Computational Approaches in Tissue Engineering

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ABSTRACT
Scaffolds designed with intricate and controlled interior architecture represent a challenging problem for tissue engineering. Various scaffold-fabricating techniques allow the creation of complex micro-structure but with irregular pore characteristics, resulting in inappropriate & asymmetrical structures not suitable for tissue engineering applications. Computer-Aided Tissue Engineering (CATE) integrates advanced technologies from Biology, Information Science and Engineering for Tissue Engineering applications. In particulars, computer-aided design (CAD), medical image processing, computer-aided manufacturing (CAM), and solid freeform fabrication (SFF) are employed for simulation, design and manufacturing of tissue scaffolds with controlled and regular pore architecture. CATE application to the design and fabrication of scaffolds can guide to improve the biomimetic and biological features of the scaffolds. This paper aims to understand the principles behind various computer aided approaches being utilized for tissue engineering applications particularly cell-scaffold implant modeling, designing and manufacturing.

Key Words
computer-aided tissue engineering, scaffold manufacturing, CAD, CAM, SFF, 3D printing

1. INTRODUCTION
Tissue Engineering has been evolved as an interdisciplinary field that utilizes principles of engineering and life sciences towards the development of biomimetic substitutes for restoration, maintenance and improvement of tissue function. [1] Traditional methodologies are mechanical replacement and organ transplantation which faces their limitations of availability and acceptance by body. Tissue engineering provides permanent solution to the problem of organ failure. Commonly adopted tissue engineering approach involves the use of cells, scaffold and oftenly bioactive agents such as growth factors and/or bio-adhesive peptides (eg. RGD peptides). [2] Cells for tissue engineering are isolated from patient or donor, cultured to a large population and then seeded in to a biocompatible porous scaffold. The cell-scaffold construct can be directly implanted into the patient or after in vitro maturation of the construct. Tissue maturation is indicated by proliferating differentiated cells, extracellular proteins and possible vascular network formation. [3] Role of scaffold is to provide physical & chemical support to guide the cell growth & organization into 3D tissues. Oftenly, a biodegradable material is chosen for scaffold preparation so as the newly formed extracellular matrix takes over its supporting function.[4] A wide range of biomaterials including polymers, ceramics in native or composite forms are generally employed for this purpose. The selection of biomaterial determines the mechanical property, degradation behavior & biological functionality of the scaffold. The shape & internal pore architecture of scaffold also influence above characteristics. [5] In vitro study shows that improper internal architecture results in scarcity of oxygen & nutrients to cells, thus affecting the depth penetration of proliferating cells inside the scaffold. Conventional methods of scaffold d fabrication produces process dependent pore architecture incapable of precisely conrol the size, shape & spatial distribution within the scaffold volume. [5-6] Cells grow over the periphery of scaffold and inhibits the deeper diffusion of oxygen & nutrients thus prevent further cell migration and vascularization.

Despite the enormous advances in tissue engineering resulting into clinically viable products such as artificial skin, several challenges still exists to prevent widespread clinical applications. Besides of regulatory and ethical issues, scientific barriers such as acquiring adequate cell source, engineering complex vascularized tissue that mimics native architecture with mechanical and metabolic function similar to normal tissues are very difficult to cope up. Utilization of computer aided technology in tissue engineering have greatly helped to model, design & manufacture scaffolds that can efficiently replace the native tissues.

