INTRODUCTION

For bone tissue engineering, various studies have been published demonstrating the bone-building capacity of progenitors, such as stem cell and other osteogenic cells [1,2]. Currently, adult stem cells, such as adipose-derived stromal cells (ADSCs) and bone marrow-derived stem cells (BMSCs), demonstrate vast potential in bone regenerative applications because they exist in an undifferentiated state and demonstrate both self-renew and differentiation into variable cell types [3-5]. In general, ADSCs and BMSCs differentiation fates are regulated by specific transcriptional regulators, which are present in low levels in undifferentiated cells, and differentiation toward one fate completely suppresses and induces genes associated with the other fates [6-8]. This phenomenon has been exploited in past research to regulate stem cell fate into osteoblast differentiation using defined media conditions with cytokines and growth factors or co-culture systems containing two or more cell types.
to more closely mimic the *in vivo* microenvironmental conditions [9].

Even though osteogenic differentiation and bone regeneration using stem cells brought about very interesting results with respect to their novel potential in regenerative medicine, research has been primarily focused on the osteogenic differentiation of stem cells in *in vitro* systems using co-culture and defined medium conditions [10-12]. Conversely, the influence of osteogenesis and osteogenic differentiation from neighboring cells in transplanted *in vivo* sites is not well characterized. In fact, the bone marrow niche environment includes various cell types, such as osteoblasts, adipocytes, hematopoietic stem cells and endothelial cells [6,13]. These cells may have an effect on the osteogenic differentiation of transplanted stem cells or on the osteogenesis process.

Specifically, recent studies have shown that adipocytes are an endocrine organ due to their secretion of various cytokines, such as adiponectin, leptin, and peroxisome proliferator-activated receptor-γ (PPAR-γ). And, it has been suggested that these cytokines interaction with osteoblasts regulates bone resorption and influences bone density, mass, and formation (Fig. 1) [14-16]. Interestingly, the effect of adipocytes on osteogenesis has been inconsistently reported in several previous studies. PPAR-γ, which is one cytokine secreted from adipocytes, suppresses osteogenesis by inhibiting Runx2 gene expression in osteoblasts [17,18], whereas adiponectin increases osteoclastogenesis [19,20]. By contrast, other studies have revealed that adipocytes increase osteogenesis through the action of adiponectin [21,22] and that leptin increases bone mass [23]. These reports have not reached a definite consensus on whether adipocytes positively influence osteogenic differentiation *in vitro* and osteogenesis *in vivo*. Therefore, the roles of adipocytes should be obvious in bone tissue engineering if they are integral to efficient bone regeneration using stem cells.

The purpose of this study was to investigate the influence of adipocytes on osteogenic differentiation *in vitro* and osteogenesis *in vivo*. To that end, the top and bottom co-culture system was used to analyze the influence of adipocytes on the osteogenic differentiation fate of ADSCs. The adipocytes and osteoblasts derived from ADSCs were co-transplanted to determine the effect of the adipocytes on new bone formation in the dorsal region of mice.

**MATERIALS AND METHODS**

**Isolation and culture of ADSCs**

Human subcutaneous adipose tissues were obtained via elective liposuction from patients who had given informed consent. Only adipose tissue that would otherwise have been discarded was used for ADSCs isolation with the approval of the Medicine Ethics Committee of Konkuk University (IACUC No. KU13125-1). The enzymatic digestion method was used to isolate ADSCs from the adipose tissues [24]. Briefly, the adipose tissue was minced and washed with phosphate buffered saline (PBS) (Sigma-Aldrich, St. Louis, MO, USA) until the blood was removed. The minced tissue was then digested with PBS containing 2 mg/mL collagenase type II (Sigma-Aldrich) and 0.2% bovine serum albumin for 45 min at 37°C with shaking. Subsequently, digested adipose tissue was filtered using a 100-μm filter and centrifuged to remove the floating adipocytes from the stromal-vascular fraction. The ADSCs isolated from the adipose tissue were cultured in Dulbecco’s modified Eagle medium (DMEM; Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% (v/v) FBS, 1% (v/v) GlutaMAX, and 1% (v/v) penicillin-streptomycin in 5% CO₂ at 37°C.

**ADSC cultured on porous membranes and differentiation**

The ADSCs (1×10⁵ cell/well) were seeded on inverted transwell cell culture inserts fitted for six-well plates and were cultured for 24 h. Following the adhesion of the ADSCs to the bottom surface of the insert, the plate was turned upright and incubated in adipogenic induction media (Gibco BRL, Gaithersburg, MD, USA) for 14 days. ADSCs grown in basal medium were used as controls. Standard oil red O stain was used to identify adipogenic differentiation of the ADSCs. Subsequently, ADSCs were seeded inside the insert. Following an additional 24 h incubation, the medium was refreshed with osteogenic induction media (Gibco BRL) for 14 days. After 30 total days of ADSC culture, fully osteogenic differentiated cells were identified using