

CHAPTER II

THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Tissue Engineering and Scaffold Materials

Many people are suffering from tissue or organ failure and are waiting for some kind of tissue or organ transplantation. Generally, the patient has been treated by autografting and allografting method (Murugan *et al.*, 2005). Although autogenic tissue transplantation is clinically considered as a gold standard, it has the limitation of donor site shortage. On other hand, allogenic transplantations are more prone to immunogenicity as well as inducing other transmissible diseases. Because of these clinical limitations, the use of tissue engineering was introduced, considerably saving numerous lives and improving the quality of life of patients (Murugan *et al.*, 2012).

2.1.1 Tissue Engineering

Tissue engineering is multidisciplinary field that applies the knowledge of engineering, material sciences and the clinical sciences in order to solve the critical medical problem of tissue loss and organ failure (Bokhari *et al.*, 2003). Tissue engineering involves the use of synthetic functional components (scaffold material), culturing them with appropriate cells that are harvested from patient or donor, and then reimplanting the engineered constructs in the patient's body where the tissue regeneration is required. There are four key factors to be considered for the success of any tissue development: (i) the cells that create tissue, (ii) the scaffold that give structural support to cells, (iii) the bioactive signaling molecules that regulate the cellular processes, and (iv) cell-matrix (scaffold) interaction that direct the tissue development and remodeling (Murugan *et al.*, 2005). Therefore, to achieve the goal of generating functional tissue *in vitro*, the specific cells, in particular anchorage dependent cells, should be combined with the right scaffold material under appropriate conditions, meaning that the cell must be subjected to conditions highly mimicking the native microenvironments that lead to tissue formation (see Figure 2.1).

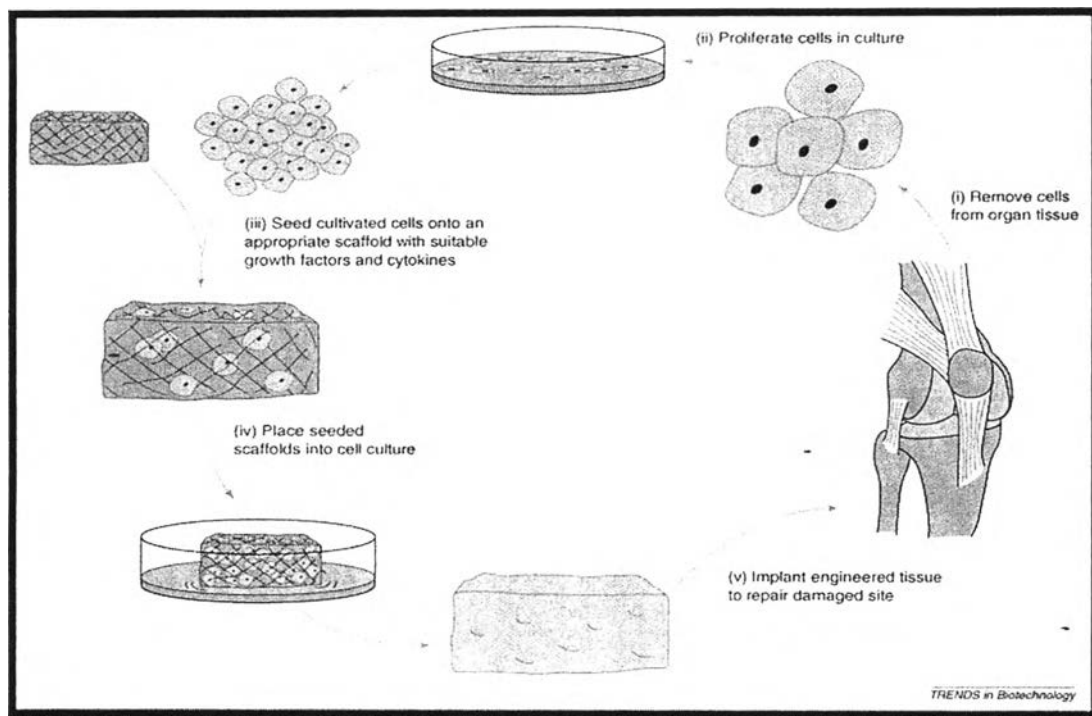


Figure 2.1 Basic principle of tissue engineering.

