported similar studies in which we constructed a cellular PHL scaffold by means of our in-house-developed micronozzle freeform deposition system. The scanning-electronmicroscopic characterization shows that thus-fabricated scaffold microarchitectures could be achieved at about the 250 μm scale level with excellent uniformity of the fill gaps, the depositing struts and the internal pore connectivity.

A microsyringe-based polymer deposition process was reported in [68]. This process is similar to 3D plotting, but uses a microsyringe and stepper motor positioner in order to achieve higher resolutions. The microsyringe had an outer diameter of 10-20 μm. A viscous solution of PLGA in chloroform was deposited by compressed air. The syringe was manipulated by a three-axis stepper motor micropositioner with a precision of 0.1 μm. The authors were able to achieve deposition resolutions of 10-600 μm diameters depending upon pressure and motor speed; 10 μm was the smallest line-width they could create. Smaller resolutions were possible, but would have required a smaller syringe tip, and would have resulted in much higher pressures than were feasible. The same group also applied soft lithography techniques to create three-dimensional structures using a micromoulding technique. Essentially, a poly(dimethylsiloxane) mould was created. Then PLGA was vacuum-moulded to the desired shape. The mask resolution was approx. 20 μm, but the actual micromoulding resolution was only 30 μm, owing to swelling of the mould. 3D structures were created by layering the molded 2D patterns on top of each other in a laminated pattern and then fusing them together with heat. However, this layering and pattern alignment seems to have to be done manually.

Issues on building 'smart' scaffolds

No 'perfect' scaffold has been developed yet. The 3D structure of the scaffold is very important, since experiments have shown that cells respond and interact to surface features. Cell adhesion and migration can be controlled by the topological characteristics of the scaffold. In addition, chemical factors can be incorporated into the scaffold itself for even greater control over adhesion, migration and differentiation [69]. For example, experiments with tissue-engineered blood vessels have shown that endothelial-cell and smooth-muscle-cell proliferation could be controlled by the choice of biopolymer and the use of bioactive proteins [70]. Future scaffolds will be intelligently designed that mimic the cell's ECM and enhance natural tissue growth. 'Mechano-active' scaffolds could incorporate encapsulated calcium-channel agonists into the scaffold along with other
elements to preferentially release growth factors in response to mechanical stresses, thus mimicking the natural body [80].

'Bioactive' biomaterials seem to be a trend for scaffold design. Scaffolds will incorporate adhesion-promoting oligopeptides and oligosaccharides based upon natural proteins such as fibronectin and laminin. Concentrations of these ligands will also be regulated to create a fine balance between overly weak and overly strong adhesion properties – thus improving adhesion without hindering cell migration rates. Cell binding specificity will also be regulated by bioactive scaffolds [71,72]. Temperature-sensitive gels designed for optimal release of chemicals or alterations in mechanical properties [73] could also be incorporated into the scaffold. With rapid prototyping, one could possibly construct a scaffold of mixed materials. A bioactive scaffold material made of a stronger structural material could be mixed with a temperature-sensitive gel that dissolves at body temperature to release biological compounds as needed. Another method of creating bioactive scaffolds is through the fusion of gene therapy with tissue engineering. Novel genetic material in the form of plasmids may be added to the scaffold – i.e. gene-activated-matrix technology. Fibroblasts migrate on to the scaffold, incorporate the DNA plasmids and begin expressing the plasmid genes [74]. It could also be possible to genetically pre-modify the cells in vitro using plasmids or viruses in order to improve cell growth during the in vitro stage or to promote scaffold incorporation and wound healing while in vivo. It would probably be preferable to do most of this in vitro, since it is more easily controlled. One would also avoid the risk of an immunological response in vivo, especially when dealing with viral vectors.

