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A Potential Approach Of Scaffolds For Drug Delivery In Tissue Engineering

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Current strategies of regenerative medicine are focused on the restoration of pathologically altered tissue architectures by transplantation of cells in combination with supportive scaffolds and bio molecules. In recent years, considerable interest has been given to biologically active scaffolds which are based on similar analogs of the extracellular matrix that have induced synthesis of tissues and organs. To restore function or regenerate tissue, a scaffold is necessary that will act as a temporary matrix for cell proliferation and extracellular matrix deposition, with subsequent in growth until the tissues are totally restored or regenerated. Scaffolds are implants or injects, which are used to deliver cells, drugs, and genes into the body. Different forms of polymeric scaffolds for cell/drug delivery are available : (1) a typical three-dimensional porous matrix, (2) a nanofibrous matrix, (3) a thermo sensitive sol-gel transition hydro gel, and (4) a porous microsphere. A scaffold provides a suitable substrate for cell attachment, cell proliferation, differentiated function, and cell migration. Scaffold matrices can be used to achieve drug delivery with high loading and efficiency to specific sites. Biomaterials used for fabrication of scaffold may be natural polymers such as alginate, proteins, collagens, gelatin, fibrins, and albumin, or synthetic polymers such as polyvinyl alcohol and polyglycolide. Bioceramics such as hydroxyapatites and tricalcium phosphates also are used. Techniques used for fabrication of a scaffold include Solvent casting/particulate leaching, Emulsion freeze drying, Rapid prototyping, Thermally induced phase separation, Gas foaming/particulate leaching and High pressure processing. These techniques allow the preparation of porous structures with regular porosity. The present review gives a detailed account of the need for the development of scaffolds along with the materials used and techniques adopted to manufacture scaffolds for tissue engineering and for prolonged drug delivery.

Keyword: Chronic Obstructive Pulmonary Disease, Metabolic Syndrome, Immunity, Treatment

1. Introduction

The field of tissue engineering has advanced dramatically in the last 10 years, offering the potential for regenerating almost every tissue and organ of the human body. Tissue engineering aims to replace or facilitate the growth of damaged or diseased tissue by applying combinations of biomaterials, cells and bioactive molecules^[1]. Every day thousands of clinical procedures are performed to replace or repair tissues in the human body that have been damaged through disease or trauma. The damaged tissue is replaced by using donor graft

tissues (autografts, allografts, or xenografts), but the main problems associated with this are a shortage of donors or donor sites, transmission of disease, rejection of grafts, donor site pain and morbidity, the volume of donor tissue that can be safely harvested, and the possibility of harmful immune responses^[2]. Compared with replacing damaged tissues with grafts, tissue engineering, or regenerative medicine, there are aims to regenerate damaged tissues by developing biological substitutes that restore, maintain, or improve tissue function^[3, 4]. In the last 2 decades, the research and development among certain

tissues in the body contain cells capable of initiating, regeneration or repair after injury^[5]. Biodegradable polymeric scaffolds for tissue engineering have received much attention because they provide a temporal and spatial environment for cellular growth and tissue in-growth^[6-8]. Scaffold is the central component that is used to deliver cells, drugs, and genes into the body. Scaffolds are defined as three-dimension porous solid biomaterials designed to perform some or all of the following functions: (i) promote cell-biomaterial interactions, cell adhesion, and ECM deposition, (ii) permit sufficient transport of gases, nutrients, and regulatory factors to allow cell survival, proliferation, and differentiation, (iii) biodegrade at a controllable rate that approximates the rate of tissue regeneration under the culture conditions of interest, and (iv) provoke a minimal degree of inflammation or toxicity in vivo . When cells are implanted or seeded into an artificial structure capable of supporting three-dimensional (3D) tissue formation, these structures typically are called “cell delivery scaffolds,” and when drugs are loaded into a 3D artificial porous structure capable of high drug loading efficiency and sustained release of a drug for longer duration, they typically are called “drug delivery scaffolds^[9].”

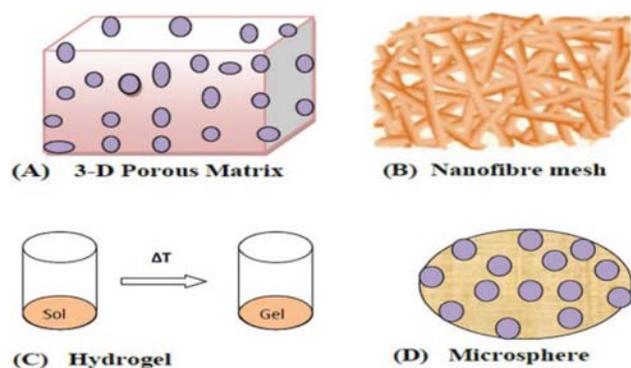


Figure 1. Matrix (A); Nanofiber mesh (B); and Different forms of polymeric scaffolds for cell/drug/gene delivery: Three-dimensional porous matrix (A), Nanofiber mesh (B), Hydrogel (C), and Microsphere (D)