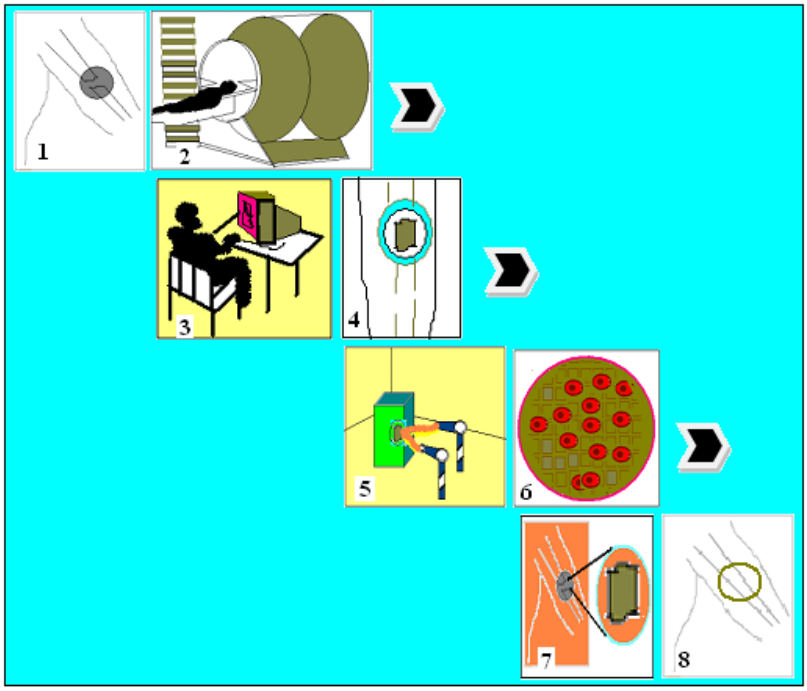

Fig 1: Principle behind computer aided scaffold-based tissue engineering. Scaffold is tailored based on medical imaging data (1-5), seeded with cells (6) and implanted into the patient (7). After few months, the scaffold is resorbed and replaced by body tissue to finally form functional tissue (8).(Modified figure from the work of W. Sun et. al)

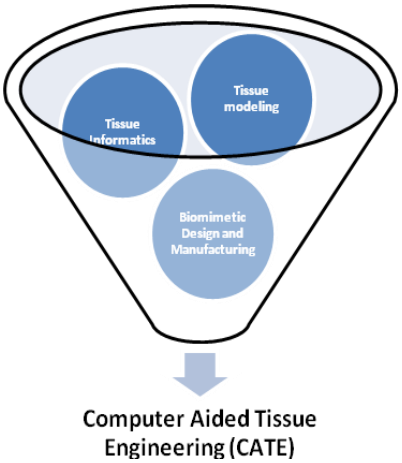

Fig 2: Components of Computer Aided Tissue Engineering

Computer-Aided Tissue Engineering (CATE) integrates advanced technologies from Biology, Biomedical Engineering, Information Technology, and modern Design and Manufacturing to Tissue Engineering applications. In particulars, computer-aided design (CAD), medical image processing, computer-aided manufacturing (CAM), and solid freeform fabrication (SFF) are employed for simulation, design and manufacturing of tissue and organ substitutes. In a broad definition, CATE embraces three major applications in tissue engineering: (1) computer- aided tissue modeling, including 3D anatomic visualization, 3D reconstruction and CAD-based tissue modeling, and bio-physical modeling for surgical planning and simulation; (2) computer-aided tissue scaffold informatics and biomimetic design, including computer-aided tissue classification and application for tissue identification and characterization at different tissue hierarchical levels, biomimetic design under multi-constraints, and multi-scale modeling of biological systems; and (3) Bio-manufacturing for tissue and organ regeneration, including computer-aided manufacturing of tissue scaffolds, bio-manufacturing of tissue constructs, bio-blueprint modeling for 3D cell and organ printing. Principle behind the computer aided tissue engineering is depicted in Figure 1 with an overview in Figure 2. Details of the applications and developments were reported in [7-9], respectively.

2. BIO-CAD MODELING

Recent developments in the computing technologies both in hardware and software side have have evolved as advances in CAD application beyond conventional design and analysis. CAD software is used in architecture, engineering and manufacturing in precision drawing or design. Now-a-days CAD is being extensively used in biomedical engineering.[8, 10-11] This development is due to advances in imaging technologies including computed tomography (CT), magnetic resonance imaging (MRI), micro CT and optical microscopy. Data derived from these technologies, computer models have been reported in published literature [8, 12-13] for simulation of human bone joints, designing implants & scaffolds etc. Modelling of human body parts in a CAD based virtual environment is BioCAD modeling. [14]

An ideal bioscaffold should be designed to mimic the architecture, biomechanics, biocompatibility and biochemical micro-environment of the native tissue. These issues as a whole can be represented as 'scaffold informatics' model. [14] Central issue of CATE approach is its ability to represent as 'Bio-scaffold informatics' model, providing the basic morphology, anatomy & organization of the native tissue to the scaffold with specific characters like cell attachment, composition, internal pore architecture and surface chemistry.

