CHAPTER I

INTRODUCTION

1.1 Background of the present study

Bone is a specialized connective tissue and a component of the skeletal system. It has several major functions, (i) mechanical function; It forms a rigid skeleton to provide a framework for the body, support for soft tissues, site of attachment for skeletal muscles, (ii) metabolic function; it acts as a central role in mineral homeostasis, not only reservoir of calcium and phosphate ions, but also of sodium and magnesium ions. (iii) protective function; it protects the internal organ and bone marrow (Kartsogiannis and Ng, 2004). The major component of bone is inorganic matrix (70% by weight), which the analog of natural hydroxyapatite (HAP) [$Ca_{10}(PO_4)_6(OH)_2$], with many impurities and usually referred to as a poorly crystalline, carbonate substituted apatite. Organic matrix is composed of 25% to provide elasticity and flexibility and determines the structural organization. Water and lipid is the minor component. The organic part of bone contains extracellular matrix components and cell components. The extracellular matrix consists of fibers, proteoglycans, glycoproteins and tissue fluid. The cell components include fibroblasts, chondrocytes (the cartilage forming cells), osteoblasts (the bone forming cells), and osteoclasts (the bone resorbing cells).

Bone is a dynamic tissue that is constantly remodeled throughout life. Bone remodeling is the process associated with growth and reshaping of bones in childhood and adolescence. Remodeling implies the continuous of bone resorption followed by synthesis of new bone matrix and subsequent

mineralization (bone formation). The maintenance of normal, healthy bone requires the coupling of bone formation and bone resorption, with intercellular communication between osteoblasts and osteoclasts to the successful of a balance between the two processes. First, for bone remodeling, the osteoblast releases collagenase to remove the nonmineralized organic matrix which covers bone surfaces. The osteoclast is then moved to this site, seals itself onto the calcified matrix, and acidifies it by pumping protons outwards, to decalcify the calcium salts. The osteoclast then releases various lysosomal enzymes to remove the exposed organic matrix (Nair et al., 1996). After resorption by osteoclasts, osteoblasts will be recruited to lie down and release bone matrix (osteoid) replace at the site of erosion (Christenson, 1997).

Osteoblasts derive from an undifferentiated mesenchymal cells and present in bone in three forms; preosteoblast, mature osteoblast, and osteocyte which trapped within the bone. One of the major function of the osteoblast is to produce the components of the bone matrix; mostly collagen type I (COLI) with lesser amounts of proteoglycans and glycoproteins, and to catalyze the calcification of the matrix. The other major function is to control the activity of osteoclasts (Nijweide et al., 1986). Osteoclast, multinucleated cells are believed to derive from the myeloid precursor cells. The osteoblasts have a close relationship to the chondrogenic cells (Aubin et al., 1995). The extracts from bone marrow stroma and calvariae from rat and mouse contain osteogenic lineage, which is called osteoprognitor cells. These stem cells are undifferentiated pluripotent stromal stem cells, which have the capacity to differentiate into lineages other than osteoblasts, including chondroblasts, fibroblasts, adipocytes, and myoblasts (Nijweide et al., 1986, Aubin et al., 1995).

