

A Review on Bone Scaffold Fabrication Methods

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Abstract - This review paper focuses on bone scaffold fabrication methods for tissue engineering applications. The suitable biomaterial and polymer for bone scaffold fabrication been discussed. The general characteristics of a scaffold are been explained in detail. The process of various fabrication method, mechanical properties and porosity of scaffold are discussed.

Key Words: Bone scaffold, Fabrication methods, Tissue engineering

1. Introduction

The damage of organs or bone could occur because of accident or the cause of certain diseases. Mostly, the affected bones are not curable to its original ability. Allografting and autografting are examples of the traditionally repairing techniques but these techniques could be painful, need long time to heal and also sometimes it will be rejected by the human body [1]. An advance field called as Tissue Engineering has been introduced in replacing and repairing of tissues in order to overcome the problems that being occurred by the traditional techniques. Tissue Engineering is a process where the cells are biopsy from the human body and being implemented biomedically by cell isolation, cultivation and proliferation and finally the cells will grow in a temporary scaffold [2].

Scaffolds act as template for tissues regeneration. The functions of a scaffold are that it transports the cells and growing factor. Besides that, the architectures and features of human tissues are being mimic and also the direction of microscopic for tissues formation. There are few characteristics that are used to judge an ideal scaffold, such as biocompatibility so that the cells can proliferate and attach, biodegradation profile, broad network of interconnecting pores for the migration, multiplication and attachment of cells are deep within the scaffolds, cells deep in the scaffold are provided sufficient oxygen and nutrients, mechanical strength and finally the waste products could be carried out easily [3].

Fabrication of a scaffold involves the consideration of biomaterial, externally and internally architectures, fabricating methods, biodegradation behaviour and mechanical properties. In addition, scaffolds had been generated by the engineers that is right physical scale and various internally porosity for the cells to grow and tissue construction in order to the perspective of encapsulate cells and proteins [4].

2. Tissue Engineering

In 1970's W.T Green, an orthopaedic surgeon from Children's Hospital had introduced a new technique to repair tissues. He began his research in tissue engineering, by inserting chondrocyte cells would help to form tissue where it will expand the growth of tissue and cells that proliferate cell production. Although this experiment had failed, but it has led to further studies in tissue engineering. A collaboration between Yannas from M.I.T and Drs. Burke from General Hospital of Massachusetts was conducted for generating skin that tissue engineered substituting matrix of collagen to maintain the growing of dermal fibroblast [5]. This example play role as seed of the new discipline now known as Tissue Engineering. The troubling of modulus-mismatch between the materials of existed implantations and bone that occurred in the analogue biomaterials intensions that was acquainted in 1980's by Bonfield was been revised again in 20th century [6]. Natural polymer or synthetic polymer and ceramics had been used in bone scaffolds for either bioresorbable or bioactive in 1970's. Biomaterials such as composites and nanocomposites has taken place in third generation where it has improved the bioactive and bioresorbable properties [7].

3. Scaffold Material

In the early orthopedic, bone implants are basically focused on metals since they provide a robust and reliable factors to support the bone structure. However, temporary reinforcement to the wound area can be provided by the metal and it also offer less therapeutic benefits where can interfere in bone remodeling by starving the new tissue of

the fluctuating loads that are important to produce strong and healthy tissue formation known as *aka stress shielding* [8]. Polymer based scaffolds can be produced by varying the parameters where the porosity and strength are significantly different. There are many techniques have been introduced in developing highly porous three dimensional biodegradable scaffolds from synthetic. The polymers that being use is biodegradable polymers such as poly (lactide-co-glycolide (PLGA) and poly (ϵ -caprolactone)(PCL). Since PLGA and PCL are approved for clinical use, both are commonly used biomaterials for tissue engineering. In the previous study, there was an unexpected result that the cells growth was affected by the mechanical properties of the films, with enhanced proliferation on films. In this case, in has an advantage in complementing the mechanical properties of a biomaterial to the intended area of implantation [9].