2.1. Bio-CAD modeling technique based on image

Bio CAD modeling starts from anatomical data acquisition from appropriate medical imaging modalities that must be capable of providing every bit of information for producing 3D views of anatomy, discrete tissue types and vascularized structures. Such image based Bio CAD modeling process involves following inevitable steps:

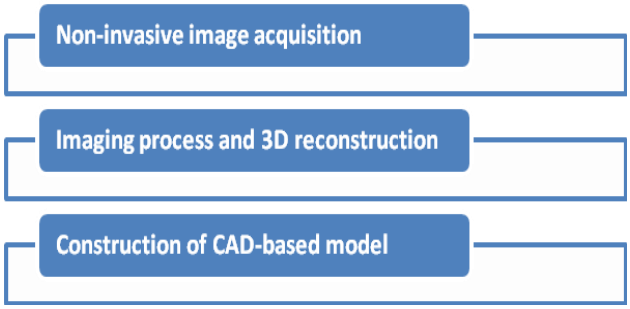

Fig 3: Different steps involved in Bio CAD modeling

2.1.1. Non-invasive imaging data acquisition

The imaging modalities used in tissue modeling are CT, MRI, optical microscopy and µCT. CT or µCT scans require exposure of a sample to small quantities of ionizing radiation, the absorption of which is detected and imaged. This results into a series of 2D images that can be stacked together to create 3D display of scanned site. The micro-CT technology can be used to access the structure and function of tissues at micro-scale. Quantification of micro structure function relationship of tissues and the designing tissue structures, including characterizing micro-architecture of tissue scaffolds by micro-CT has helped to design and fabricate of tailor-made tissue microstructures. Recent advances in micro-CT imaging [15], such as faster detectors, slip-ring technology, and dedicated rodent scanners, have made it possible to obtain high-resolution CT images with an isotropic spatial resolution of 0.075– 0.15 mm [16-17]. Non-invasive imaging of ventilation has been shown to be sensitive to a variety of lung diseases [18]. Wilfred W. Lam applied micro-CT to imaging of ventilation in rat lungs. The technique presented were found ideally suited for measuring changes in regional ventilation that result from heterogeneous lung disease in rat models.[19] Due to high resolution, CT and micro-CT as an imaging modality for tissue engineering purposes have got wide applications. MRI provides images both for soft tissues as well as for hard tissues. This imaging modality is much superior due to its capability to differentiate the tissue sites with similar density. Dhenain et al. performed micro-MRI scans on mouse embryos and resolution achieved was 20–80 micron voxels. Using simple region growing techniques and mimics software, the resulting segmentation isolated each of the major developing organs in the embryo. Sun W et. al. developed a 3D representation for the central nervous system, heart, and kidneys of the subject as reported in literature.[8] Mark Dewey et al detected coronary artery stenoses noninvasively with multislice CT or MRI [20] J.A. Detre et al evaluated the utility of cerebral blood flow (CBF) in conjunction with pharmacologic flow using magnetic resonance imaging (MRI). This ability to provide information inexpensively and noninvasively during a structural examination extends the diagnostic potential for MRI. [21]

