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Optimisation of Human Osteoblasts and Osteoclasts Co-Culture Model

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Abstract

Osteoblasts (OBs) and Osteoclasts (OCs) are two main specialised cells involved in the bone-remodeling process which are responsible for new bone formation and aged bone resorption respectively [1]. However, these cells do not act independent of each other since several communication pathways have been identified between them [2]. Macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL) are mainly produced by OBs in the bone play a central role in OCs differentiation (osteoclastogenesis) [3]. Therefore, OBs/OCs co-culture has provided a new approach for studying bone diseases *in vitro* since the cellular environment is closer to *in vivo* environment compared to monoculture [4]. However, establishment of OBs/OCs co-culture model are often time consuming. A study concluded that optimal osteoclast differentiation is between 9 and 16 days with M-CSF and RANKL stimulation [5]. Therefore, the objective of this study is to determine the optimal time-point for osteoclastogenesis in OBs/OCs co-culture without external stimulation to establish a novel, reliable and replicable human OBs/OCs co-culture system.

Human foetal osteoblastic cell (hFOBs 1.19) was used as the source of OBs while human peripheral blood mononuclear cell (PBMC) was used as the source of OCs. PBMCs were isolated from the blood of healthy donors after going through density centrifugation process. OBs were maintained in a T25 culture flask until 90% confluency. Three thousand cells/well of OBs was seeded in the 6 well plate. After 3 days, 1500 cells/well of PBMCs were directly added to OBs. Tartrate-resistance acid phosphate (TRAP) staining was conducted using a TRAP staining kit to validate the OCs maturation after 9 to16 days of co-culture. Quantitative analysis of TRAP activity in culture supernatants was measured using microplate reader at 540 nm optical density (OD).

Qualitative examination of OBs/OCs co-culture after 9 days of co-culture showed reddish appearance indicating TRAP-positive cells (Figure 1). This validates the differentiation of PBMCs into mature functioning OCs at day 9 of cultivation without any external stimulation. However, TRAP-positive cells are the most abundant at day 11 of co-culture. This is supported by quantitative analysis of TRAP activity in the co-culture supernatant which exhibited the peak of the TRAP activity at day 11 (Figure 2). A longer

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cultivation period did not improve TRAP activity. In previous report, TRAP-positive cells were observed at day 14 of co-culture without any artificial inducer [6]. However, in this study TRAP-positive cells already presence after 9 days of co-culture and the most abundance at day 11. This might be different depending on the cell concentration and size of the culture flask or well plate used.

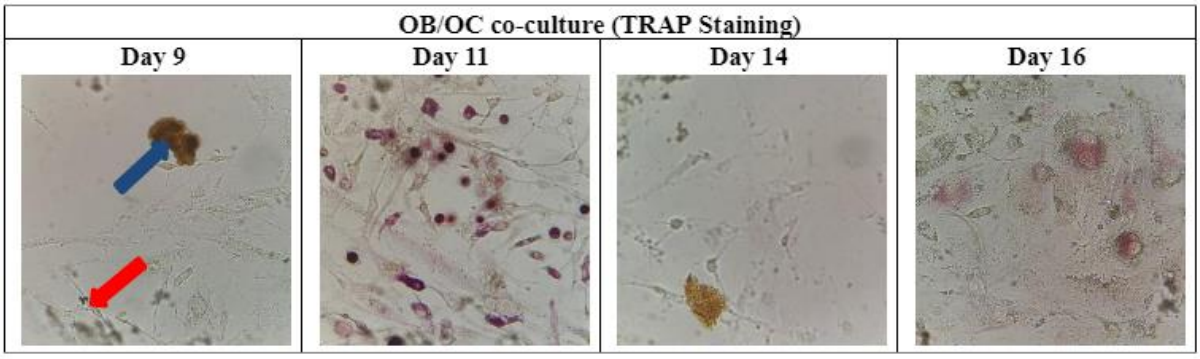

Figure 1: Characterisation of the osteoblasts/osteoclasts (OBs/OCs) co-culture at Day 9, 11, 14 and 16. Osteoblasts (OBs) and osteoclasts (OCs) were pointed by red and blue arrows, respectively (40X magnification). Day 11 was chosen as the best time-point for OCs differentiation because abundance of TRAP-positive cells has been observed.

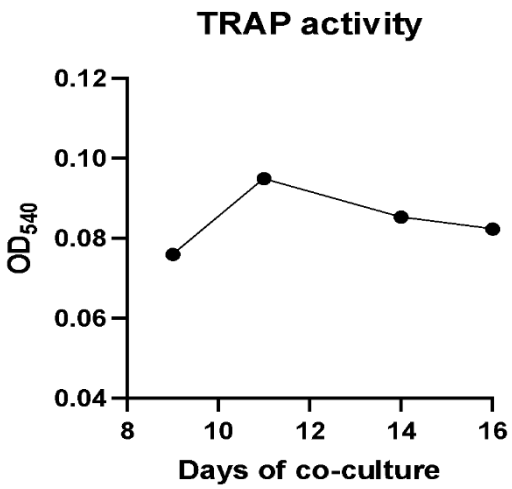

Figure 2: TRAP activity in the osteoblasts (OBs) and osteoclasts (OCs) co-culture supernatants at day 9, 11, 14 and 16. The peak of TRAP activity can be observed at day 11 of co-culture. Data are presented as mean values of optical density (OD) at 540 nm.

This study demonstrates that OCs differentiation of PBMC could be reached after 9 days of co-culture. However, the optimum OBs/OCs co-culture duration was determined at 11 days since the TRAP-positive cells were more abundance and TRAP activity in the supernatant was higher. The longer co-culture period did not increase the TRAP-positive cells and TRAP activity. Therefore, a reliable bone cell co-culture system capable of mimicking the bone-remodelling process in humans could be achieved after 11 days of co-culture with 3000 cells/well of OBs and 1500 cells/well of PBMCs in 6 well plate.

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Keywords
Osteoblasts, Osteoclasts, Co-culture

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