CHAPTER II
LITERATURE REVIEW

2.1 Emulsions

An emulsion is a heterogeneous system consisting of two immiscible liquid phases. One phase is dispersed in the other phase as drops of microscopic or colloidal size. There are two types of emulsion, oil-in-water (O/W) and water-in-oil (W/O) emulsion.

The O/W emulsion can be found in mayonnaise, ice cream and milk. On the other hand, W/O emulsion can be found in butter and margarine. Emulsions result from mixing of pure immiscible liquids. Emulsions are usually very unstable and can break rapidly. For this reason, an emulsifying agent or surfactant is necessary for stabilizing the emulsions. Surfactants decrease the interfacial tension and the total free energy of the interface between two immiscible phases, which can facilitate emulsion formation and improve emulsion stabilization. The surfactant molecule consists of two structures: hydrophilic head groups and hydrophobic tail groups. The hydrophilic group makes the surfactant soluble in non-polar solvents and oil.

A type of emulsion (W/O or O/W) is affected by preferential solubility of the emulsifying agent in one of the phases (Bancroft *et. al.,* 1913). According to Bancroft's rule, the phase in which the surfactant is predominantly dissolved tends to be the continuous phase. In O/W emulsions, surfactants are more soluble in water than in oil. In W/O emulsions, surfactants are more soluble in oil than in water. However, the type of emulsion has been interpreted quantitatively, according to the hydrophilic-lipophilic balance (HLB) classification. HLB number is a measure of hydrophilic and hydrophobic moieties within a non-ionic surfactant (Griffin *et. al.,* 1949). The W/O emulsions require low HLB surfactants. In contrast, the O/W emulsions often require higher HLB surfactants.

Emulsions may breakdown by various mechanisms such as coalescence, Ostwald ripening, creaming, and sedimentation as shown in Figure 2.1.