Different forms of polymeric scaffolds for cell/drug delivery are available: (1) Highly porous well interconnected open pore structure (2) A nanofibrous matrix that is prepared by

electro spinning (3) A thermo sensitive sol-gel transition hydro gel (4) A porous microsphere. These are already widely utilized as sustained protein-release formulations and have been applied in tissue engineering for the potential use as a cell delivery carrier or supportive matrix^[10-11] of the polymeric scaffolds listed above, a typical 3D porous matrix and nanofibrous matrix are the implantable forms and a thermo sensitive sol-gel transition hydro gel and porous microsphere are the injectable forms.

1.1 Scaffold for tissue engineering (cell delivery) should possess the following properties

1. Mechanical properties that is sufficient to shield cells from tensile forces without Inhibiting biomechanical cues
2. Desired volume, shape, and mechanical strength^[12].
3. Acceptable biocompatibility
4. A highly porous and well-interconnected open pore structure to allow high cell seeding density and tissue in-growth
5. Bio adsorption at predetermined time period
6. Biocompatible chemical compositions and their degradation products, causing minimal immune or inflammatory response^[13].
7. Physical structure to support cell adhesion and proliferation, facilitating cell– cell contact and cell migration^[14].

2. Design strategies for cell and drug delivery systems

Although prefabricated scaffolds are most widely used for tissue regeneration as well as drug delivery purposes, different forms of polymeric scaffolds for cell/drug delivery are also available. These forms can be classified as (1) a typical 3D porous matrix, (2) a nanofibrous matrix, (3) a thermo sensitive sol-gel transition hydro gel, and (4) a porous microsphere. Of these, the typical 3D porous matrix and nano fibrous matrix are the implantable forms and the thermo sensitive sol-gel transition hydro gel and the porous microsphere are the injectable forms. Injectable scaffold materials formed in situ have received much attention recently because they can be

administered using a syringe needle and thus avoid surgery. To mimic the topological and micro structural characteristics of the ECM, a biomaterial must have a high degree of porosity, a high surface: volume ratio, a high degree of pore interconnection, appropriate pore size, and geometry control^[15]. These properties can be well controlled in an injectable scaffold. Some of the drug/cell delivery systems and their design strategies are given in the following sections:-

A.) Hydro gel-Based System

Hydro gel matrices are physically or chemically cross-linked, water-soluble polymers, which swell to form a gel like substance on exposure to water^[16]. Hydro gels are appealing for biological applications because of their high water content and biocompatibility. Hydro gels can be made from naturally occurring polymers such as collagen, chitosan, and gelatine or synthetic polymers such as poly(ethylene glycolide) and poly vinyl alcohol. Growth factors are released from hydro gels through diffusion of the growth factor A Novel Carrier for Cell and Drug Delivery through the highly hydrophilic scaffold, mechanical stimulation, or hydrolytic degradation of the scaffold or upon swelling in response to an environmental stimulus. For example, gelatine and dextran can be fabricated as an interpenetrating polymer hydro gel for drug delivery and can exhibit an intelligent property of degradation in response to dual stimuli. Release behaviour can be regulated by controlling the chemical and physical properties of the gels from a few days to several months^[17]. Above critical concentrations, these hydro gels show a sol state at room temperature, but change into a gel state at body temperature^[15], hydro gels can be administered in a minimally invasive manner and therefore they are used in tissue engineering strategies as a potential cell and protein delivery vehicle^[11] Additional advantages of hydro gels are that they may protect drugs, peptides, and especially proteins against the potentially harsh environment in the vicinity of the release site; they enable enhanced residence times, sustained delivery, and/or targeted drug delivery ; and they have significant potential in wound healing

applications, though pore size and degradation properties must be optimized. For example, injectable poly (N-isopropylacrylamide) physical hydro gels encapsulating cells have been prepared for cartilage and nerve regeneration^[18, 19]. Pluronic/heparin composite hydro gels delivering growth factor also have been studied to induce angiogenesis. Photo crosslinked poly(ethylene glycol) (PEG)-based hydrogels have been utilized for delivery of chondrocytes and osteoblasts^[19-21]. Bone morphogenic protein introduced into the hydrogel material (temperature-sensitive chitosan-polyol salt combination) has been effective in promoting de novo bone and cartilage formation in vivo. Poly (lactic acid-glycolic acid) (PLGA) grafted with PEG and PEG grafted with PLGA hydrogels capable of sustained insulin delivery and cartilage repair were synthesized. Pluronic copolymers at a higher concentration (more than 20% [w/v]) have been used to encapsulate chondrocytes and produce engineered cartilage^[20].