2.2.2 Scaffold Materials

Scaffold materials is significant for tissue engineering since they serve as matrices for cellular ingrowth, proliferation, and new tissue formation in three-dimensional (3D). Generally, scaffold materials serve at least one of the following purposes: First, it should be allow cell attachment and proliferation. Second, it can deliver and retain cells and biochemical factors. Third, it can enable diffusion of vital cell nutrients and expressed products. Fourth, it preserve apply certain mechanical and biological influence to modified the behavior of the cell phase. Scaffold for tissue engineering have been developed through a variety of techniques, and have been fabricated from natural and synthetic materials (Pakeyangkoon *et al.*, 2013). There is a lot of research try to design the scaffolds that must be followed by extensive clinical trials, so the opportunities for the use of scaffold for tissue engineering will surely use for human soon.

2.2.3 MTT Assay

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT: Sigma Aldrich, USA) assay is a quantitative method and standard colorimetric assay (an assay which measures changes in colour) for the measurement of cell viability and growth. The reduction of yellow tetrazolium salt in metabolically active cells to form an insoluble purple formazan crystal product by the dehydrogenase enzymes secreted from the mitochondria of viable cells. This assay can also be used to determine the cytotoxicity of potential medical agent and other toxic materials. Firstly, the cell-contained polyHIPE foam was washed two times with PBS to remove any unattached cells, and then a 300 μ l MTT solution (5 mg/mL in medium without phenol red) was added to each well and incubated at 37 °C for 30 min. After incubation of the cell with the MTT solution, a buffer (100 μ l/well) was placed in each well in order to extract the purple formazan crystal and determine their amount by using a UV visible spectrophotometer at a wavelength of 570 nm (Pakeyangkoon *et al.*, 2013).

2.2 High Internal Phase Emulsion Polymer (PolyHIPE)

Polymerization of high internal phase emulsion (HIPE) is a method to produce highly open porous polymeric foam with interconnecting pore networks. The internal or dispersed phase occupies more than 74% of the total volume and commonly contains water, an initiator and stabilizers. The external or continuous phase generally constitutes for less than 26% of the total volume. It usually consists of the monomer, a crosslinking comonomer and emulsifier (surfactant) (Cameron *et al.*, 1996).

By introducing droplet of aqueous phase in the organic mixture, under mechanical stirring, highly internal phase emulsion is produced. When polymerization of continuous phase occurred highly porous materials with interconnected and very low density will be formed (see Figure 2.2). For polyHIPE porous polymer, styrene (S) with divinylbenzene (DVB) based polyHIPE is the most popular one for produce polyHIPE porous materials. However, many polymer compositions such as methacrylate or acrylic acid are possible such as

poly(GMA/EGDMA)polyHIPE and poly(DVB/VBC)polyHIPE (Barbetta *et al.*, 2000). According to their unique properties such as high porosity, high surface area and degree of interconnectivity, polyHIPE porous materials are being considered attractive for many application such as poly(S/DVB)polyHIPE modified with bioactive molecules are being used as a scaffold for biomedical applications (Akay *et al.*, 2004, Busby *et al.*, 2001, Hayman *et al.*, 2005), poly(GMA/EGDMA)polyHIPE are used as a separation media (Krajnc *et al.*, 2005).