2.1.2. Reconstruction for 3D image representation

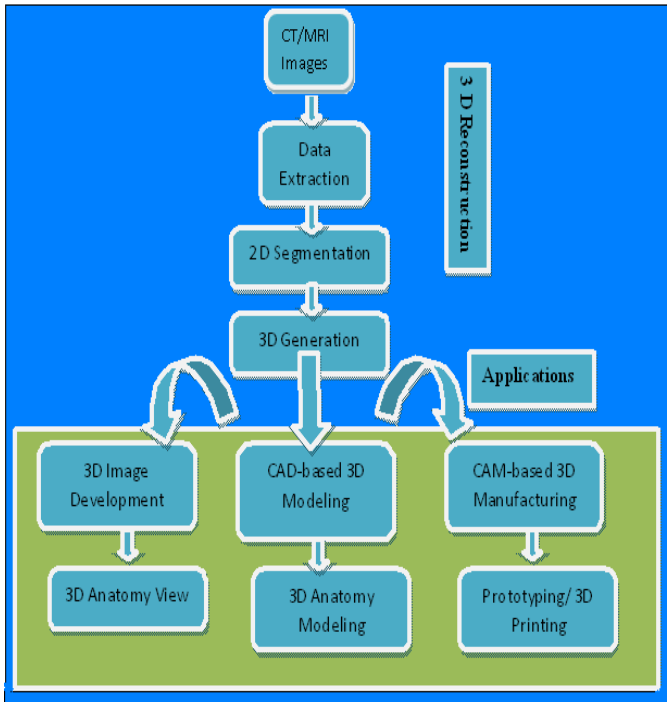

Fig 4: Flow Chart for 3D Reconstruction of Medical Images & Applications

The medical images (CT/MRI images) are segmented into 2D information and integrated into 3D region growth. These volumetric image data yield more meaningful, three-dimensional anatomic view.of body parts. The volumetric information of body parts of patient, their representation and anatomical view helps in medical modeling. The shades around the surface of 3D objects are removed by widespread processing of image data for more clear computer representation of object. Even though many visualization issues cannot be resolved by CAD models inspires for the construction of a prototype model. In Prototype modeling, constructive processes are involved as opposed to subtractive processes. Such models helps in model assisted applications like in in computer assisted surgery (Preoperative planning & intra-operative planning).

2.1.3. Construction of CAD based biomodeling

The 3D tissue descriptions produced by non-invasive modalities, such as CT, Micro- CT, MRI and Optical Microscopy are accurate, however, the voxel-based anatomical imaging representation cannot be effectively used in many biomechanical engineering studies. For example, 3D surface extraction requires either a large amount of computational power or extreme

sophistication in data organization and handling; and 3D volumetric model on the other hand producing a realistic 3D anatomical appearance, does not contain geometric topological relation. There are three different process paths for generating a CAD model from medical imaging data (Table1) [14]:

a. MedCAD interface approach,
b. Reverse engineering interface approach,
c. STLtriangulated model converting approach.

3. BIOMIMETIC DESIGNING

Scaffolds designed with regular and controlled internal architecture represents a challenge for tissue engineering. Various scaffold-manufacturing techniques allow the creation of complex structure but with no or very little control over the main features of scaffold such as the size, shape, and interconnectivity of pores, resulting in inappropriate & irregular structures not suitable for tissue engineering applications. The combined application of computer-aided design (CAD) and manufacturing techniques allows a higher degree of control over these scaffold characteristics with very few limitations in terms of achievable complexity. The designing of complex pore architecture with interconnected channel networks in CAD is itself a tedious and time consuming since it requires modeling hundreds of different geometrical elements with different pore characteristics, oftenly it takes several days to design a single scaffold architecture manually. An automated design methodology has been proposed to overcome these limitations. [14] This approach involves interaction of CAD program with novel software algorithms and permits the automated designing of several geometrical elements, with particular characteristics. This methodology has been used by Sun W. et al to design five cubic scaffolds with interconnected pore channels that range from 200 to 800 mm in diameter, each with an increased complexity of the internal geometrical arrangement.[22] Clinical case study of craniofacial implant consisting of an integration of one of these geometries has also been presented.

TABLE 1: Comparison of different process paths for generating CAD model from imaging data

MedCAD interface	Reverse engineering interface	STLtriangulated model
Intended to bridge the gap between medical imaging and CAD design software.	Uses a 3D voxel model produced by segmentation.	The 3D voxel model converted to the STL file and this STL file can then be imported into reverse engineering software for surface refinement and NURBS surface generation.
Follows IGES (International Graphics Exchange Standard), STEP (Standard for Exchange of Product (STEP) or STL format. It can export data from the imaging system to the CAD platform and vice versa	3D voxel model is converted to point data form and the points are loaded into a reverse engineering software. The points are then triangulated to form a faceted model	Differs from the reverse engineering approach in utilization of STLtriangulated surface as modeling input rather than the point clouds data.
Provides limited ability to model a freeform surface. Incapable to capture detailed and complexed tissue anatomical features, particularly with complex geometry.	Takes comparatively longer processing time, resulting into significantly better output than the other two methods	Process time is more efficient, Inherit all limitations of STL format
Easiest and quickest approach	Suitable for complex shapes since control is achieved at every level.	Quick method to arrive at a CAD Model
Not suitable for complex models	Longer process	May not work if triangulated surfaces contain errors

3.1. Biomimetic design of scaffolds for hard tissue regeneration

Besides the biological and anatomical requirements, the load bearing tissues need to satisfy mechanical and geometrical constraints. Characteristics of such scaffolds include:

a. **Anatomical requirement**—the scaffold must have an appropriate geometric size that exactly fits the site of replacement.
b. **Mechanical requirement**—the 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 that have the required mechanical stiffness and strength of the replaced structure;
c. **Biological requirement**—the 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;

Now it is possible to fabricate tissue scaffolds will all the above design requirements using Bio-CAD modeling, medical image processing and solid freeform fabrication. For example, scaffolds with designed internal architecture and specified geometry with selected biomaterials.