3.1 General Characteristics of Scaffold

The bone scaffold must meet certain requirement. The ideal bone scaffold should be biocompatible and osteoconductive, osteoinductive factors to enhance new bone ingrowth and osteogenic cells to begin secreting new ECM. In general, the required characteristic of bone scaffold can be classified into four related aspects:

- i. **Biological properties:** - the scaffold material must be biocompatible and promote cell adhesion, migration and ingrowth. As the cells produce their own ECM, the synthetic matrix should degrade into non-toxic components that can be eliminated from the body.
- ii. **Internal pore structure:** - both cell seeding and bone ingrowth normally are well developed with high porosity, typically among 50-90%. In general the pore size falls within a certain critical range to promote cell seeding and ingrowth both upper and lower bounds are computed by different factors. Cell size controls the lower bound; the specific surface area via the availability of binding site decides the upper bound. Klawitter *et.al's* study showed that the optimal pore size for bone ingrowth is in the range of 100-250m cell in growth and nutrients transportation are interconnected with porosities.
- iii. **Mechanical properties:** - the primary bone tissue has relative high compressive strength that supports the body weight. So the scaffold must provide mechanical support during the reconstruction process. Mechanical integrity for the scaffold design has to the sufficient to resist handling during implantation and 'invivo' loading. An ideal scaffold would be biomechanically similar to the type of bone being replaced in order to function quickly as a synthetic bone replacement. The compressive modules are in the range of 0.01 to 2.0 GPa for trabecular bone, and

14 to 18 GPa for cortical bone. The scaffold should be able to maintain sufficient mechanical properties until newly formed bone can assume a structured role and then the scaffold can be degraded and resorbed in the process of bone regeneration. Numerous studies have demonstrated profound effects of mechanical forces (strain) on cells using 'invivo' and 'invitro' models. The mechanical properties of the substrate are significant factors affecting biological response, as the mechanical environment of the contained cell is determined by these properties.

- iv. **Precise three- dimensional shape:** - The scaffold must be manufactured to an arbitrary complex 3-D shape, which can match that of the tissue to be replaced at both the microscopic and macroscopic levels.

4. Manufacturing Methods

There are several methods for manufacturing a scaffold used in tissue culture. Some of them are reviewed here in this section.

4.1 Fiber Bonding

An excellent surface area to volume ratio for cell enhancement can be exhibited from polymer fibers to make them a viable option for scaffold attachment. This fiber bonding technique is introduced so that at the point of intersection, the fibers will be bonded for enhancing the mechanical integrity for utilizing in regeneration of organ [10]. Originally the fiber bonded scaffolds example fabricated from PGA and PLLA polymers [11]. Briefly, the PGA fibers will be arranged in nonwoven mesh and the fibers will be bond at their contact points at above polymer's melting point temperature. The PGA fibers are encapsulated due to heat treatment for prevention of melting polymer structural collapse. While for PLLA, it is dissolved in methylene chloride and cast over the meshed fibers and dried, in order to produce PGA-PLLA composite matrix. Methylene chloride is used to dissolve the PLLA and the solvent removal is done by vacuum drying after the heat treatment and fiber bonding. There are similar techniques exist for other biocompatible polymer fibers. For the fiber bonding scaffold fabrication and PLGA solution, at the contact point, the polymer solution is build up on the PGA fibers and been bonded. By this technique, PGA mechanical properties will be provided and expose cells to surface of PLLA and PLGA. The disadvantages of fiber bonding is the porosity and pore size could not be controlled, lack of availability of suitable solvents and required appropriate melting temperatures of polymers. Rotter, 2002 studied both the Ethisorb and a PLLA fleece with degradation of 9 to 12 months. However, for load-bearing tissues such as bone and cartilage, the challenge for the cell/tissue construct is to have mechanical

properties similar to those of the host tissue. The Fig.1 below shows the fabricating method and tissue engineered cartilage analyzing from nasal septum cartilage from human patients by [12].

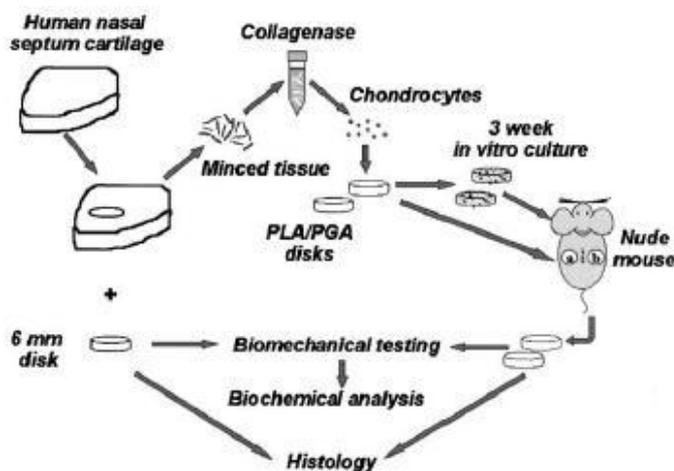


Fig -1: Fabricating method and tissue - engineered cartilage analyzing from nasal septum cartilage from human patients [12].

