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Review Paper

A review on scaffolds used in tissue engineering and various fabrication techniques

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Abstract

Developments in the field of tissue engineering has replaced the earlier practice of allograft transplantation by modern approaches like tissue engineering using scaffolds. This has eliminated the occurence of GVHD, which generally occurs in patients who have received tissue grafts. Scaffolds can be fabricated from various materials like hydrogels, natural polymers, hydroxapatite, etc, depending on the tissue to be engineered. They can be engineered using various techniques such as rapid prototyping, electrospinning, etc. Scaffolds of appropriate porosity, fabricated from biocompatible materials can increase the efficiency of cell proliferation. Scaffolds have been used in the fabrication of bone ,cartilage, muscle, neural and skin tissues. They can be seeded with various types of cells such as fibrobalsts, osteoblasts, etc depending on the tissue of interest.

Keywords: graft, polymer, proliferation, scaffolds.

Introduction

The human body consists of a number of tissues which are masses of cells which perform specific functions. They can be of various types such as muscular, epithelial, nervous, connective, etc. They act as building blocks of various organs. These tissues stop functioning normally when they are damaged due to injury, ageing or disease. The damaged tissue has to be replaced to ensure proper functioning of the respective organ. For example, the skin which is responsible for innate immunity of the body by protecting it from harmful microorganisms, chemical substances, etc. can be damaged by disease, burns and other accidents. The damaged skin has to be replaced by healthy skin cells to ensure effective defence against pathogens.

The conventional methods involve tissue transplant which is classified into various types [1] autografts, which involve replacement of the damaged tissue of an individual by healthy tissue from the same individual, allografts, which involve replacement of the damaged tissue by healthy tissue from a non identical individual of belonging to the same species, xenografts, which involve replacement of the damaged tissue by healthy tissue from an organism belonging to a different species and isografts, which involve replacement of the damaged tissue by healthy tissue from an organism which is genetically identical and from the same species.

The number of limitations associated with these methods exceeds the number of benefits. Firstly, the probability of acceptance of these grafts especially allografts and xenografts is very low. The acceptance of a graft is governed by MHC (Major Histocompatibility Complex) [2] which is a protein located on the cell membrane. This complex is called HLA (human leukocyte antigen) in human beings. A graft is accepted if the MHC/ HLA of the donor matches with

that of the recipient. If the graft is rejected, it is subjected to necrosis which results in tissue death.

Secondly, there is a risk of infections caused by microorganisms, which can be transmitted from the donor to the recipient. Lymphocytic choriomeningitis which is caused by Lymphocytic Choriomeningitis virus (LCMV) [3] , cytomegalovirus (CMV) infection which occurs during liver transplant[4], HIV[5] and candidiasis, caused by the fungus *Candida albicans*[6] are some infections caused by viruses and fungi.

Thirdly, there is a chance of occurrence of skin cancers, such as squamous cell carcinoma, especially after heart transplants, which causes changes in the skin [7].

Development in the field of tissue engineering has led to the creation of scaffolds which function as templates onto which cells of interest can be seeded. Under appropriate conditions, these cells develop into the tissue of interest. Scaffolds can be made of different types of materials, including polymers, bioactive glass, chitosan and collagen.

Before selecting a scaffold, a number of criteria should be taken into consideration. Firstly, the scaffold should get degraded within the body after the new tissue has been formed [8]. This eliminates the need of removing the scaffold by surgical procedures. Secondly, it should not create any inconvenience to the user in the form of pain or infection. Also, it should not exhibit toxicity within the user [9]. Thirdly, it should be preferably made of a low cost material so that it can be afforded by a wide spectrum of users. Finally, it should have interconnected pores with an appropriate pore size. Also, it should have enhanced mechanical properties [8].

Methods used in fabricating scaffolds

Electrospinning

In this technique, a drop of the desired polymer solution is created by forcing it through a capillary tube. The drop formed at the tip of the capillary is converted into a solution jet by increasing the electric field in such a way that it overcomes the drop's surface tension. The jet is made to accelerate onto a collection target. A polymer fiber is formed by the evaporation of the solvent from the polymer jet [9].