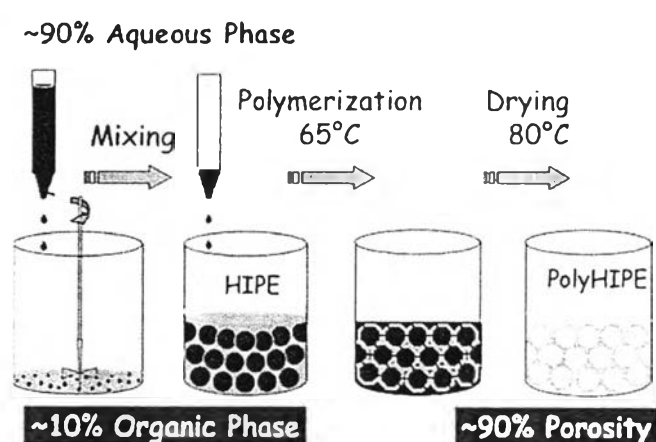


Figure 2.2 Scheme for typical preparation of polyHIPE.

Source: <http://matwww.technion.ac.il/Silverstein/HIPE.html>

For better understanding, some important wordings that will be used are defined here. Firstly, 'Pores' corresponds to the big spherical cavities within the material. Secondly, the small holes between adjacent pores and referred to as 'inter-connected pores' (see Figure 2.3).

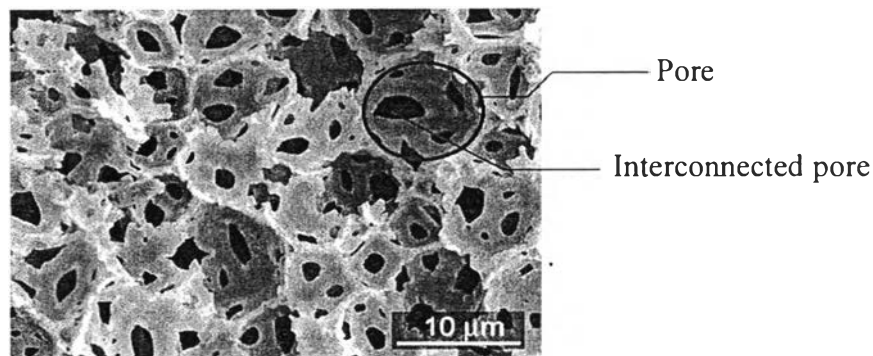


Figure 2.3 SEM picture showing typical structure of polyHIPE monolith. Pores and interconnected pores are marked.

Source: <http://matwww.technion.ac.il/Silverstein/HIPE.html>

2.2.1 Controlling the Morphology and Properties of PolyHIPE

It is possible to optimized and modified the structural and properties of polyHIPE through the regulation of pore sizes, interconnecting holes, surface area and other parameters by controlled the emulsion stability of system. The stability of polyHIPE emulsions is strongly influenced by many factors, a better understanding of these factors will have important impact for selecting type of oils or emulsifiers to obtain suitable system. Until now, there are many researches involve in the ways to increase emulsion stability of polyHIPE foam.

William and co-worker (1988) have demonstrated that the surfactant concentration affects the morphology of polyHIPE. They found that slightly higher surfactant levels lead to increase emulsion stability and a small opening in the cell wall, known as interconnected pores, are formed. However, at the surfactant level above 50% of the total organic phase volumes, the structures of the resulting materials were not maintained. The polyHIPE foam generated under these conditions would has no walls and the structure of the emulsion would collapse. From this work, they concluded that fine cellular morphology and small interconnected with very smooth surface area were observed at around 20% surfactant used.

Generally, polyHIPE polymer with closed cell type will be formed because of rest monomers, residual surfactants, and porogenic solvent in the continu-

ous phase are trapped within the cellular structure of polyHIPE porous polymer. This lead to produced polyHIPE materials with low surface properties. To overcome this problem, the Soxhlet extraction technique is needed to remove the residual materials from pore structure of polyHIPE foam and resulting materials with high surface properties would be obtained. Additionally, the effect of Soxhlet extraction time on the resulting materials was investigated by Morgan and co-worker (2003). They showed that the extraction time was found to have an effect on the mechanical and flammability properties of the resulting polypropylene nanocomposite. In year 2008, Pakeyangkoon *et al.* showed the effect of soxhlet extraction and surfactant system on morphology and properties of PolyHIPE.