4.2 Electrospinning

Electrospinning is a method to create porous scaffolds composed of nano and microscale biodegradable fibers. It involves the technology of electrostatic spraying of polymer coatings known as electrostatic fiber spinning or electrospinning. A highly porous scaffold of nonwoven and ultra-fine fibers is fabricated using electrospinning. Biocompatible such as PGA, PLGA, and PCL can be electrospun into nano fibers scaffolds with porosities more than 90% [13]. An appropriate solvent been selected by dissolving particular polymer for an example PCL dissolved in chloroform. A syringe is used to load the polymer solution and at a constant rate by syringe pump and through a metal capillary it expells. The polymer is charged using a high voltage around 10-15kV that is applied to the capillary and ejects the polymer toward a grounded collecting surface. The solvent evaporates once the thin fibre gather on the plate and results in a nonwoven porous scaffold. As for changing the thickness of fibre, diameter of scaffold, and average diameter of pore, there are few factors need to be considered. Such as, the concentration of polymer, solvent choice, rate of ejection, voltage applied, diameter of capillary, material of collecting plate, distance between the collecting plate and capillary. Electrospun has a good result in mesenchymal stem cell culture for cartilage and bone tissue engineering [14].

4.3 Solvent Casting

Scaffold fabrication using solvent casting method is very simple, easy and not expensive. In this method, large

equipment is not required, it is only involved evaporation of the solvent and forming of scaffolds involves either one from the two routes. There are two methods, one of them is polymeric solution is used for dipping the mold and solution will be drawn with sufficient time given. The result is a polymeric membrane layer is formed. Another method is polymeric solution as added into a mold, giving sufficient time for the solvent to evaporate, so that formation of a polymeric membrane layer which adhering to the mold is formed [15]. One of the disadvantages of this method is, the protein is denatured by the toxic solvent and it can affect other solvent. Besides that, it is possible that scaffolds that been fabricated by this method may contain toxicity. As a solution, the toxic solvent is being removed from the fabricated scaffolds are drying the scaffold fully using vacuum process. Since this method needs a lot of time, some researchers have done combination of solvent casting method with particulate leaching method [11]

4.4 Particulate Leaching

This technique is widely used for scaffold fabrication and also one of the famous techniques among all the techniques. The pores or channel are created using porogens such as salt, wax or sugar. In this method, salt is made to small pieces as preferred size by grinding and poured into a mold and also porogen is filled into it. The salt filled mold is then used for casting polymer solution. Water is used to leach out the salt crystals once the solvent is evaporated in order to form the scaffold pores. Porogens plays role in controlling the pore size, this is done by controlled porogens amount, sizing and shaping of porogen. This method is easy to be done. The scaffolds fabricated from this technique produce ~500µm pore size and 94%-95% porosity percentage and required crystallinity. Although this method does not require much polymer for scaffold fabrication, but it has problem in controlling the shaping of pore and openings of interpores [16].

4.5 Phase Separation

Scaffold fabrication using phase separation technique involves separation of two phase polymeric solution that is lean phase polymer and rich phase polymer. Polymer lean phase is where the concentration of polymer is low while the rich phase polymer is where the polymer concentration is high. Phenol or naphthalene is used in dissolving the polymer and the biologically active molecule from the solution is dispersed. The separation of phase is done by lowering the temperature of liquid and two phase solid is formed by quenching extraction, evaporation, and sublimation process is done to remove the solvent to form a porous scaffold that the structure is integrated by bioactive molecules [17].Phase separation technique can be combined with other fabricating technology in designing controlled pore morphology three dimensional structures. For creating nano fibrous

scaffolds, this method can be joined with rapid prototyping [18]. *Gong, 2005* have shown in their study that average pore diameter of scaffolds starting from few micrometers to $\sim 300\mu\text{m}$ can be fabricated by a special technique of elaborated phase separation without involving any electrolytes or additives. Type of coarsening method have been clarified considering the domain growth and phase separation. A normal process of coarsening can produce scaffold with diameter averagely from few micrometers to $150\mu\text{m}$ according to the time of coarsening, in assistance with the close pore morphology. The scaffold's porosity value is abit smaller than the theoretical calculation around 93%. This is due to the partial collapse. Findings reported that as the time coarsening extended, pore diameters increased. The pore size rate of growth was escalating rapidly 0-2 hours due to the rapid coalescence and polymer droplets formation. After 3 hours, the pore size grew to $\sim 100\mu\text{m}$ and the droplets became isolated and decrease the pore growth rate. A slow pore size rate of growth was found to be at the coarsening time to 3-5hours. The figure below shows the changes in microstructure in thermally induced phase separation process by [19].