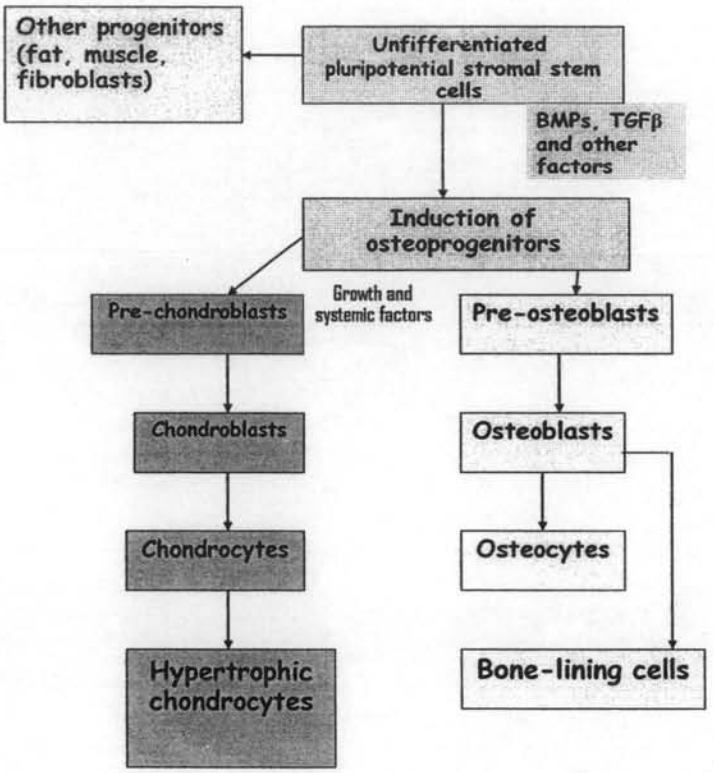

Figure 1. Origin of cells of the osteoblast and chondracyte lineages. (modified from Kartsogiannis and W.Ng,2004)

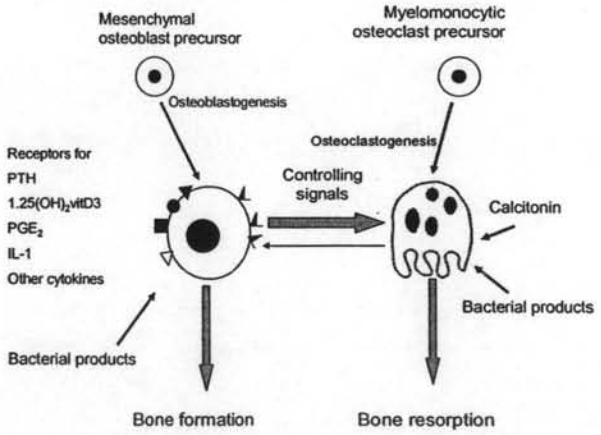

Figure 2. Interactions between the two major bone cell populations and mediators of bone remodeling including bacterial components. The osteoblast produces the extracellular matrix of the bone and also plays a pivotal role in controlling the activity of the osteoclast. (Nair et al., 1996)

Cell cultures are derived from either primary tissue explants or cell suspensions. The growth of explants taken directly form the living organism is also known as primary cell culture, whereas continuous cell lines are, by definition, often transformed cell lines.

Cell cultures in bone cell biology are mainly used to determine (a) the expression of phenotypes of osteoblasts, chondrocytes and osteoclasts; (b) regulation of differentiation of relatively undifferentiated mesenchymal cells along different lineages; (c) signaling pathways relevant to osteoblast, osteoclast and chondrocyte functions; (d) the effects of over-expression and under-expression of particular gene products on cell function; (e) bone formation and mineralization *in vitro*; (f) interactions between osteoblasts and osteoclasts, particularly in the regulation of osteoclast formation *in vitro* (Kartsogiannis and Ng, 2004). Most of the studies in bone research field used the osteoblastic cell lines as models. The osteoblastic cell lines derived either from bone tumors (ROS 17/2.8 or UMR 106; human MG-63 or SaOS-2) or from primary bone cell cultures (MC3T3-E1, UMR 201 and RCJ cell lines). UMR 106 and the ROS 17/2, from rat osteosarcoma, were the most widely used.

Jawbones are functionally subdivided into basal bone and alveolar bone. The basal bone is housing of nerves and blood vessels and functions as a site of muscle attachment. The alveolar bone is a tooth supporting area. The development of its is depend on root formation. The tooth-bearing surfaces of jawbones are woven bone. The internal wall of the alveolar bone is called the cribriform plate because of the many openings for blood vessels and nerves that communicate between the marrow spaces and the periodontal ligament (PDL). Turnover rate of alveolar bone is more rapid than those of other parts of

the skeleton. The turnover rate is related to normal remodeling to accommodate the mesial and occlusal shifting of teeth. The lesions of jawbone due to odontogenic infections, is mostly present in alveolar bone. Osteoblasts and osteoclasts in alveolar bone may play an important role response to the stimuli from odontogenic infection.