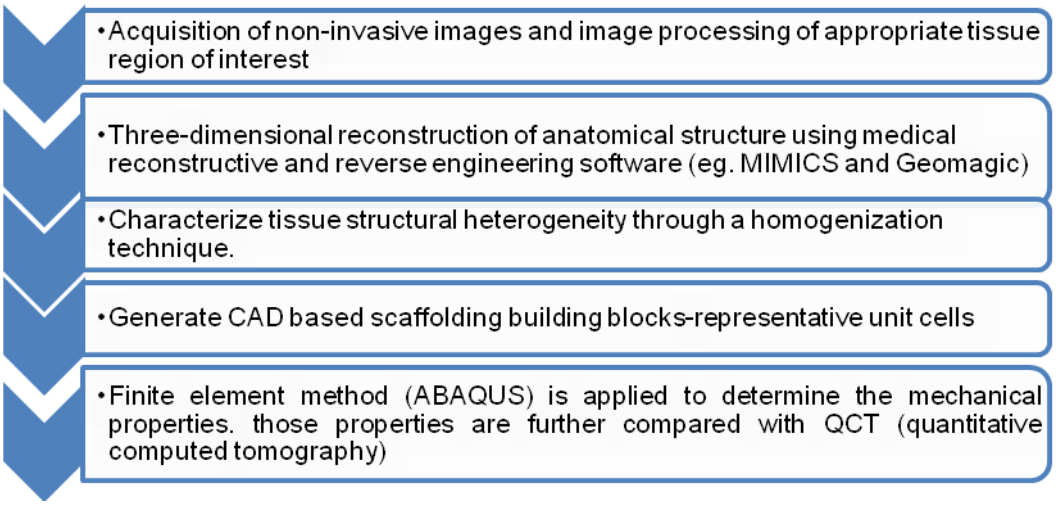

FIGURE 5: Flow chart for designing process of tissue scaffold

4. SCAFFOLD FABRICATION BY SFF

Conventional techniques for preparing porous structures to use as tissue engineering scaffolds comprise porogen leaching, phase-separation/freeze-drying and gas foaming processes. However, in the last decade, solid freeform fabrication (SFF) methods have increasingly been applied for this purpose. SFF refers to the computer-controlled fabrication of parts in an additive process, from a computer-aided design. Using these techniques, it is possible to generate individual products or small series of products with complex structures in a fast way.[23] The first intended and most common use is rapid prototyping (RP), in which SFF techniques speed up and improve the design process by enabling the making of physical models of designed products in a matter of hours. For high-end applications such as tissue engineering, the relatively high cost per produced part can be acceptable to employ SFF techniques for manufacturing purposes. As a result of the development of these technologies, tissue-engineered constructs can be prepared that contain a controlled spatial distribution of cells and growth factors, as well as engineered gradients of scaffold materials with a designed microstructure.[24]

Advanced mouldless manufacturing techniques, commonly known as solid free-form fabrication (SFF), rapid prototyping (RP) or, more colloquially, art to part technology. Layer wise deposition of added materials is guided by computer program. Each layer represents the cross sectional model of scaffold at specified level. RP techniques can be easily automated and integrated with imaging techniques to produce scaffolds that are customized in size and shape allowing tissue-engineered grafts to be tailored for specific applications or even for individual patients. Rapid prototyping is a class of standard techniques to manufacture prototypes from complex 3D datasets. Several rapid prototyping machines are available commercially with their pros and cons. Important techniques for Rapid prototyping are stereolithography, fused deposition modeling, selective laser sintering, and 3D printing.[25] Due to the capability to fabricate complex 3D shapes with controlled internal architecture, the use of rapid prototyping to generate scaffolds is a promising area. Several studies have been done to investigate the application of different rapid prototyping technologies for the manufacturing of scaffolds for tissue engineering applications.[26].