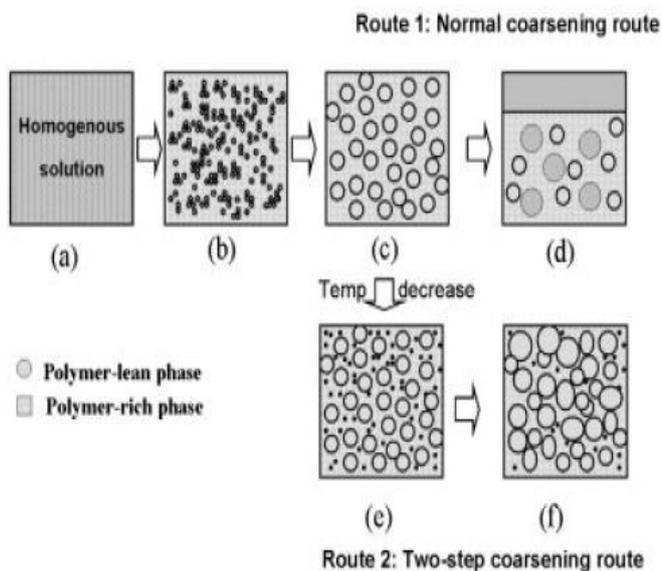


Fig -2: Microstructure change in thermally induced phase separation process [19].

4.6 Melt Molding

Basically, the interconnectivity and geometry of a scaffold is very important for the exchange waste and nutrient among the pores. Melt molding method is developed to fabricate a scaffold with highly interconnectivity and geometrical. In melt molding method, mold filled with teflon and PLGA powder and gelatin microspheres which has particular diameter is involved. Next, the mold is heated above the PLGA glass transition temperature and

pressure is applied to the mixture. By doing this, all the PLGA particles will be attached together. Gelatin microspheres are dissolved by using water to immerse the mixture once the mold is removed and finally the scaffold produce is dried. The shape of scaffolds that being produce by this technique will be same as the mould's shape [20] *Park, 2008* fabricated highly functionalized polymeric 3D scaffolds by a combination of PMD and electro-spinning. The process parameters such as pore sizing, interconnectivity of the pore, diameter of fiber and data of CAD modelling are considered for every layer in fabricating scaffold in the polymer melted deposition method. Direct polymer melt deposition method was used in building the first layer according to the desired processing parameter. Then, spreading of matrix of polymeric nanofiber on the layer was done. Repetition laminating on combination of matrices of nanofiber and layers of microfibers were done to the previous layer. In order to fabricate a three dimensional hybrid structured scaffold. Meanwhile the direct polymer deposition method is being run, extruding of half molten microfilament is done to the previous layer. Once the layer is solidified after a moment, another layer is attach to come up with a completely three dimensional hybrid structured scaffold. This state can help in improving cytocompatibility as the nanotopography in the scaffold structure, composition of the chemical and larger inner surface for the attachment and growing of cell [21]

4.7 Fiber Mesh

Woven or interwave is known as individual fiber. One of these individual fibers is used for scaffold fabrication which is three dimensional or different pore size using fiber mesh technique [22]. Polymer that is biocompatible and biodegradation such as PGA is utilized as the thread of suture synthetic where the polymer is spun into the fiber. The scaffold fabrication is done by depositing the polymer solution on a non-woven mesh of other polymer continued by evaporating [3]. The plus point of utilizing this method is the scaffold fabricated is very good in attachment of cell due to large surface area. Other than that, the cells deep in the scaffold have good survival and growth since it has fast nutrient absorptions [23]. The disadvantage of this technique is has low structure stability. The structure pattern and crystallinity can be improved by hot drying the PLLA fiber.