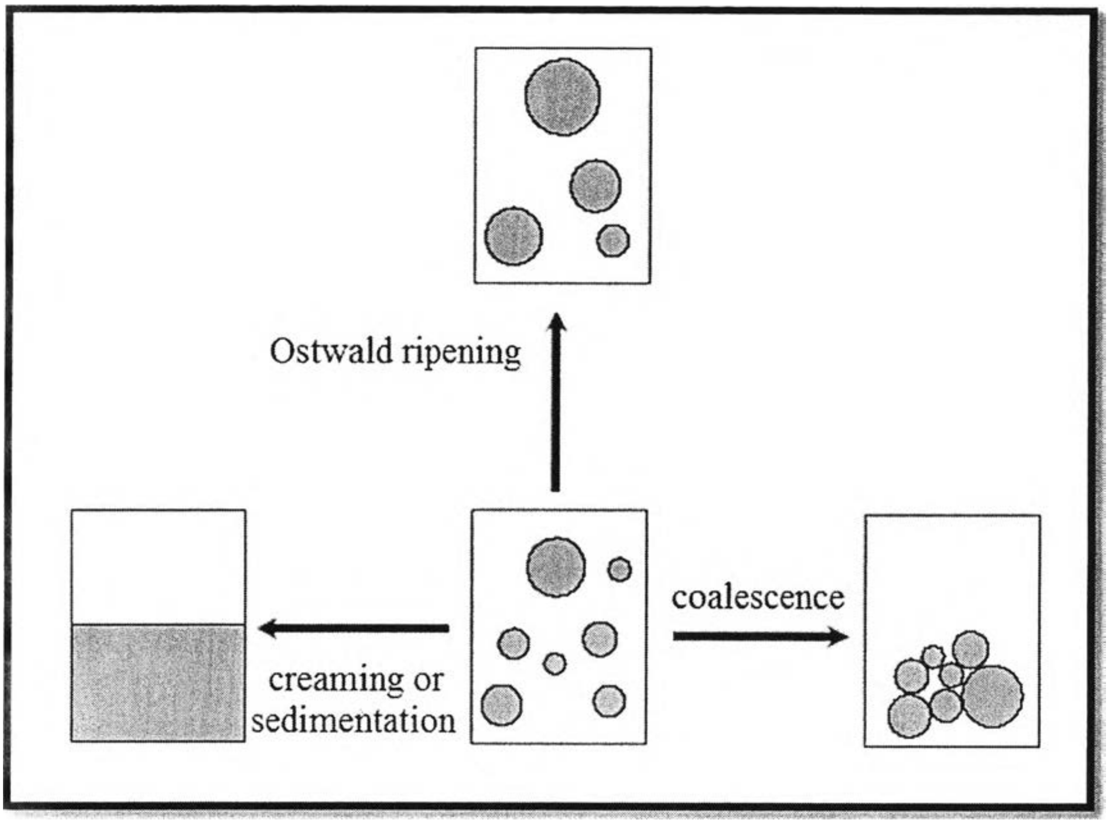


Figure 2.1 Schematic representation of the emulsion breakdown.

Irreversible coarsening in emulsions can occur by two different mechanisms: coalescence and Ostwald ripening. Coalescence is the irreversible process in which two or more emulsion droplets fuse together to form a single bigger droplet. The fusion is controlled by nucleation within the thin film separating droplets. Conversely, Ostwald ripening is the process by which components of the discontinuous phase diffuse from smaller to larger droplets through the continuous phase. In Ostwald ripening the growth occurs by diffusion of the droplet phase through the continuous phase one molecule at a time. Creaming means floating of oil droplets in an O/W emulsion due to different densities of oil and water. The process in a W/O emulsion is called sedimentation. In this case water droplets settle down to the bottom of the sample.

2.2 High Internal Phase Emulsion Polymerization

The first fundamental principle for the formation of HIPEs is the presence of two immiscible liquids. One of which is water or aqueous solution and the other is organic or oil phase. Lissant defined the high internal phase emulsions (HIPEs) as emulsions which have internal phase volume above 74.05% of total emulsion volume (Lissant *et. al.,* 1974). This number represents the maximum volume which internal phase can be occupied by uniform, undeformed spherical particles (Cameron *et. al.,* 1996). HIPE can be found in either normal (oil-in-water, O/W) or inverse (water-in-oil, W/O) forms. Hydrophobic polymers synthesized within water-in-oil emulsions and hydrophilic polymers synthesized within oil-in-water emulsions. PolyHIPEs are formed by an emulsion consisting of two phases: the continuous phase or non-droplet phase, which contains the monomer(s), and the internal phase or droplet phase, which is responsible for the porosity of the material (Barbetta *et. al.,* 2004). Preparation of the high internal phase emulsions requires an appropriate surfactant which is completely insoluble in the droplet or dispersed phase. The suitable surfactant must prevent the emulsion from inversion at high internal phase volume fractions. In addition, the surfactant stabilizes HIPEs which will help HIPE formation.

Morphology of polyHIPE; the large spherical cavities are called “pores”, whereas the circular holes connecting adjacent pores to one another are known as "interconnected pore". The pore size of polyHIPE porous foam can be changed from sub-micron range to a few hundred microns by changing the chemical composition of emulsion and the processing conditions. It has been indicated in previous studies that the pore sizes can be obtained in the range of 0.5–10,000 mm (Bokhari *et. al.,* 2003). A minimum pore size that allows cell to migrate and tissue to penetrate into the scaffold is 50 µm. An optimum pore size of 100–400 µm preferred by osteoblasts because it provides the optimum compression and tension on the osteoblasts (Boyan *et. al.,* 1996).

Porosity allows cells to migrate through the pores, and allows nutrients transport to cells (Karageorgiou *et. al.,* 2005). Cell migration and tissue penetration into the scaffold require a minimum pore size of 50–100 μm, an optimal in growth of osteoblast cells requires pore sizes of 200–400 μm (Davies *et. al.,* 2000).