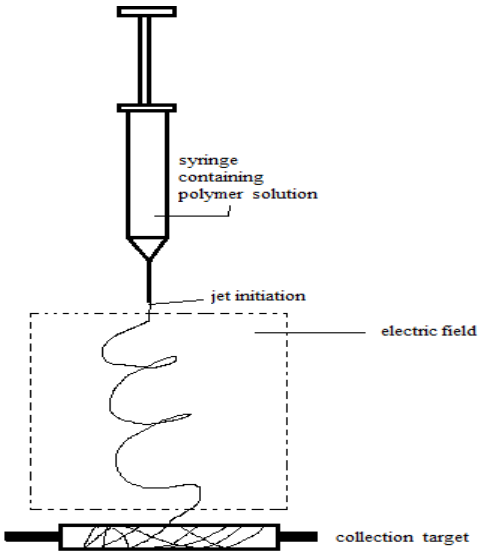

Figure 1: Illustration of electrospinning

Particulate leaching

In this method, salt particles of small size, are coated on a mould into which the desired polymer solution is loaded. The size of salt particles determines the size of pores in the

scaffold. After the evaporation of solvent from the polymer solution, the salt particles are removed by leaching with water[9].

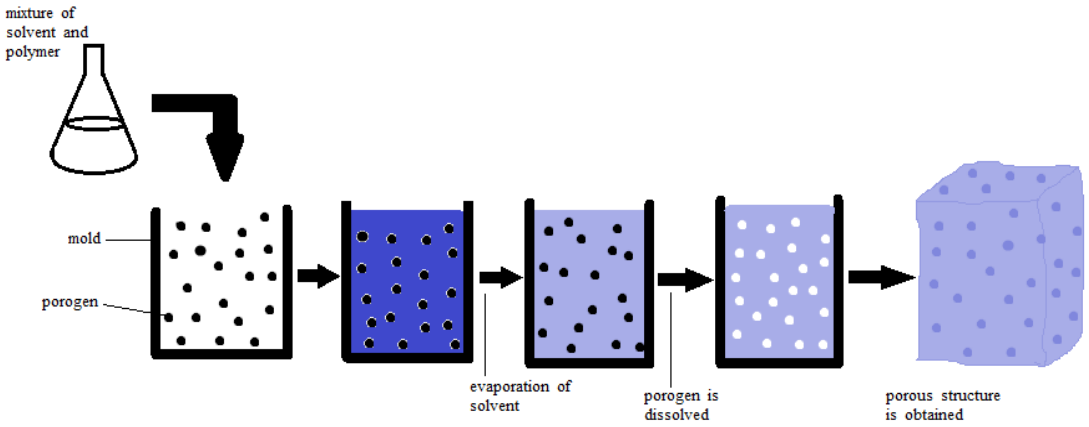

Figure 2: synthesis of scaffold by particulate leaching

Phase separation

In this method a polymer solution is made to separate into two phases, a polymer lean phase and a polymer rich phase. Solidification of the polymer rich phase occurs on removing the solvent. This techniques can be of various types, namely, liquid- liquid phase separation and solid- liquid phase separation [9].

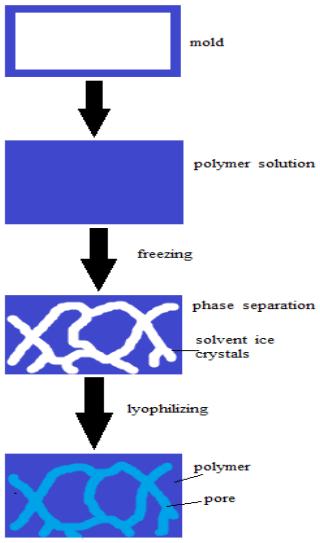

Figure 3: scaffold synthesis by phase separation

Inkjet printing

This technique involves the use of an electronically controlled chamber, containing alginic acid and an ink cartridge containing $CaCl_2$ solution. The $CaCl_2$ solution is printed onto the alginate in the form of layers. Gelling of alginate results in the formation of 3D structure. The crosslinked structure is immersed into fresh alginic acid solution and the next layer of crosslinked alginic acid is synthesised. The process is repeated a number of times [10].

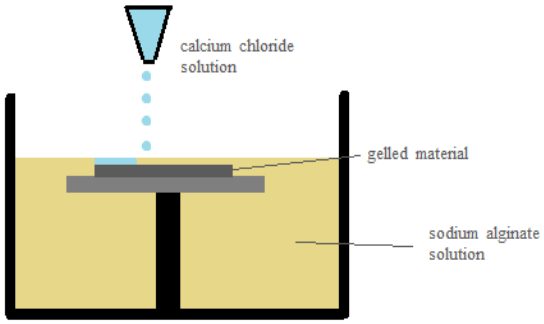

Figure 4: illustration of inkjet printing

Rapid prototyping

In this process, a 3D image of the scaffold is created by Computer Aided Design using various software. This image file is then converted to a stereolithographic format, in which virtual slicing in various cross- sections is carried out. The file is then sent to an rapid prototyping machine, which fabricates the scaffold [11]. Rapid Prototyping can be of various types such as Fused Deposition Modelling, 3D printing, selective laser sintering.