For large tissue implants, blood supply is crucial. If it takes too long for blood vessels to be formed, the bioengineered tissue may die before it can incorporate itself into the host. Growth factors and seeding the tissue scaffold with endothelial cells may accelerate this process. Also, in large tissue implants, structural strength is vital. To manage this problem, heterogeneous scaffold materials and structures should be used – one to maintain structural integrity and the other for incorporating cells and chemical factors. For building large tissue substitutes [75], integration of both biological tissue and non-biological artificial elements, such as syringes, drugs, tubes, sensors, electronics, and nano- or micro-scale biodvices into the design and fabrication of scaffold is critically important for designing the next generation of 'smart' scaffolds.

3D cell and organ printing

3D cell and organ printing advances SFF to construct a 3D object with living biological species, such as specific tissues or organisms. A fundamental requirement of this process is its capability of simultaneously delivery scaffolding materials, living cells, nutrients, therapeutic drugs, growth factors and/or other important chemical components at the right time, right position, right amount and within the right environment to form living cells/ECM (or scaffold) for in vitro or in vivo growth. Cell and organ printing, like any other SFF process, requires: (1) a blueprint model, which is a software representation containing bio-information, physical and material information, anatomical and geometric information of the to-be-printed tissue or organ; (2) a process model, which is also a software representation that contains the printing operation control commands, process planning and toolpath generated for the bio-blueprint model and machine hardware and control system; (3) a process machine, which is a hardware representation that possesses the functionality of the printing; and (4) a tissue/organ culture system which can maintain and grow the printed living objects. A conceptual definition for computer-aided jet-based 3D tissue engineering of living human organs was proposed in [76]. The authors described the 3D organ printing process as three major steps: (i) pre-processing or development of 'blueprints' for organs; (ii) processing or actual organ printing; and (iii) post-processing or organ conditioning and accelerated organ maturation.

Bio-blueprint model for 3D cell and organ printing 3D cell and organ printing requires a description and representation of details of organ anatomy, morphology, tissue heterogeneity and vascular systems at different tissue/organ organizational scales. For example, cell deposition in 3D cell and organ printing is controlled through a process planning program. In the printing process, the toolpath guides the printing head(s) to deposit cells as needed to form a 3D tissue or organ construct. In order to print a specific organ, the toolpath program needs to know detailed data of the geometry of the to-be-printed organ, the organ internal architectures, the boundary of the heterogeneous tissues within the organ, and the organ vasculature and its topology. In addition, the toolpath program should also contain the information on cell compositions so it can guide the printing heads to deposit the right cells at the right time and at the right location. The above information often leads to an extremely complicated database, and in most cases it can only be processed (i.e., information storage and retrieval) by a CAD-based model owing to the specific requirements on the geometry and topology. We define such a computer model as a 'bio-blueprint model for 3D cell and organ printing'. Specifically, the functions of the bio-blueprint model will: (1) describe anatomy, geometry and internal architecture of a organ (or tissue) of interest, including the tissue heterogeneity, the individual tissue geometry and the boundary distinction within the organ of interest; (2) define a vascular network and the 3D topology in a organ of interest; and (3) provide a needed database on organ/tissue geometry, heterogeneity
and the associate vascular network that can be used for toolpath generation of 3D cell and organ printing.

The framework of development of a biomimetic model is outlined through the following major steps: step 1, development of a computer modelling representation of a 3D organ; step 2, development of a 3D vascularization network; step 3, development of a CAD-based organ bio-blueprint model.

The development of a bio-blueprint model starts from the 3D reconstruction of organ anatomy (without vascular system) from the given modalities (medical imaging data), and on the development of a CAD-based modelling representation that can be used to explicitly describe organ geometry, topology, and individual tissues. The model development will involve using state-of-the-art 3D reconstruction [16], reverse engineering [21], a CAD platform [77] and the in-house heterogeneous modelling algorithms [78, 79]. We generate such a bio-blueprint model from non-invasive medical imaging (obtained from the public domain and [80] and/or from patient-specific CT, MRI, optical image, X-ray, micro-CT, etc.) for capturing and replicating organ/tissue anatomy, including detailed internal and external morphology, geometry, vascularization and tissue identification. Therefore all following three hierarchical scales, as shown Figure 9, need to be considered in the construction of the bio-blueprint model: (1) the scale of the organ (to consider the organ's macrostructure tissue types, vasculature, ducting and anatomical compatibility); (2) the scale of the tissue, or suborgan (to consider the heterogeneity of tissue with appropriate type of cells and their interaction); and (3) the scale at the cellular level (to consider the selection of ECM or scaffold materials and to divide bio-blueprint model into small blocks to enable local definition of ECM/scaffold materials).