PolyHIPEs can easily be modified by addition of chemicals with the desired properties, e.g. hydroxyapatite. Hydroxyapatite is a calcium phosphate (CaP) ceramic which is considered as the most suitable ceramic materials for bone reconstruction. Hydroxyapatite has been extensively used because of its biocompatibility and osteoconductivity, which can promote cell growth (Dorozhkin *et. al.,* 2002). Hydroxyapatite also has excellent chemical biological affinity with bony tissues to encourage new bone formation with bone without any need for the presence of other connective tissue.

2.3 Preparation of PolyHIPE Materials

PolyHIPEs are prepared by dripping the droplet phase into the continuous phase, which is composed of the monomer(s), crosslinking agents, and the suitable surfactants. After the addition of the droplet phase, the continuous phase around the droplets polymerizes. The droplets then create voids within the foams and result in a highly porous and permeable morphology. The obtained material is then washed in a Soxhlet apparatus and then dried.

In practice, liquids that can be used to prepare the polyHIPEs material must be either hydrophilic or hydrophobic, in order to generate a stable emulsion. Stable emulsions can be formed from two immiscible liquids when an emulsifier or surfactant is used. Such emulsions will not undergo phase separation after a change in conditions like temperature or over time. This process is shown in Figure 2.2 (Cameron *et. al.,* 2003).

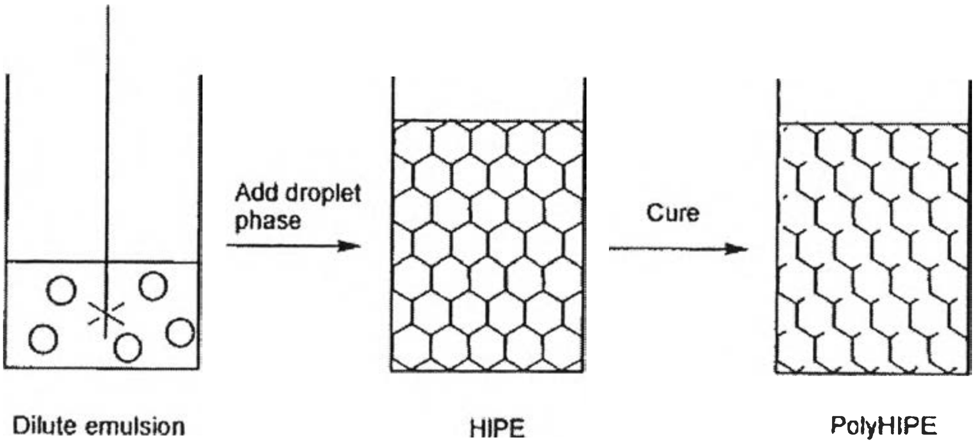


Figure 2.2 Scheme for preparation of polyHIPE by high internal phase emulsion polymerization technique.

2.3.1 Controlling the morphology and properties of polyHIPEs

2.3.1.1 Cellular structure

PolyHIPE porous foams are can be obtained as either closed or open cell materials. Williams *et.al* (1988) have demonstrated that the amount of surfactant affects the structure of polyHIPE pores. Below 5% surfactant, closed cells are produced and lead to high density polymers. Above 7% surfactant, open cells are formed and lead to low density polymers (Figure 2.3).