In fused deposition modelling a thermoplastic is heated and forced out through a nozzle, placed over an x- y plane, which is controlled by a computer. This helps in creating a model of the scaffold [12].

3D printing involves the creation of an initial layer of powdered metal or ceramic. This is then layered by a binding liquid, which is forced out through the nozzles of an inkjet printer [12].

Selective laser sintering is a process in which a layer of a powder of a particular polymer is created and a laser beam (CO_2 laser) is focussed on this layer. Increase in temperature, above the glass transition temperature of the powdered material leads to its fusion and converion into a solid mass [13].

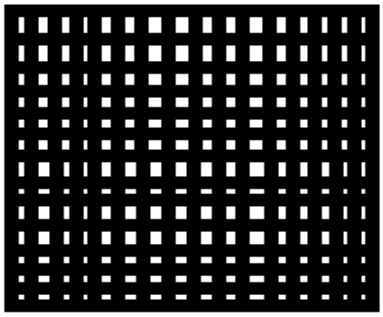

Figure 5: structure of scaffold produced by rapid prototyping

Computer Aided drawing in the fabrication of scaffolds

This process is done by initially obtaining an image of the desired tissue by CT scan [12-14]. CT scan provides images with various shades of gray, depending on the Hounsfield units, which is based on attenuation coefficient. The image consists of a number of pixels which are considered as voxels, as they represent an amount of volume in the body of the individual. CT scan produces images of the tissue in various cross sections, in the form of slices (2-dimensional image). The 2- dimensional images can be converted into contours, and can be assembled to form a 3- dimensional structure. The CT scan data can be converted into 3D CAD, using software like mimics [15]. AutoCAD [15] is used to create the architecture of the scaffold.

Software like Quickslice TM [15] are used to design the pores of the scaffold. The 3D structure of the scaffold thus obtained is converted into STL file format and is transferred to a rapid prototyping machine, which fabricates the scaffold.

According to the tissue of interest, scaffolds can be classified into various categories:

Scaffolds for bone engineering

The bone tissue can be classified as compact and spongy bone. Compact bone consists of a number of haversian systems, enclosed by a periosteum. Each haversian system consists of layers of bone called lamellae, which are separated by osteocytes enclosed in lacunae. At the centre of the haversian system, there is a central canal through which arteries and veins run. The spongy bone is organised in the form of trabeculae which contain alternating, concentric layers of lamellae and osteocytes. The space in between trabeculae are filled with red bone marrow [16].

The bone tissue can be destroyed by bone diseases, tumour formation or injury caused by accidents. Since bones are responsible for providing a structural framework to the body, defective bones have to be replaced by healthy bone cells by the aid of scaffolds.

According to a research by [17] composite foams made of β- tricalcium phosphate (β- TCP), hydroxyapatite (HA) and poly (L- lactic acid) (PLA) can be used as scaffolds for bone engineering. HA and PLA [18] are known to precipitate calcium phosphate from stimulated body fluid and have enhanced mechanical properties. Also, they help in the proliferation of osteoblasts. PLA can be easily degraded and excreted after being metabolised within the body. BCP (mixture of β-TCP and HA) scaffolds provide 70% porosity[19-20] and Zn- doped β- TCP scaffolds account for high compressive strength[19].

Recent studies have shown that bioactive glasses like borate, phosphate and silicate bioactive glasses, on account of their high elastic modulus, compressive strength, bone forming ability and chemical affinity to bone can act as potential raw materials for scaffold fabrication [21].
Silk based scaffold materials[22] like porous silk sponges with very high porosity (>92%) ensure efficient transport of nutrients and premineralised silk scaffolds[23] with enhanced osteoconductivity and mechanical properties provide an appropriate environment for bone cell proliferation.

Hydrogels used in scaffold fabrication are created by covalent or ionic cross linking of molecules. These hydrogels are either made of natural polymers like chitosan, or synthetic polymers like polyvinylalchohol [24].

Various materials, other than those mentioned above are used in bone tissue engineering. Some of them include collagen- glycosamine (produced from collagen and chondroitin -6- sulphate) [25], ultra-short single-walled nanotubes created from polypropylene fumarate [26], polycaprolactone, a polymer which is bioresorbable [27] and biogenic polyphosphate and biogenic silica which are obtained from sea sponges [28].