SFF techniques have several advantages over conventional techniques:

a. Excellent control over (pore network) design and properties; optimization is possible
b. Excellent reproducibility; small sample-to-sample variations
c. Improved mechanical properties of the scaffolds
d. Higher pore interconnectivities, enhanced permeability of the scaffolds
e. Better suited for modeling (computational flow dynamics, cell proliferation and differentiation behavior)

4.1. Tissue engineering of patient-specific implants

This innovative treatment protocol uses medical imaging, computational modeling and bioresorbable scaffold fabricated with rapid prototyping (RP) technique. The patient specific scaffold designing and manufacturing has been represented through a flow chart (Figure 6).

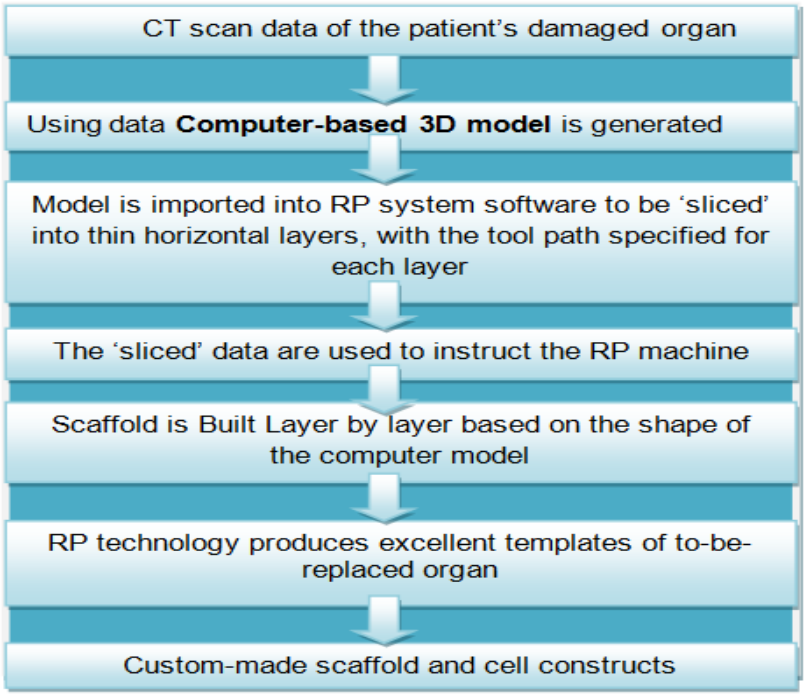

Fig 6: Flow chart for SFF of tissue scaffold using RP technology

4.2. 3D Printing technology

3D printing (3DP) technology, developed at MIT has became one of the most investigated SFF techniques in tissue engineering and drug-delivery applications. 3D printing of cells or biomaterials has been used to fabricate 3D tissue constructs. 3DP provides layer wise deposition of cells or biomaterials in a rapid fashion to create construct. A fundamental requirement of 3DP is its capability to simultaneously deliver scaffolding materials, living cells, nutrients, therapeutic drugs, and 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/extracellular matrix (or scaffold) for in vitro or in vivo growth. Conventional printing methods are laser printing and inkjet printing. Disadvantages of the laser printing are heat generated during the process which may affect the cells viability and it is unsuitable for larger structures involving thousands of cells.

Cell/Organ printing, requires:

a. **A blueprint model**, which is a software representation containing bio-information, physical and material information, anatomic and geometric information of to be printed tissue or organ;
b. **A process model**, which is also a software representation containing the print operation control commands, process planning and toolpath generated for the bio-blueprint model and machine hardware and control system;
c. **A process machine**, which is a hardware representation that possesses the functionality of the printing;
d. A **culture system** which can support the *in vitro* growth and proliferation of printed living objects.

