3D Culturing is Superior to 2D Conventional Culturing in Examining The Osteogenic Potential of Stem Cells *In Vitro*

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Introduction

In stem cell and tissue engineering fields, researchers often need to conduct in vitro osteogenic assays to verify the "stem" nature of their stem cells [1-3]. Although many studies have shown that *in vitro* osteogenic differentiation of stem cells, such as mesenchymal stem cells, is better under 3 dimensional (3D) conditions [4-7], these *in vitro* assays are frequently performed within 2 dimensional (2D) tissue culture plates because of the lack of standard 3D cell culture devices and protocols. These functional assays for stem cells are very important as their results will determine whether those stem cells can be used further for product development or research use. Aimed at providing a more sensitive and suitable assay which can be standardized for determining the osteogenic potential of stem cells, we have developed standard 3D scaffolds, 3D Insert^{TM}-PCL made from polycaprolactone, which can be conveniently used with current polystyrene tissue culture plates (Figure 1). Compared to standard 2D polystyrene cell culture plates, using 3D Insert^{TM}-PCL will provide a more favorable osteogenic differentiation condition and hence the osteogenic marker production will be enhanced and easily detected. These standardized products will make it possible for researchers from different labs to compare their experiment results when they use the same standard 3D Insert^{TM}-PCL in their studies.

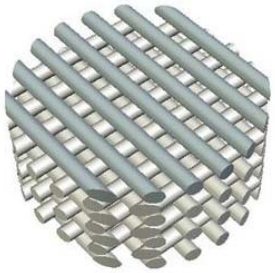

Figure 1. Porous Structure of 3D Insert^{TM}-PCL

Materials and Methods

Human mesenchymal stem cells (hMSCs) (Lonza) and mouse 7F2 osteoblasts were seeded and cultured under both 2D and 3D conditions, which included 2D polystyrene (PS) tissue culture plates (TCPs) , and 3D Insert^{TM}-PCL（fiber diameter: 300μm; spacing: 500μm）. Briefly, the cells were seeded at a density of approximately 5 x10^4/cm^2 (cell number over surface area) onto 2D TCPs and 3D scaffolds which were placed in wells of 96-well culture plates. After incubated in an incubator at 37 ^oC and 5% CO2 for 3 hours, the 3D scaffolds were transferred to wells of new 96 well TCPs. All scaffolds were replenished with fresh maintenance medium and cultured under 37 ^oC and 5% CO2 condition. After 24 hours, the maintenance medium was replaced with osteogenic induction medium to continue the culture. The osteogenic differentiation of these seeded cells was evaluated with following assays at various time points.

Results and Discussion

-Cell Proliferation by MTT

Cell proliferation assay showed that hMSCs proliferated in both 2D and 3D conditions (Figure 2). Cell number reached peak by one week earlier in 3D PCL scaffold than in 2D PS TCPs, while cell proliferation peaked higher in 2D condition than in 3D conditions. After 2 weeks of osteogenic induction, cell number from 2D PS culture quickly decreased to a level that is comparable to 3D PCL scaffolds, indicating reduced cell viability probably due to over-confluent of cultured cells. By contrast, cell number stayed at its highest level after proliferation reached peak in 3D PCL scaffold (Figure 2). These findings suggest that hMSCs proliferate faster in 3D culture than in 2D culture.

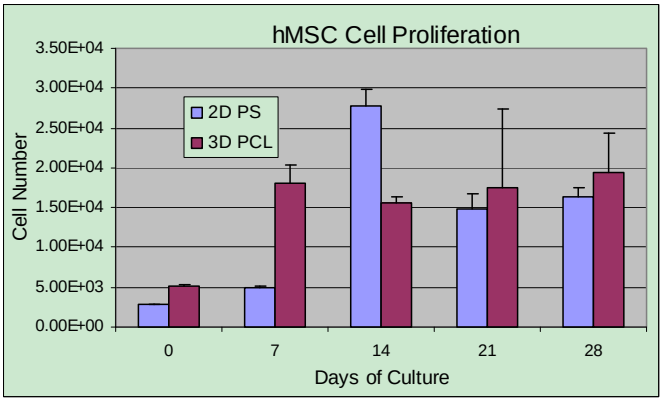

Figure 2: Cell proliferation of hMSC from the culture of 2D PS TCPs and 3D InsertTM-PCL scaffolds was examined using MTT proliferation assay kit (Cayman Chemical).