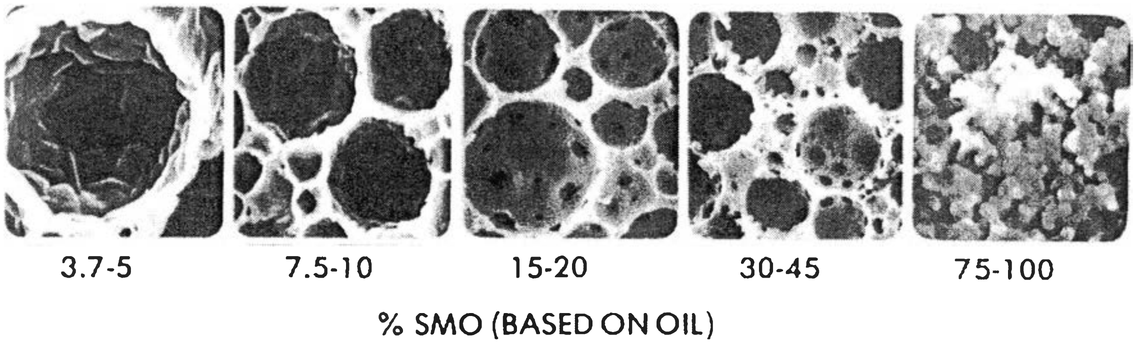


Figure 2.3 Scanning electron microscope shows the microstructures of emulsion foams with varying percentage of surfactant.

2.3.1.2 Pore size

Williams *et.al.* (1990) have studied the effect of ionic strength on the pore size and indicated that increasing amount of salt (potassium sulfate) reduced the pore size of the polyHIPE porous foams (Figure 2.4).

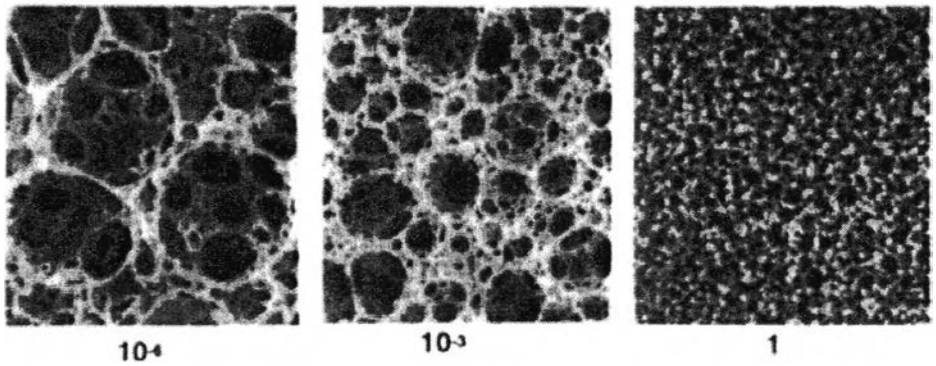


Figure 2.4 Microstructure of polyHIPEs contain varying amount of salt $K_2S_2O_8$ (the numbers indicate the grams of salt per 100 mL of water).

Moreover, this group also discovered that divinylbenzene helped to diminish the pore size. Since divinylbenzene is more hydrophobic than styrene, increasing the amount of divinylbenzene causes the emulsion to become more stable, which lowers the rate of droplet coalescence and leads to a smaller poresize (Figure 2.5).

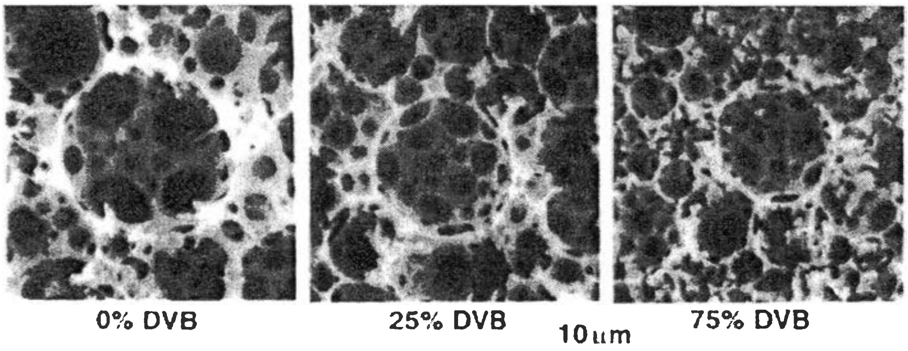


Figure 2.5 Microstructure of polyHIPEs varying amount of divinylbenzene which is cross-linking agent.