2.2.2 PolyHIPE for Tissue Engineering Application

Three dimensional highly open porous polymeric foam, produced from a high internal phase emulsion (HIPE) technique is one of the most widely used synthetic materials in tissue engineering application. Poly(styrene/divinylbenzene)polyHIPE foam has successfully been tested for its ability to support the growth of cells, and it has good biocompatibility between osteoblasts and solid supports in the *in vitro* environment (Akay *et al.*, 2004).

Moreover, biomaterials based polyHIPE foam has been studied by Busby *et al.*, (2001) They prepared highly porous polyHIPE foam containing poly(ϵ -caprolactam; PCL) by the free radical polymerization of a PCL macromonomer. PolyHIPE porous foam containing the PCL macromonomer can act as a substrate for the growth of human fibroblast. These materials are sufficiently biocompatible to support cell function and growth over a period of 2.5 days. A general property demanded of all substrate materials in tissue engineering application is biocompatibility or even bioactivity. The substrate materials must be compatible, non-toxic to living cells, and able to support cell growth, adhesion, and proliferation. Usually, the interaction between the substrate and living cells on hydrophilic materials is better than hydrophobic materials.

However, polymeric scaffolds synthesized via HIPE technique have been made of hydrophobic polymers such as polystyrene (PS), which by nature has hydrophobic characteristics. This is improper for supporting the cell-scaffold

interaction, and poor adhesion between cell and substrate is obtained since an aqueous media has usually been used in cell culturing. Therefore, it is difficult for the culture media to penetrate the porous scaffold structure throughout and attach to the biomaterial surface, consequently not promoting cell spreading, seeding, and growth. Numerous reports have indicated that ethylene glycol dimethacrylate (EGDMA) and its derivatives have been widely used in the fabrication of scaffolds in tissue engineering applications. Substrate materials prepared from EGDMA, which act as the crosslinking agent, have been shown to be biocompatible, have high water uptake, and have relatively low cytotoxicity. (Lin-Gibson *et al.*, 2005)

In 2007, Christenson *et al.*, (2007) reported that HIPE technique can be used to generate highly porous biodegradable scaffold with interconnected pores for tissue successful prepared by HIPE technique containing the macromer poly(propylene fumarate) (PPF) and the cross-linker propylene fumarate diacrylate (PFDA).

However, polyHIPE foam HIPE technique usually produces from synthetic materials such as polystyrene, which is hydrophobic in nature and lack of cell recognition signals. This is improper for supporting the cell-scaffold interaction, and poor adhesion between cell and substrate is obtained since an aqueous media has usually been used in cell culturing. Therefore, the investigation of scaffold materials with desirable surface chemistry properties was concerned in order to improve cell adhesion, support cell growth, proliferation and also differentiation. There are several methods have been developed in order to enhance the biological response to tissue fluids, hydrophilic groups and wetting characteristics, including physical, mechanical treatment, conventional coating processes (i.e. spraying or dipping), vacuum deposition technique, wet chemical treatment (i.e. plating, grafting, or bonding). Pekeyangkoon *et al.* (2013) investigated surface modification via atmospheric pressure plasma treatment. They found that this surface modification was successfully used to enhance the hydrophilic properties of the polyHIPE foam surface, as well as improve the interaction between the living cells and the polyHIPE substrate.

2.3 Layer-by-Layer Technique

Layer-by-Layer (LbL) method is a technique used in surface modifications has received much attention in the field of electrochemistry as a very promising tool for the fabrication of nanostructured films with high organisation at the nanoscale level fabricated films through specific interactions of organic functional groups for multilayer growth, and the films deposited onto a solid substrate were obtained with high stability. For the growth of multilayer films, the adsorption occurred as a result of alternating the immersion process of a solid substrate (for example, quartz and hydrophilic or hydrophobic glasses) in a cationic and anionic polyelectrolyte solution and the excess of weakly adsorbed polyelectrolyte is washed in a solvent solution and dried with nitrogen gas/air flow (see Figure 2.4).