The bio-blueprint model is represented in a CAD format which adopts 'boundary representation' by which an organ or tissue anatomy and topology can be explicitly described by the enclosed and adjacent boundaries through mathematically defined NURBS functions [81]. Reducing memory usage of the bio-blueprint or of interpolating structures not visible in medical imaging, repetitive or patterned structures can be done by reducing to combinations of feature primitives. This method also smoothens out irregularities in continuous features caused by noise in the imaging modality. For instance, a feature primitive based reconstruction method for vascular networks is used to generate a 3D biological vascular system for organ growth. In this primitive feature modelling approach, the basic vascular primitives (e.g., crotch or segment) characteristic parameters are determined from patient-specific CT/MRI images (Figure 10), and further use of Boolean operation algebra forms a high-level vessel assembly. The vascular feature primitives are represented as NURBS bases, and the parameters in the NURBS equations can be determined through measuring the spatial positions of the vascular CT/MRI images at different projections. Procedures of this reconstruction are schematically illustrated in Figure 11.

Strategies of applying bio-blueprint model for tissue engineering

The bio-blueprint model not only provides the needed biological information for organ anatomy, tissue heterogeneity and vascular networking, but also facilitates and introduces the design or bio-manufacturing intent, such as the biological intent of the cell types, combination of cell-growth factor, and tissue heterogeneity; the biophysics intent of the designed cell deposition, ECM and structural configuration, vascular design, and intended neovascularization post-implantation; and the biochemical intent of the ECM surface treatment and desirable cell–cell and cell–matrix interaction within one single database. The model at different scale levels can also be used to create small units of specialized tissue types as organ building blocks and to assemble these bricks in a time-dependent and order-specific pattern throughout the macro-structure of the tissue or organ to be printed, and to generate process planning for entire tissue or organ printing through CAD/CAM interface [4, 82].

The construction or printing of an organ in general involves at least three distinct scales. At the organ level, considerations must be given to the organ's input and output
vasculature and ducting, its connections to the nervous system, and its size and shape to maximize its compatibility with the prospective host, and assurance of anatomical compatibility for implantation. At tissue, or suborgan level, the intended volumetric domain must be seeded with the appropriate type of cells in the correct areas, and consideration must be given to how the cell types will interact with each other. In addition, macroporosity and channels for vasculature must be added to the tissue to enhance cell growth. Modification of the CAD blueprint is able to solve these problems. For example, the CAD blueprint can be divided by cell type as per signal segmentation, either allowing the two cell type regions to be grown separately then reassembled or by automated deposition of the cells in the correct pattern on a single tissue scaffold [83]. Finally at the cellular level, one considers the scaffold surfaces to which cells will readily bind. These must possess appropriate microporosity and growth factors to guide cell growth in the correct way. While this level must be addressed by selection of the scaffold material itself, the division of the CAD model into small blocks enables local selection of scaffold material rather than a single material for the entire organ scaffold. For instance, adjacent blocks might use totally different scaffold materials: one with a low microporosity for mechanical load properties, the other with high microporosity and embedded angiogenic factors for enhanced growth of soft tissue and vasculature.

With a 3D CAD-based bio-blueprint model of the desired tissue, subdivided through a knowledge base by manufacture requirements, tissue-culture requirements and feature primitive descriptions of patterned structures such as vasculature, a number of tissue-manufacture strategies become apparent. The bio-blueprint model may be modified by simple Boolean operations, split into any number of components or elements while conserving features to high precision. As these elements are independently exportable to freeform-fabrication technologies, the bio-blueprint model enables multi-component manufacture and assembly of tissue scaffolds. Strategies towards tissue engineering exploiting this capability include heterogeneous tissue block assembly and laminate assembly as described as follows.