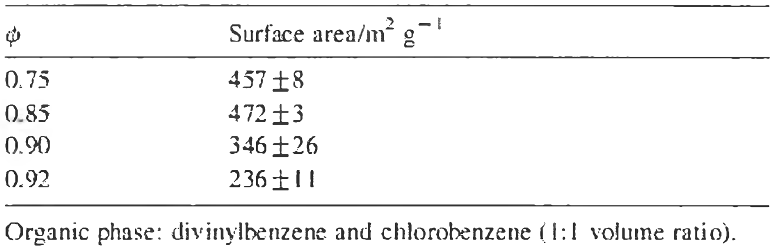
Furthermore, the presence in the aqueous phase of small quantities of organic additives promotes Ostwald ripening. *Tetrahydrofuran* (THF) is a organic additive which is capable of partitioning between the two emulsion phases was found to increase the average pore and interconnected pore diameters. Addition of 1% (v/v) THF to the aqueous phase of a styrene/DVB HIPE leads to the increasing of pore sizes in the range 50–150 μm (Hayman *et. al.*, 2004).

Increasing the temperature of the aqueous phase also increases the average pore and interconnected pore size (Ross *et. al.*, 2006). When increase the temperature of the aqueous phase, frequency of contact droplets will increase and result in a higher probability of droplet coalescence.

2.3.1.3 Surface Area

Cameron *et.al.* (2000) have proposed a method to increase surface area by using hydrophobic solvents instead of some part of monomer(s) in the organic phase. The resulting material has smaller porous structures, but lager pores due to phase separation during polymerization. Furthermore, the internal phase volume (ϕ) has an effect on the surface area (Hainey *et.al.*, 1991). The surface area of polyHIPE porous foam decreases with increasing the volume of internal phase (Table 2.1).

Table 2.1 The surface area of PolyHIPE materials according to volume fraction (ϕ) of the internal phase



ϕ	Surface area/m^2 g^{-1}
0.75	457 ± 8
0.85	472 ± 3
0.90	346 ± 26
0.92	236 ± 11

Organic phase: divinylbenzene and chlorobenzene (1:1 volume ratio).

2.3.1.4 Thermal Stability

PolyHIPE porous foams have a low thermo-oxidative stability. The thermo-oxidative stability of these materials increases with increasing concentration of maleimide (Hoisington *et. al.*, 1997). Another work (Duke *et. al.*, 1998) illustrated that N-substituted maleimides can be copolymerized with polyHIPE in order to enhance thermal properties. N-cyclohexylmaleimide and N-ethylmaleimide maximize Tg of polyHIPE materials. However, N-propylmaleimide and N-butylmaleimide minimize Tg of polyHIPE materials (Cameron *et. al.*, 1997)

2.3.1.5 Mechanical Properties

The mechanical properties of poly(styrene/divinylbenzene) polyHIPE are similar to mechanical properties of gas-blown polystyrene foam, but poly(styrene/divinylbenzene) expresses higher compressive strengths (Williams *et. al.*, 1988). At a high stress rate, polyHIPEs will collapse. Crush strength decreases with decreasing density of foams. (Figure 2.6) Furthermore, the concentration of surfactants present in the system has an impact on the mechanical properties as well since, the stress-strain behavior is sensitive to surfactant concentration (Williams *et. al.*, 1990).

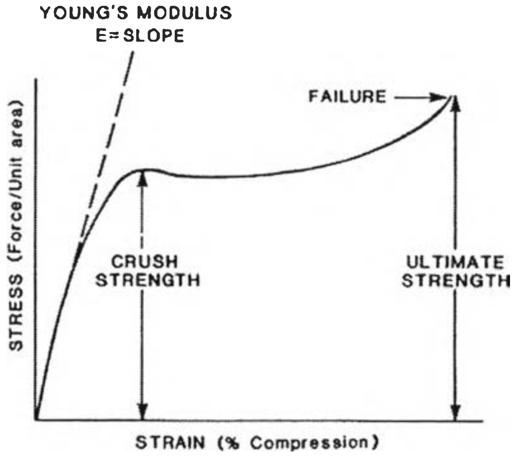


Figure 2.6 Stress/strain curve of poly(styrene/divinylbenzene) polyHIPE material.