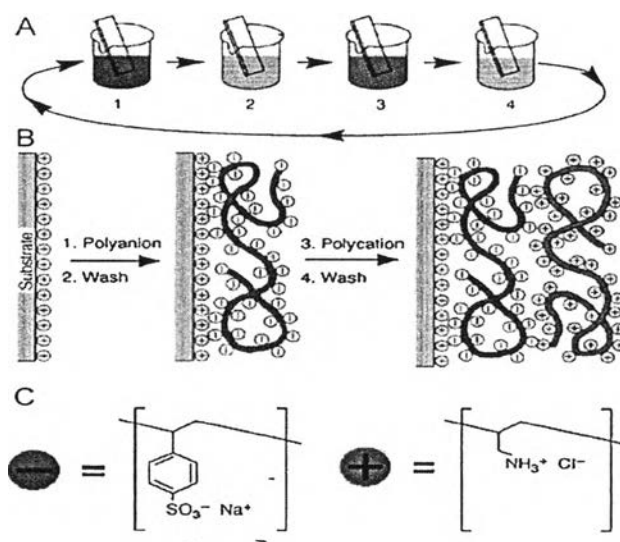


Figure 2.4 (A) Schematic of the film deposition process using slides and beakers. (B) Simplified molecular picture of the first two adsorption steps, depicting film deposition starting with a positively charged substrate. (C) Chemical structures of two typical polyions, the sodium salt of poly(styrene sulfonate) and poly(allylamine hydrochloride).

Source : <https://sites.google.com/site/arshadtu/layerbylayer>

The LbL method becomes very advantageous because numerous different materials can be employed in the fabrication of films. Also, LbL is more versatile than other fabrication techniques and provides the possibility of using a large range of different materials in films construction, including polyelectrolytes (Clark *et al.*, 1997), dendrimers (DENs) (Zhang *et al.*, 2003) and carbon materials (Olek *et al.*, 2004). Moreover, parameters such as roughness, thickness, and porosity of films can be controlled by changing experimental conditions such as pH, temperature, polyelectrolyte concentration, and ionic strength of the media (Schönhoff *et al.*, 2007).

McCarthy and co-worker (1997) reported that Layer-by-layer deposition of polyelectrolytes onto poly(ethylene terephthalate) (PET) film as a method for polymer surface modification. PET samples that had been surface modified to contain carboxylate (PET-CO₂⁻) and ammonium (PET-NH₃⁺) functionality. The individual layers are extremely thin (2-6 Å), and this thickness is affected by the substrate surface chemistry and can be controlled by adjusting the ionic strength of the polyelectrolyte solutions. They found that the layer-by-layer deposition process is quite forgiving and proceeds under a variety of conditions. Peel tests indicate that the multilayer assemblies show good mechanical integrity, no failures were observed in the multilayers.

In year 2009, Dubas *et al.*, (2009) studied the coating of polyelectrolyte multilayer thin film on nanofibrous scaffolds to improve cell adhesion. They found that coated fiber show more cell attachment, proliferation, and spreading than the uncoated one. From this result can be suggested that surface modification with PEMs is an effective technique for increasing cell adhesion.

2.4 General Properties of Chemical that Used in this Research

2.4.1 Polystyrene (PS)

Polystyrene is a vinyl polymer, produced from free radical vinyl polymerization of petroleum based styrene monomers, a hydrocarbon material. Its structure consists of a long hydrocarbon chain with phenyl groups (aromatic rings) besides, along the chain.