B.) Microsphere- and Micro particle-based Systems

Delivery has several potential advantages, such as the inherent stability of plasmid DNA, reduced fabrication costs, extended shelf-life, a more economical use, and application in skin repair. Application is pellets incorporated with basic fibroblast growth factor- loaded microspheres into alginate porous scaffolds to enhance vascularization after implantation in the rat peritoneum. Chitosan scaffolds loaded with basic fibroblast Microspheres and micro particles have attracted attention as carrier matrices in both the biomedicine and bioengineering fields and could satisfy the need of delivering biomolecules such as growth factors, genes, and cells^[21]. Prior to injection, the porous structure (30 μ m) would allow sufficient cell seeding in and out of the matrix. After injection in vivo, the porous matrix would permit infiltration of cells and in growth of tissue from the host, facilitating the regeneration process. Micro particles also can be used as injectable scaffolds to support cell growth and proliferation directly and as vehicles of growth factor, and to enhance cell proliferation and

expansion simultaneously. Microsphere based technology has an application for tissue engineering as well as gene therapy. Gene growth factors contained in gelatin microparticles were effective in accelerating wound closure Critical Reviews™ in Therapeutic Drug Carrier System of pressure ulcers^[22]. Biodegradable PLGA microspheres have been studied for delivery of chondrocytes for cartilage engineering. Nanofabricated particles could offer better delivery properties to direct cell fate and to regulate processes such as angiogenesis and cell migration.

C.) Membrane-based Systems

Human skin is considered the gold standard for treatment of skin wounds. However, skin grafts are not always the perfect solution. They are limited in terms of the conditions needed for tissue availability, graft rejections, and conformability with the surrounding tissue with respect to thickness and pigmentation^[23]. Current strategies for wound dressings have been aimed at the development of the bilayer-structured membrane, with incorporation of growth factors into these matrices for improved healing. For example, gelatine hydro gel containing epidermal growth factor-loaded microspheres has an enhanced effect on re-epithelisation, improving the healing of the wound area. Antibiotics should be incorporated into the membranes to prevent infections because sustaining a sufficient drug concentration at the site of infection is important for the treatment of an infected wound. For example, a bilayered membrane combines silver sulphadiazine and a laminin-modified collagen membrane, which was shown to facilitate the dermal wound healing process.

3. Biomaterials for scaffolds formation

Many different materials (natural and synthetic, biodegradable and permanent) have been investigated. Most of these materials have been known in the medical field before the advent of tissue engineering as a research topic, being already employed as bioresorbable sutures. Examples of these materials are collagen and some polyester. New biomaterials have been

engineered to have ideal properties and functional customization: injectability, synthetic manufacture, biocompatibility, non-immunogenicity, transparency, nano-scale fibers, low concentration, resorption rates, etc. A commonly used synthetic material is PLA-poly(lactic acid). This is polyester which degrades within the human body to form lactic acid, a naturally occurring chemical which is easily removed from the body. Similar materials are polyglycolic acid (PGA) and polycaprolactone (PCL): their degradation mechanism is similar to that of PLA, but they exhibit respectively a faster and a slower rate of degradation compared to PLA. Scaffolds may also be constructed from natural materials: in particular different derivatives of the extracellular matrix have been studied to evaluate their ability to support cell growth. Proteic materials, such as collagen or fibrin, and polysaccharidic materials, like chitosan^[24] or glycosaminoglycans (GAGs), have all proved suitable in terms of cell compatibility, but some issues with potential immunogenicity still remains. Among GAGs hyaluronic acid, possibly in combination with cross linking agents (e.g. glutaraldehyde, water soluble carbodiimide, etc.), is one of the possible choices as scaffold material. Functionalized groups of scaffolds may be useful in the delivery of small molecules (drugs) to specific tissues. Another form of scaffold under investigation is decellularised tissue extracts whereby the remaining cellular remnants/extracellular matrices act as the scaffold.

4. Scaffolds Fabrication Techniques

Many different techniques that are used to fabricate scaffolds for tissue engineering are summarized in the following sections.

- A. Solvent casting/particulate leaching,
- B. Emulsion freeze drying,
- C. Rapid prototyping,
- D. Thermally induced phase separation,
- E. Gas foaming/particulate leaching,
- F. High pressure processing,

A. Solvent casting/particulate leaching

Solvent casting/particulate leaching method is the most convenient method for preparing porous scaffolds. It involves the casting of polymer/salt/organic solvent mixture solution followed by solvent evaporation and dissolution of the salt particulates in aqueous solution^[25].