(a) Heterogeneous block assembly approach. With a 3D CAD-based bio-blueprint model that can be subdivided by tissue type and reshaped as needed, a new approach to the manufacture of 3D organs becomes apparent. By dividing up the CAD blueprint into a number of small blocks, it becomes possible to construct all these
small brick-like components of an organ in parallel and then assemble them into the whole structure. This method is totally dependent upon the CAD model’s ability to be subdivided, or diced, with minimal loss of data. This approach to organ manufacture would alleviate some of the difficulties at each of the three scales of organ tissue scaffold design. Rather than attempting to find a universal scaffold material or assembling an entire organ through a low-pass approach and trusting to self-assembly to sort out the appropriate cell types and lay down vasculature, it may be advantageous instead to use a more piecemeal approach to constructing an organ. One suggested approach to organ manufacture is to create small units of specialized tissue types and to lay down these bricks in a time-dependent and order-specific pattern throughout the macrostructure of the organ to be manufactured. In doing so, the first step would be to develop a bio-blueprint model as described. Secondly, this model would be divided into the various tissue patterns that will be required to construct the organ. This pattern need not be truly biomimetic to the original. For simplified construction, it may be expedient to use the bio-blueprint model only for the external shape of the organ and use an artificial pattern of vasculature and tissue type in the manufacture process and finished product. The bio-blueprint should then be subdivided into small brick-like units. These units would then be constructed out of quartz or another material that cells will not readily attach to, or perhaps a common material simply coated in Teflon. These placeholder bricks should be stacked to create a quartz block replica of the organ to be constructed.

The large vessels, small vessels and capillary beds for the organ should be cultured outside of the quartz replica. The larger vessels and smaller vessels should be placed into the quartz frame, their scaffold units being swapped in to replace the quartz blocks. The initial capillary beds should be laid down immediately surrounding the initial vasculature. The vessels should then be fed with appropriate medium for tissue culture. Subsequently, over periods of weeks, based on the maturation rate of the tissue cultures, specialized cell types in scaffolds specialized for their development would be swapped in for the small quartz bricks around the vasculature. The vasculature would be permitted to mature with each additional ‘ring’ of tissue around it, and the capillary beds should expand through the new tissues. While this method would take several months, the result should be a more functional tissue construct.

The schematic 2D slice of the kidney in Figure 12 shows the process of heterogeneous tissue block assembly. First by creating a biomimetic structure of quartz blocks, the eventual shape of the organ is laid out. Then, appropriate blocks are removed to insert in vitro-grown vessels. Sculpted tissue scaffolds, the tissue already mature, are placed around the vasculature and given time to integrate. Subsequently, as the tissue blocks attach to each other, more quartz blocks are removed and replaced with tissue blocks, until a complete organ is constructed. The procedures are outlined in Figure 13. There are numerous potential advantages to the heterogeneous tissue-block-assembly approach. By laying the vasculature down first and allowing the vessels time to expand slowly through successive additions of tissue blocks, this technique should prevent cell death due to starvation and hypoxia. The additional tissue blocks would never be more than a centimetre from existing vasculature, so the cells in the scaffolds could survive through diffusion until the vascular tissue invades. Also, by creating the tissue in small, specialized blocks, each block is subject to specialized care to ensure that it forms the correct cell types in the correct geometry. In addition, these cell types will be nearly mature at the time of implantation, so will not be permitted to grow into other tissue types, as might happen were the organ printed in one unit. In addition, the slow addition of small units will apply incremental mechanical loading to the organ during manufacture, not the instantaneous heavy loading that would occur in a single printing of the entire organ and might crush the tissue or high-porosity scaffolding.

(b) Laminate assembly approach. An alternative less biomimetic approach is laminate assembly. Much like
heterogeneous tissue block assembly, laminate assembly requires culture of individual tissue components followed by assembly post-culture. The component structure for laminate assembly is individual slices of tissue of approx. 500 μm thick, enabling oxygen diffusion during culture. These tissue laminates would be stacked in turn with layers of vascular beds laden with angiogenic factors. An illustration of laminate assembly is displayed in Figure 14.

