

## CHAPTER III

### EXPERIMENTAL

#### 3.1 Materials

Styrene (S; Sigma-Aldrich Chemical) and ethylene glycol dimethacrylate (EGDMA; Sigma-Aldrich Chemical) were used as monomer to produce polyHIPE porous material. The surfactant was sorbitanet monooleate (Span80; Sigma-Aldrich Chemical). The initiator and stabilizer were potassium persulfate ( $K_2S_2O_8$ ; Fluka Chemie) and calcium chloride ( $CaCl_2$ ; Fluka Chemie) respectively. Tetrahydrofuran (THF) and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich Chemical. Hydroxyapatite (HA) was used as received.

#### 3.2 Methodology

##### 3.2.1 PolyHIPE Preparation

Poly(styrene/ethylene glycol dimethacrylate) polyHIPE porous foams were prepared by using the high internal phase emulsions technique. The oil phase (10 vol% of the total volume) contains styrene (S) as a monomer, ethylene glycol dimethacrylate (EGDMA) as a crosslinking agent and a surfactant (sorbitan monooleate; Span80 2 ml). The aqueous phase consists of water (89 ml), THF (1 ml),  $K_2S_2O_8$  (0.2 g) and  $CaCl_2$  (1 g) then varying the ratio of styrene and ethylene glycol dimethacrylate by volume at 90:10 80:20 70:30 60:40 and 50:50 respectively. After that, stir the aqueous phase and transfer it to a dropping funnel. The aqueous phase is added drop-wise into the organic phase under constant mechanical stirring at 300 rpm. Dosing rate of the aqueous phase is 1.77 ml/s. After finish dropping, pour the resulting emulsion into vials. The resulting materials are polymerized by placing them in a water bath at  $60C^{\circ}$  for 24 hours. After that, the solid materials were extracted with isopropanol for 6 hours and washed with water for 24 hours to remove some

residue from pores of polyHIPE. Then place the solid materials in vacuum oven 60 C° until a constant weight was obtained.

### 3.2.2 Hydroxyapatite Modification

Poly(styrene/ethylene glycol dimethacrylate) polyHIPE porous foams were prepared by using the high internal phase emulsions technique. The oil phase (10 vol% of the total volume) contains styrene (S) as a monomer, ethylene glycol dimethacrylate (EGDMA) as a crosslinking agent (S:EGDMA; 70:30 ratio by volume) and a surfactant (sorbitant monooleate; Span80 2 ml). The aqueous phase consists of water (89 ml), THF (1 ml), small amount of phosphoric acid, K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (0.2 g), CaCl<sub>2</sub> (1 g) and varying the amount of hydroxyapatite between 0.1% to 1.0% w/v. Then stir the aqueous phase and transfer it to a dropping funnel. The aqueous phase is added drop-wise into the organic phase under constant mechanical stirring at 300 rpm. Dosing rate of the aqueous phase is 1.77 ml/s. After finish dropping, pour the resulting emulsion into vials. The resulting materials are polymerized by placing them in a water bath at 60C° for 24 hours. After that, the solid materials were extracted with isopropanol for 6 hours and washed with water for 24 hours to remove some residue from pores of polyHIPE. Then place the solid materials in vacuum oven 60 C° until a constant weight was obtained.

### 3.2.3 Poly(S/EGDMA) PolyHIPE Foam Characterization

The physical properties such as phase morphology, surface area and wettability of the polyHIPE porous foam were measured by scanning electron microscope (SEM), surface area analyzer (BET), and contact angle measurement respectively. The chemical property on the surface of polyHIPE was investigated by fourier transform infrared spectroscopy (FT-IR). A Universal Testing Machine (Lloyd model LRX) was used for mechanical properties of polyHIPE foams.

### *3.2.3.1 Phase Morphology*

The scanning electron microscopy was performed with a Hitachi S-4800 field emission scanning electron microscope operating at 10 kV with  $\times 50$ ,  $\times 100$  and  $\times 400$  magnifications in order to investigate phase morphology and determine pore size of the poly(S/EGDMA) porous foam. All specimens were coated with platinum under vacuum before analysis.

### *3.2.3.2 Surface Area Measurement*

N<sub>2</sub> adsorption-desorption isotherms were obtained at -196 °C using a BET model Quantachrome, Autosorb 1MP. The samples were degassed at 100 °C for 18 hours in vacuum furnace before analysis. Determination of surface area was measured using the dynamic seven point BET analysis method.

### *3.2.3.3 Contact Angle Measurement*

The static contact angle measurement was performed by using a Kruss (model DSA 10) contact angle measuring instrument at ambient temperature to determine wettability of the surface of polyHIPE scaffolds. A 10  $\mu$ L droplet of deionized water was vertically dropped with a micro-syringe onto the polyHIPE surface. The average contact angles were measured by using shape analyzer program.

### *3.2.3.4 Mechanical Properties*

The mechanical properties of all polyHIPE porous scaffolds were measured in compression mode, according to ASTM D822. Cylindrical test specimen (2.54 cm in diameter and 2.54 cm in length) were prepared. A speed of 0.127 cm/min and a 2.5 KN load cell were used for all measurements. The compressive strength was defined as the maximum load registered, divided by the initial cross-sectional area of the scaffold. The compressive modulus was calculated by measuring the maximum slope of the elastic region of stress-versus-strain curve immediately from the toe-in region.