B. Emulsion freeze drying

Emulsion freeze drying method emulsion is prepared by dispersing water phase in organic continuous phase containing biodegradable polymer. This can give rise to porous scaffolds with various pore sizes^[26].

C. Rapid prototyping

Rapid prototyping (RP), also known as solid free form fabrication has recently introduced a new method in fabricating well-designed tissue engineering scaffolds.

D. Thermally induced phase separation

The phase separation technique is based on thermodynamic demixing of homogeneous polymer solvent solution into polymer poor phase, exposure of the solution to another immiscible solvent. Particularly thermally induced phase separation (TIPS) uses thermally induced phase separation. The polymer solution is quenched below the freezing point of the solvent and subsequently freeze dried, producing a porous structure.

E. Gas foaming/ particulate leaching

In gas foaming/particulate leaching method effervescent salt is used as gas foaming agent. Binary mixture of polylactide(PLA) solvent gel containing dispersed ammonium bicarbonate particulate was cast in mold and subsequently immersed in hot water. The evolution of ammonia and carbon dioxide gas, along with leaching out of ammonium bicarbonate particulates from the solidifying polymer matrix, resulted in the formation of pores with high interconnectivities.

F. High pressure processing

High pressure processing is also known as the supercritical fluid technology. It is performed by applying the gas such as carbon-dioxide to a dry polymer at high pressure, which forms single phase polymer gas solution. The pressure is then reduced to create thermodynamic instability of the dissolved carbon dioxide and result in nucleation and growth of gas cells to generate pores within polymer matrix.

5. Application of scaffold

Scaffold is used mainly to deliver the cell/drug/gene into the body, and application of Scaffold is mainly categorized into those three categories: (1) cell delivery, (2) DNA/ Gene delivery and (3) drug delivery.

5.1 Scaffold for cell delivery

In these, the cells with growth factor are encapsulated or seeded into the scaffold and administered into the body. Local and sustained delivery of paracrine factors, either by inducing or inhibiting cell proliferation, survival, migration, and/or differentiation, may greatly enhance tissue remodelling or organogenesis^[27]. Growth factors can be incorporated into the scaffold matrix by bulk encapsulation, specific or nonspecific surface adsorption, and addition of microspheres encapsulating them

5.2 Scaffold for DNA/gene delivery

The gene encoding a growth factor to target cells has been suggested as an effective approach for enabling continuous expression and release of the growth factor in the local tissue site to avoid protein instability problems encountered during the harsh formulation process and the short half-life after release in the body fluid^[28] when growth factor-releasing scaffolds used. Polymer scaffolds have been designed to release the genetic material continuously as naked DNA or in the form of polyplexes, thereby transfecting to seeded cells and expressing the growth factor to stimulate morphogenesis of specific cells to form the desired tissue.

5.3 Scaffold for drug delivery

Low molecular weight drugs that control proliferation or differentiation of cells can be incorporated into biodegradable scaffolds to induce cellular differentiation and tissue remodeling. For example, dexamethasone, a steroidal anti-inflammatory drug, was loaded into the bulk phase of PLGA scaffolds for sustained release^[29]. It was observed that sustained release of dexamethasone effectively induced differentiation of bone marrow stem cells to osteoblasts or chondrocytes.

6. Conclusion

Scaffolds have been well investigated with respect to the material requirement, properties, and technology for the production of scaffolds. The field of biomaterials has played a crucial role in the development of tissue-engineered products. In spite of this, few scaffolds are available commercially, particularly for cell/drug delivery. Most of the scaffolds studied are still in the investigation stage and are yet to be approved for clinical use. Looking into convenience and practicability, there is immense scope in developing injectable gel-sol scaffolds because they are easy to use, versatile, and involve the use of safe adjuvants; many of them are already listed in the Generally Recognized as Safe list or even have been approved by the Food and Drug Administration. New biodegradable polymers need to be developed to meet all requirements for surgically implantable scaffolds. New approaches targeting cell/drug delivery are the need of the hour, particularly for tissue regeneration, postsurgical management, cancer, and congenital malformations. Scaffold drug delivery based on the use of biodegradable materials. By using the biodegradable material tissues or organs are prepared by simply implantation of the scaffolds are used.

7. Abbreviations

1. DNA- Deoxyribonucleotide
2. ECM- Extracellular materials
3. GAG- Glycosaminoglycans
4. PCL- Polycaprolactone
5. PEG- Polyethylene glycol

6. PGA- Polyglycolic acid
7. PLA- Polylactic acid
8. PLGA- Poly Lactic glycolic acid
9. RP- Rapid prototyping
10. TIPS- Thermally induced phase separation

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