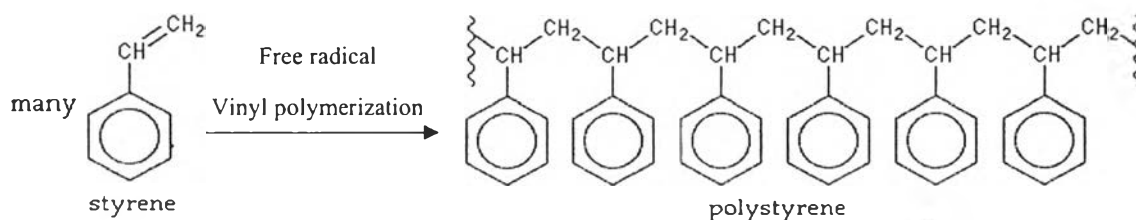


Figure 2.5 Free radical vinyl polymerization reaction of polystyrene.

Source: Wikipedia.org

Polystyrene is classified as a thermoplastic material, since it turns into liquid when heated above 185°F (85°C) and freezes to a rigid state when cooled sufficiently, which means that at room temperature it is solid. Due to the many aromatic rings in its structure, polystyrene is a material that possesses high fire resistance, good resistance to acids and bases, good electric insulating properties, high shock-resistance, and excellent surface characteristics. Due to these outstanding properties, polystyrene can be considered as a unique and potentially useful material. A further advantage of polystyrene is that it is an inexpensive raw material. Hence, it is an excellent material for producing consumer products.

2.4.2 Ethylene Glycol Dimethacrylate (EGDMA)

Ethylene Glycol Dimethacrylate or 2-(2-Methyl-acryloyloxy)ethyl 2-methyl-acrylate (IUPAC name) is a diester produced from two equivalents of methacrylic acid and one equivalent of ethylene glycol. It is a clear liquid and typically used as a functional monomer for polymers as well as a free radical copolymer crosslinking agent (high purity crosslinker with bridging/specialty capability) between the molecular chains of polymers and elastomers.

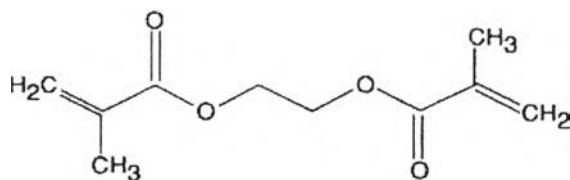


Figure 2.6 Structure of Ethylene Glycol Dimethacrylate.

2.4.3 Polystyrenesulfonate (PSS)

Polystyrenesulfonate or PSS are polystyrene sulfonic acid polysalts, usually in form of sodium and calcium. PSS is polyanion, white powder and soluble in water. They have widely used as ion-exchange resins.

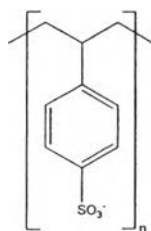


Figure 2.7 Structure of Polystyrenesulfonate.

Source: Wikipedia.org

2.4.4 Poly(Diallyldimethylammonium Chloride) (PDADMAC)

Polydiallyldimethylammonium chloride (shortened polyDADMAC or polyDDA) is a homopolymer of diallyldimethylammonium chloride (DADMAC). The molecular weight of polyDADMAC is typically in the range of hundreds of thousands of grams per mole, and even up to a million for some products. PolyDADMAC is usually delivered as a liquid concentrate having a solids level in the range of 10 to 50%. It is a high charge density cationic polymer. The charge density makes it well suited for flocculation.

PolyDADMAC is used as a coagulant in water purification. It is effective in coagulating and flocculating inorganic and organic particles such as silt, clay,

algae, bacteria and viruses. At high concentrations the organic polymer can remove natural organic matter such as humic and fulvic acids resulting in fewer disinfection by product precursors and less color.

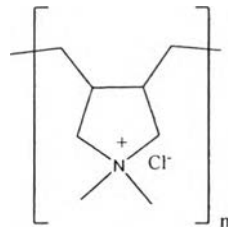


Figure 2.8 Structure of Poly(diallyldimethylammonium chloride)

Source: Wikipedia.org