The enabling cell/organ printing system. The cell printer described in [76] showed its capacity to print gels, single cells, and cell aggregates. It was reported that the layer-by-layer sequentially placed and solidified thin layers of a thermo-reversible gel could serve as a 'printing paper' in this technology. The closely placed cell aggregates or embryonic tissue explants can fuse within 3D gels and morph into ring-like, tubular structures was presented in [76] to demonstrate the feasibility of proposed organ printing technology.

Development of cell printing was also reported by using the Bioplotter (Envision Technologies, Marl, Germany). The Bioplotter basically converts a CNC Automation (Amherst, NH, U.S.A.) milling machine with a liquid dispenser driven by compressed air for material deposition. Various materials can be deposited via syringe needles with a diameter of 0.1–2 mm. The preferred inner diameter in experiments was reported to be around 0.25 mm for good resolution and speed. The syringe/cartridge set-up gave it great versatility and simplicity, since one only had to switch the cartridges to change materials. The maximum resolution, however, was only about 200 μm and was controlled by air pressure, nozzle inner diameter and properties of the resin system.

One of the main advantages of the Bioplotter was that there was no heating involved that could kill cells or denatured proteins and growth factors [84]. Complex 3D structures can be built using Bioplotter by creating the scaffold with a liquid that has the same density as the scaffold material. This aqueous environment allows the seeding of living cells into the scaffold. Various materials can be used for the scaffold — pastes, resins, gels, bone cements, cellulose, silicone, polyurethanes, hydrogels, and ceramic powders. Hydrogels are interesting scaffold material in that they could be used for cell seeding, drug-delivery systems, and for integrating growth factors [85].

The 3D plotting machine reported in [86] is very similar to Bioplotter, and uses a desktop robot and a pneumatic syringe assembly. The Teflon-coated nozzle had an inner diameter of 150 μm. Three different materials (chitosan, chitosan/20% HA and chitosan/40% HA) were contained in three different 30 ml barrels. The 3D plotter could also handle STL files, an advantage over the Bioplotter system in Germany, as claimed in [86]. The setup was used to create a chitosan/HA scaffold. The scaffold material was dispensed as a hydrogel. They were able to form continuous internal channels, and create pore sizes of 200–400 μm in the chitosan/HA scaffolds. The scaffolds were seeded with fibrin glue and osteoblast cells for in vitro studies. The cell seeding of the scaffold was successful and showed healthy cells with good migration throughout the scaffold.

It may be possible to implant electronics and circuits into the bioartificial organ for monitoring purposes. Metal jets are potentially capable of creating 3D circuits [97], and biosensors built using inkjet technology are already possible. Perhaps a multi-head rapid-prototyping machine could incorporate active electronic technology into the tissue-engineered implant. Simple off-the-shelf ink-jet printers
Conclusions

The present Review outlines an emerging field of CATE and its three major categories of computer-aided tissue modeling, computer-aided tissue informatics, and computer-aided tissue scaffold design and manufacturing. Some enabling computer-aided technology and its application to tissue engineering have been described. New developments in the CATE categories, particularly on 3D reconstruction, computer-aided tissue scaffold manufacturing, bio-blueprint model development and its application to 3D cell and organ printing have been introduced.

Application of CATE allows one to explore many novel approaches in modeling, design and fabrication of complex tissue scaffolds that have enhanced functionality and improved interactions with cells. Central to CATE is its ability to represent pertinent tissue biological, biomechanical and biochemical information as a computer, and in most cases, a CAD-based, bio-tissue informatics, model. This model can be used as a communication tool between biologists and tissue engineers, and the database of the model serves as a central repository to interface design, simulation and manufacturing of tissue substitutes. In this regard, CATE facilitates the advance of tissue engineering from its segmental disciplinary and empirical laboratory-based study to integrated empirical, laboratory and computer modelling- and simulation-based multi-disciplinary research.

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