2.3.2. Poly(styrene/ethylene glycol dimethacrylate) polyHIPE

Poly(styrene/ethylene glycol dimethacrylate) polyHIPE highly porous foam was prepared from using high internal phase emulsion technique. Poly(S/EGDMA) was modified by atmospheric pressure plasma treatment was used to improve hydrophilic properties and scaffold/cell interaction. Poly(S/EGDMA) fulfils the requirements for used as scaffold in tissue engineering applications (Pornsri *et. al.*, 2012). Pronsri *et. al.* demonstrated that the efficiency of attachment and proliferation of the L929 fibroblast-like cells to the poly(S/EGDMA) polyHIPE increases with the use of the atmospheric pressure plasma surface modification technique. The level of cell attachment of the treated poly(S/EGDMA) polyHIPE foam was higher, compared to the untreated specimen (Figure 2.7).

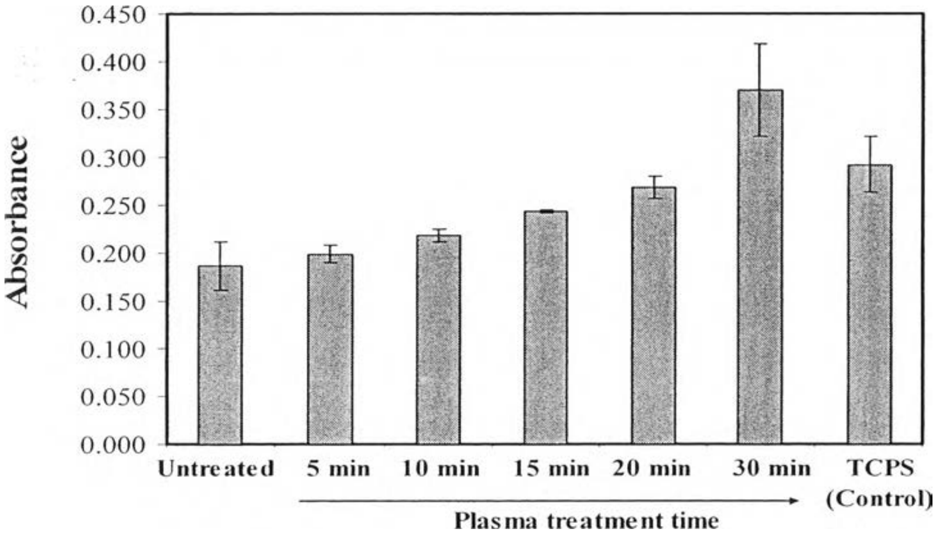


Figure 2.7 The effect of plasma treatment time on cell attachment of poly(S/EGDMA) polyHIPE foam scaffold.

The proliferation of the fibroblast-like cells on the treated poly(S/EGDMA) polyHIPE foam was better than that on the poly(S/EGDMA)polyHIPE foam without being subjected to plasma surface modification (Figure 2.8).

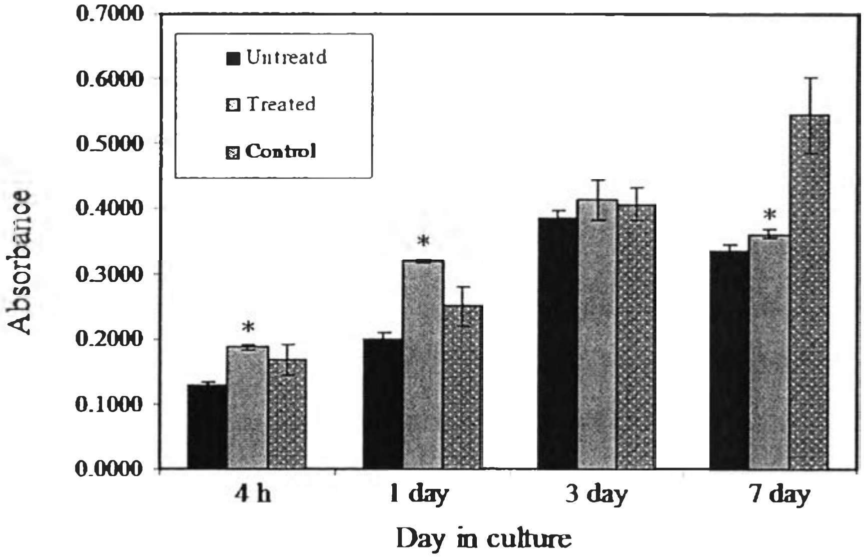


Figure 2.8 The effect of plasma treatment time on cell proliferation of poly(S/EGDMA) polyHIPE foam scaffold.