Scaffolds for skin engineering

The skin defends the body against pathogens, toxic compounds, dust, etc. It helps in the excretion of waste to the outer environment in the form of sweat. It comprises three layers, the outermost epidermis, dermis which is the middle layer and the innermost subcutaneous tissue. The epidermis is made of keratinocytes which help in the production of a fibrous protein named keratin. Collagen and lipocytes form the dermis and subcutaneous tissue respectively [29]. Sebaceous and sweat glands are also present in the skin. Wounds with significant loss of skin require efficient regeneration with improved skin appearance by the minimization of scars. Also, the dressing of deep wounds should be done with a material which will protect them from infection.
Scaffolds fabricated from fibers of micro or nano diameter, produced by electrospinning can be used as bandages to dress deep wounds. Electrospun scaffolds are made of materials like poly(lactic acid- co- glycolic acid) abbreviated as PLAGA [30] poly[(D, L- lactide)- co- glycolide]

and collagen [31]. Maximum proliferation of human fibroblasts was obtained using PLAGA fibers of diameter between 350 and 1100 nm.

Biodegradable scaffolds are produced from natural polymers like collagen, chitosan, crosslinked collagen and chitosan [32], crosslinked gelatin and montmorillonite- chitosan [33], and alginate which is obtained from seaweeds [34]. Plant derived human collagen, produced from transgenic tobacco plants can be used as efficient scaffolds for the proliferation of fibroblasts, keratinocytes and endothelial cells.

Recently, sandwich type scaffolds have been fabricated with the upper layer formed by poly (ε- caprolactone) [PCL] nanofiber with structural cues and arrayed, square shaped microwells and the lower layer formed by nanofibers placed radially. Small tissue masses are placed in microwells in between the two layers. It was observed that the wound was replaced by a significant amount of normal epithelial cells 21 days after surgical implantation of the scaffold [35]. Other materials used in fabricating scaffolds for skin regeneration include Gelatin-Chondroitin 6 Sulphate-Hyaluronic Acid [36], poly (L-lactic acid) [37] and polyurethane which facilitates the release of platelet derived growth factors [38].

Scaffolds for muscle tissue engineering

Muscle tissue which helps in motion of the body are of various types, namely skeletal muscle, cardiac muscle and smooth muscle. Skeletal muscles [39] are voluntary muscles which help in the motion of bones, whereas smooth muscles, found in the gastrointestinal tract and cardiac muscles, located in the heart myocardium are involuntary.

Proliferation of cardiomyocytes has been facilitated by the fabrication of scaffolds formed from polyglycolic acid (PGA) [40] and poly (2- hydroxyethylmethacrylate- co- methacrylic acid) [pHEMA-co- MAA]. Neovascularisation was maximum in porous (pHEMA- co- MAA) [41] scaffolds of pore diameter 40 and 80 nm. It was observed that sphere templated scaffolds, used in acellular cardiac implants showed maximum neovascularization. Human embryonic stem cell derived cardiomyocytes can be proliferated using scaffolds made of poly-L- lactic acid (PLLA) and polylactic -glycolic acid (PLGA) [42].

Smooth muscle cells can be seeded in P(LLA- CL), a copolymer of PLLA and PCL, and PGA scaffolds which are produced by electrospinning [43]. Polyethylene glycol (PEG), PLLA, PLGA and PGA can act as Extracellular Matrices (ECM) which are processed into sponges, fibers and tubular structures [44][45]. Polydimethylsiloxane (PDMS), poly- (glycolide- co- caprolactone) [PGCL] [46] and poly(lactide- co- caprolactone) [PLCL] [47] scaffolds, on the account of biocompatibility are excellent materials for the fabrication of scaffolds for seeding of vascular smooth muscle cells.

Skeletal muscle cells can be engineered from a mixture of mouse myoblasts and human embryonic endothelial cells or from Human Embilical Vein Endothelial Cells (HUVEC), which could be seeded on PLLA and PGLA sponge scaffolds [48]. Polyesterurethane [49] membranes are used to proliferate murine myeloblast cell line (C2C12), rat myeloblast cell line (L6) and primary HSCs (Human Satellite Cells). Due to the efficiency of polyesterurethane membranes, they could prove as promising scaffolds in the engineering of skeletal muscle.

Discussion

The increasing number of accidents and diseases, has increased the probability of tissue damage in individuals. Replacement of the damaged tissue through transplantation is a tedious process. The probability of acceptance of the graft is very less due to the occurrence of graft versus host disease. Obtaining a healthy tissue also involves time consuming processes like blood group matching and HLA typing. Nowadays, the lost tissue is regenerated by proliferating cells obtained from the same individual using scaffolds. The design of scaffolds has been accelerated through computer aided design. Rapid prototyping and inkjet printing has made scaffold fabrication an automated process. Also, the product obtained is biodegradable and biocompatible.

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