-Assay for Alkaline Phosphatase (ALP) and Osteocalcin

ALP assays were performed in cell lysate to assess the osteogenic differentiation of both hMSCs and osteoblasts in both 2D and 3D cultures. Data showed that the activity of ALP, a marker of osteogenic differentiation at early stage, was doubled in cells cultured in the 3D Insert^{TM}-PCL scaffolds as compared to 2D culture at week 1 (Figure 3). In addition, production of osteocalcin, another marker of osteogenesis at late stage, was dramatically enhanced in the supernatant of cells grown on 3D Insert^{TM}-PCL at week 3 (Figure 4). On the other side, osteocalcin secretion remained at a relatively low level and a slight increase was only observed at week 4 in 2D culture.

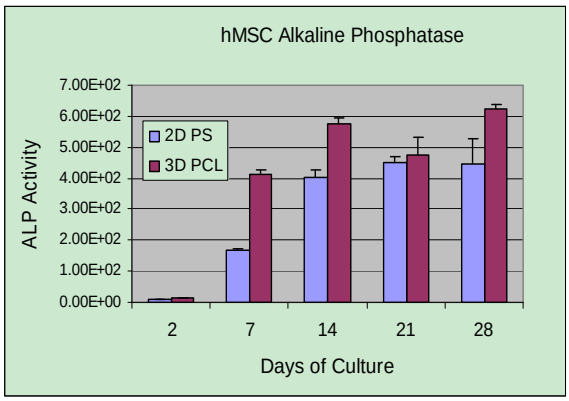

Figure 3: ALP activity of hMSC was measured using the p-Nitrophenyl Phosphate Liquid Substrate System (*Sigma*). The result is expressed as the 4-nitrophenol release.

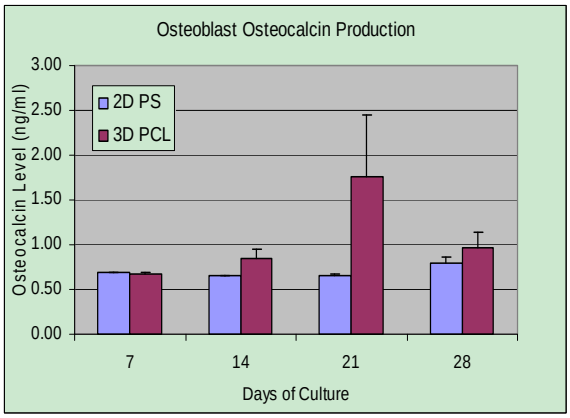

Figure 4: Osteocalcin production of 7F2 osteoblast over culture course was measured using the mouse osteocalcin EIA kit (*Biomedical technologies*).

-Von Kossa Staining

Von Kossa staining of both hMSC and mouse osteoblasts indicated that cells grown on 3D PCL scaffold underwent more extensive mineralization at late stage of osteogenesis than 2D environment (Figure 5).

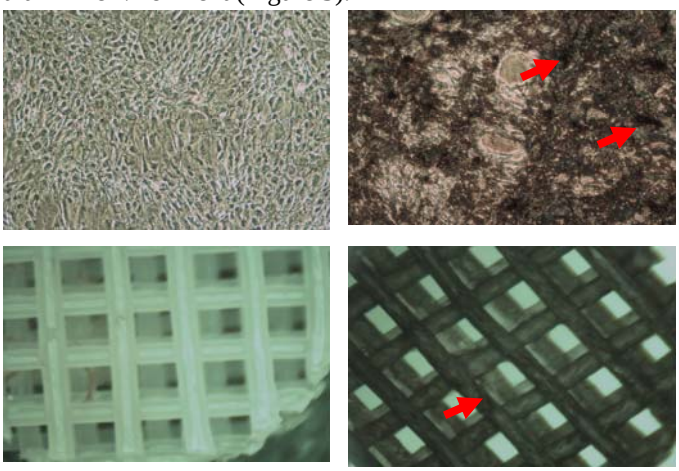

Figure 5. Von Kossa staining (Sigma) of hMSC in 2D PS plate (upper) and 3D PCL scaffold (lower) on day 7 and day 28 of culture. The dark brown-black staining (indicated by red arrows) shows positive staining of mineralization spots on day 28. No apparent positive staining was observed on day 7.

Conclusions

Our results demonstrated that 3D Insert^{TM}-PCL scaffolds provide a better 3D culturing environment for *in vitro* osteogenic differentiation of hMSCs and osteoblasts than conventional 2D polystyrene TCP because the osteogenic marker production of cultured cells was greatly enhanced and easily detected. These 3D Insert^{TM}-PCL scaffolds are easy to use and will be a useful tool for conducting standard 3D osteogenic assays in controlling the quality of harvested and expanded stem cells and osteoblasts.

References

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