2.3.3 Modification the Internal Surface of PolyHIPEs by Deposition

The thermal stability of poly(ST/DVB)polyHIPE can be enhanced by including small amount of organic antioxidants. Park *et.al.* (1992) deposited a conducting polymer, either pyrrole or thiophene, followed by an oxidant, like iron trichloride onto the internal surface of poly(ST/DVB) polyHIPE materials. There are many techniques to modify surface of polyHIPE. For example, nickel-mesh electrodes were embedded into polyHIPE to increase mechanical properties. Sotiropoulos *et.al.* (1993) has performed electroless plating of Ni from solutions into polyHIPEs. Akay *et.al.* (2004) have modified poly(PS/DVB) polyHIPE porous foam by the deposition of hydroxyapatite onto the internal surface. Deposition of hydroxyapatite onto poly(PS/DVB) polyHIPE leads to greater cell penetration and a higher number of attached cells. There are very important features for biomedical applications.

The result reveals that when polymers are modified with hydroxyapatite there is a significant increase in penetration of cells into the polymer and increased in formation of rat osteoblasts compared to unmodified polyHIPE polymer (Figure 2.9).

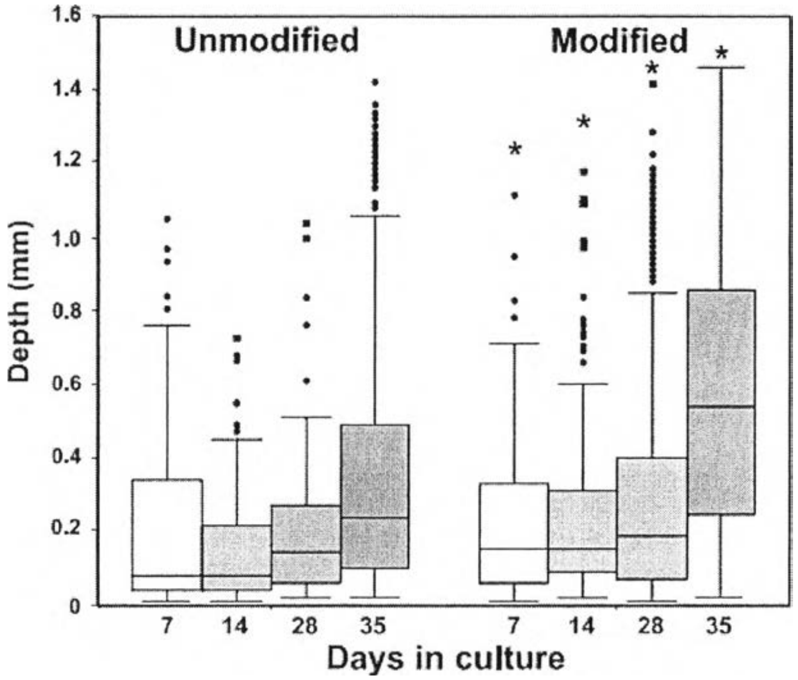


Figure 2.9 The plot shows depth of penetration of rat osteoblasts cultured on modified or on unmodified polyHIPE.

OBJECTIVES

- To study high internal phase emulsion polymerization technique
- To study the effect of hydroxyapatite on poly(S/EGDMA)polyHIPE system
- Evaluate the morphology and properties of poly(S/EGDMA)polyHIPE
- Evaluate the effectiveness of hydroxyapatite