4.8 Freeze Drying

Fabricating a porous scaffold, freeze drying method is very useful [24]. Sublimation is the main to be considered in this technique. Producing a solution that the concentration desires, dissolvment of polymer to a polymer is needed. Lyophilisation is involved to remove the solvent once the solution is freeze. For this process, vacuum with high power is needed in order to come out with a scaffold which has high porosity and inter connectivity. By controlling the rate of freeze and pH, the pore size also is

controlled, where small pores are a result of the rate of freezing is fast. The plus point of this technique is that it has no need of high temperature or separate leaching process. In this technique, there is also minus point where fabricating scaffold needs more time and the pore size is small [25].

4.9 Solid Free Form

Solid freeform technique is used to fabricate a scaffold that is complex that is a part to art technology. In this technique, mould is not required and it is different from normal machines where materials are removed constantly. In a short briefing on how the solid free form techniques operates start from spreading a thin layer of polymer powder for example PLGA over a piston surface. Then, a binding liquid is dispensed according scaffold layer pattern that is desired through an inkjet where the binding liquid acts as a polymer solvent. After a while, the piston is lowered depending on how thick is the single layer, the posterior powder layers and also application of binding liquid. The polymer is remained unbounded in the network while the process of fabricating is ongoing in order to uphold the layers that are disconnected from the sections [26]. In recent days, solid free form is the efficacious way in producing scaffolds in desired properties and the scale that is large. Additionally, this technique is famous in creating scaffolds that is highly reproducible architecture and variable in composition.

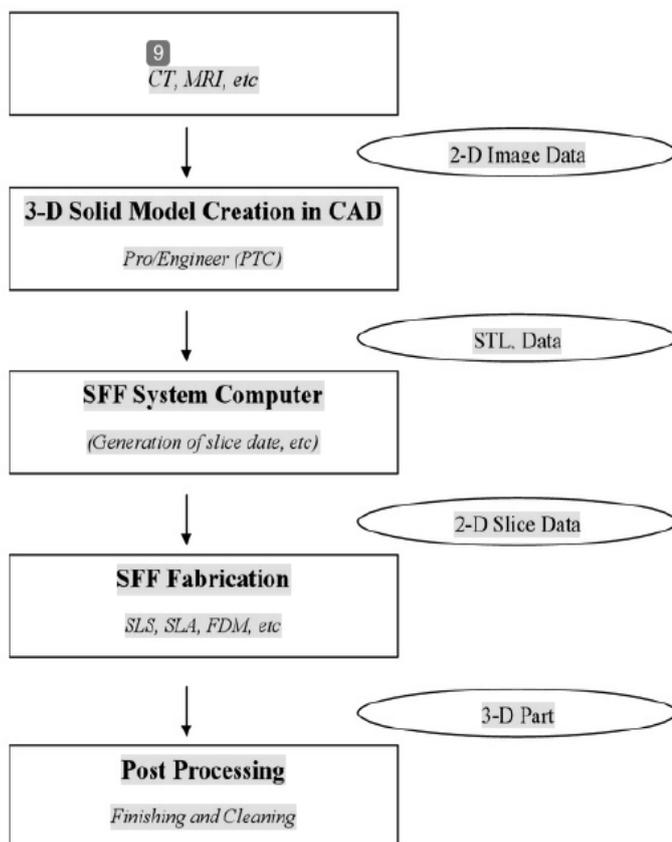


Fig-3: Solid Free Form Processing Flow [26].

5. Future Perspective

As been discussed in the review, there are various methods or techniques involved in developing scaffold. For every methods or techniques, they have their own process parameters that need to be considered such as the materials were used, temperature, pressure applied and time retained that will result in a good scaffold. If there are any changes in those process parameters, it may conclude in a scaffold that the microstructure property is inconsistent. In order to improve the available design of scaffold, the pore sizing, interconnectivity of pore, pore and porosity characteristics those are appropriate for diffusion of nutrient and attaching of cell. Other than that, the composition of chemical in the material for fabricating scaffold, roughness of surface, interaction of the material and cell and degradation also plays role in the characteristics of the scaffold surface. To overcome this, the expertise knowledge from the different field is required such as biomedical engineering and chemical engineering field. This might be taken into consideration in the future for enhancement of tissue engineering field and applications.

6. CONCLUSIONS

Basically, scaffolds are used as a site in attaching the cells, proliferating, differentiating and migrating by considering the protein synthesis and growing factors. Scaffolds plays role in providing mechanical support, delivering cells or molecules that are inductive to the affected site. Other than that, scaffolds help in controlling the tissues that are newly form in their structuring and functioning. There are many characteristics that need to be considered in a fabricated scaffold in providing good function. A scaffold can be fabricated by various methods where the methods provide the architecture that is regular.

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