Bacterial infections are known to involve in bone pathology. To date, there are three possibilities how bacteria cause pathological bone loss: (i) bacteria directly destroy the noncellular components of bone by liberating acid and proteases, (ii) bacteria promote cellular processes, via osteoblasts and osteoclasts, that stimulate the degradation of bone, or (iii) bacteria inhibit the synthesis of bone matrix (Nair et al., 1996). Mechanisms (ii) and (iii) may be either direct effects of components released by bacteria or a consequence of the induction of host factors. For example, cytokines or prostaglandins act on osteoblasts, and then osteoblasts will send the controlling signals through osteoclasts. Many bacterial factors have been described as osteolytic factors, such as, endotoxin and lipopolysaccharide (LPS), lipoteichoic acid, lipid A-associated proteins, porins, cell wall components of many bacteria, *P. gingivalis* fimbrial protein, and capsular polysaccharide of *A. actinomycetemcomitans* (Nair et al., 1996).

LPS is a major component of the outer membrane of gram-negative bacteria (Medzhitov and Janeway, 1997). Although it has ability to stimulate bone resorption *in vitro*, many studies demonstrated that LPS failed to directly stimulate the osteoclasts. LPS produces bone resorption by activates osteoblasts to release factors which recruit and /or activate osteoclasts (Nair et al., 1996). Gram negative bacteria, such as *Porphyromonas endodontalis*,

P. gingivalis and *Prevotella intermedia*, have been found in human dental pulp and periapical lesions (Sundqvist et al., 1989, van Winkelhoff et al., 1985, Jin et al., 1989). LPS could be involved in inducing periapical lesions.

As mentioned above, osteoblasts are important in periapical lesion development. However, studies of human osteoblast functions *in vivo* are limited because of the ethical reason. *In vitro* study using human osteosarcoma cell lines may not be a good representative for the clinical situation. Study using primary osteoblastic cells should be a better model for studying the non-malignant human alveolar bone functions and diseases.

1.2 Objectives

The primary osteoblastic cells from human alveolar bone are good models to study in physiology and pathology that response to medicaments, cytokines or growth factors. The purpose of this study is to isolate and culture the cells derived from human alveolar bone and characterize their osteoblastic phenotypes.

1.3 Hypothesis

The cells derived from human alveolar bone have the osteoblastic phenotypes and can be used as a model for further research *in vitro*.

1.4 Field of Research

To investigate the RNA expression of osteoblastic phenotypes, alkaline phosphatase activity and nodule formation of cells derived from human alveolar bone.

1.5 Inclusion Criterions

1.5.1 Cells were grown from alveolar bone from patients undergone surgery of alveolar bone.

1.5.2 Cells were grown with standard culture media added 50 μg/ml of ascorbic acid and 10 mM of β-glycerophosphate for 14 days. Only cells that positive stained to substrate of alkaline phosphatase were included.

1.6 Limitation of Research

The number of bone samples investigated in this study is restricted due to the limitation of time and grant support.

1.7 Application and Expectation of Research

Many studies attempt to isolate the osteoblasts from bone fragments. The long bone and calvariae from fetal/neonatal rats and mice were popularly used in the primary cell culture systems. These culture systems imitate osteoblastic differentiation *in vivo*, thus providing perceptions into the regulation of gene expression in osteoblastic cells. However,murine

osteoblasts may demonstrate the different osteoblastic patterns from the human osteoblasts. To date, only few studies used the primary human osteoblasts. The use of osteoblasts derived from human alveolar bone as a model for studying *in vitro* has been reported in two studies. In one study, primary human osteoblasts from oral cavity were used to study mechanisms of internalization of Staphylococcus aureus. This study did not mention about the obtaining process or osteoblastic characterization of these cells(Jevon et al., 1999). The other study revealed 2-week profile of Runx2/Cbfa1 and some of bone markers expression after added inducing agent, in cells derived from human alveolar bone (Perinpanayagam et al., 2006). Isolation and culture of human primary osteoblastic cells from alveolar bone is potentially a useful model, especially for studying the response to cytokines of human alveolar bone osteoblasts. In addition, these cells may help to develop the new root canal filling materials or medications which promote the healing of the periapical lesion, and also to elaborated physiopathogenesis of alveolar bone diseases.