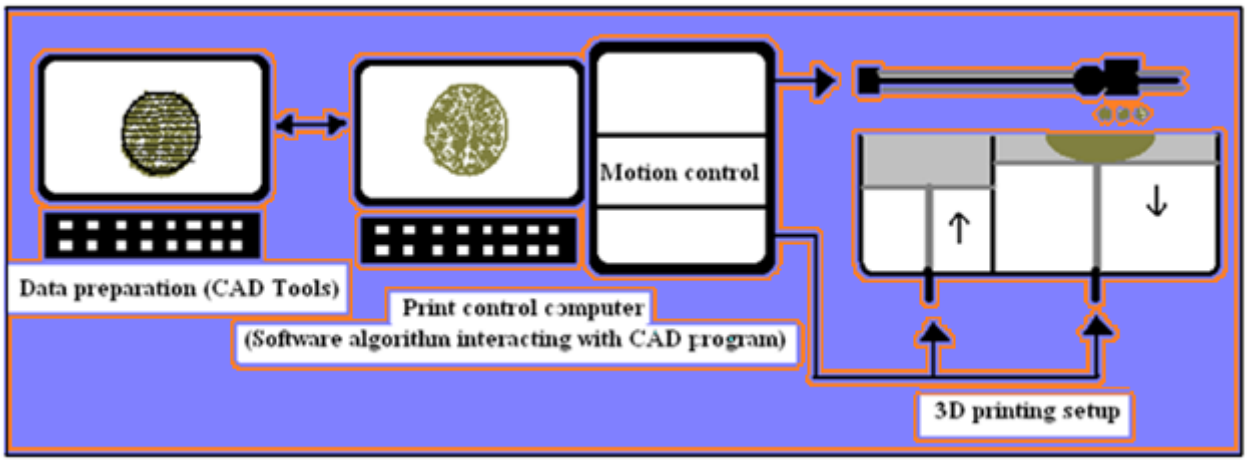

Fig 7: Schematic representation of 3D printing technology

Detailed information about the organ anatomy, morphology, tissue organization with vascular networks are utilized in cell and organ printing. In 3D printing of cells and organ, the process is contolled through planning program. During printing process, the toolpath controls the motion of printing head(s) to deposit cells as required to form a 3D construct. For printing specific organ, the toolpath program needs detailed data of the geometry of the to-be-replaced organ, internal architectures, heterogeneous tissues within the organ and vascular network. Besides these, the toolpath program should also have information of cell compositions to guide the printing heads for depositing the right cells at the right time and at the right place. The 3D printer constructs the 3D model by first spreading a fresh powder over a platform and depositing the binder solution onto the powder bed by 'inkjet' print head. After printing of each 2D layer, a fresh layer of powder is laid down followed by deposition of binder solution. The process continues until the whole construct is completed. After the binder dried in the powder bed integrating each layers, the final product is retrieved after removing unbound powder. Boland *et al*. created complex cellular structures by automated direct inkjet printing of primary embryonic hippocampal and NT2 cells in fibrin gels layer wise. Analyses revealed the healthy and functional printed cells. [27] Ilkhanizadeh *et al.* found that Inkjet-printed macromolecules remained biologically functional when printed on poly-acrylamide-based hydrogels, and influences the differentiation of multipotent primary fetal NSCs in an efficient and well-controlled manner. [28]
Piezoelectric-based droplet ejectors, harmless to biological samples have been used for continuous or drop-on-demand ejection of the fluid. [29] The non-contact piezoelectric based printers are in use but with few limitations because of pressure induced cell damage. Acoustic-based non-contact printing overcomes some of the shortcomings of the previous technologies. Research have shown that focused and high-intense sound beams can be used to eject droplets from free liquid surfaces.[30] This process has capability to produce picolitre drops without nozzles. Besides non-contact, this printing is devoid of any cell damage due to heat, pressure and shear. Demirci *et al*. used acoustic-based printers to print picoliter droplets of approximately 37 µm diameter with single to few cells in each droplet. Various cell types, including mouse embryonic stem cells, fibroblasts, hepatocytes and cardiomyocytes encapsulated in acoustic picolitre droplets were injected and viability were studied. The viability across various cell types was found more than 90% in printed sample.[31]

Despite these impressive technologies and results which show the promising scope of organ printing, several challenges need to be overcome before the full potential can be achieved.

5. CONCLUSION & FUTURE

The fusion of biomaterials and computer technologies and their application to tissue engineering offer new opportunities to overcome the challenges faced by existing technologies to fabricate scaffolds. The various applications of computational softwares and software controlled machinery have revolutionized the controlled fabrication of complex artificial tissue scaffolds. The scope of the 3D tissue printing has expanded to organ printing in recent years due to the advancement in the printing technology. 3D organ printing is still emerging to overcome the main challenges in tissue engineering, mimicking the complexity of the tissues and providing vascularization. In future, it may be possible to directly print the new tissues at injured site of patient by depositing *in vitro* cultured cells and tissues utilizing endoscopic techniques. It is believed that with the rapid growth of this promising research field will transform the conventional tissue engineering approaches and greatly contribute to the therapeutic potential of tissue engineering.

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