Comparison Of Osteogenic Cell Differentiation Within 2D And 3D Culture Systems

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Abstract

The physiology of different cell types has been shown to vary remarkably within three-dimensional (3D) environments compared with two-dimensional (2D) monolayer in vitro cultures. Chemically synthesized 3D scaffolds, which offer a variety of features to match tissue specific applications, are currently receiving tremendous attention in the bone tissue engineering field. Currently, there are only a few reports comparing osteoblastic differentiation of stem cell progenitors within 2D and 3D culture systems. Therefore, the objective of this study was to compare the growth pattern of hMSCs and osteoblast cells within 2D and 3D culture systems. Human mesenchymal stem cells (hMSCs) and 7F2 osteoblasts were cultured on 3D porous PCL scaffolds and 2D tissue culture polystyrene (TCP) plates. Cell proliferation, ALP activity, osteocalcin secretion and collagen type I, II, and III secretion were performed on 2D surfaces such as tissue culture plates/flasks because of the ease, convenience, and high cell viability of 2D culture. These conventional 2D culture systems have notably improved the understanding of basic cell biology. Nevertheless, 2D plastic substrates are considerably limited in reproducing the complexes environments existing in vivo. In addition, cells grown on 2D plastic substrates are forced to adapt to an artificial flat, rigid surface, resulting to some degree, a misrepresentation of findings, including altered mechanotransduction and decreased functionality [1-3]. Cells cultured in 2D systems have been shown to differ remarkably in their morphology, proliferation, and differentiation than those growing in 3D environments [4,5].

Materials and Methods

Cells

hMSCs were purchased from Lonza Walkersville, Inc (Cat#: PT-2651), and re-suspended in MSCGM Basal Medium (Lonza, Walkersville, MD) with 10% fetal bovine serum (FBS), 2mM of L-glutamine and 100uM pen-strep. 7F2 cells were cultured in alpha-MEM with 10% FBS and 2mM L-glutamine. Both cultures were kept in a humidified 5% CO2 incubator at 37°C.

Schematic Scaffold Fabrication and Cell Seeding

PCL was purchased from Sigma-Aldrich and the 3D PCL scaffold was fabricated using 3D precision micro-fabrication technology. Within the scaffold, the struts within each layer were oriented 90° relative to the struts of the layer immediately below (Figure 1). Fiber diameter and spacing are approximately 300µm and 500µm, respectively. Scaffolds were sterilized by soaking in 70% ethanol for 15 min. The scaffolds were then flooded with 200 µl of maintenance medium and kept in culture until use.

Osteogenic Differentiation

24 hour after the cell culture, the media were replaced with osteogenic differentiation induction media, which contains basal medium supplemented with 50µM ascorbic acid and 10mM β-glycerophosphate. 0.1µM of dexamethasone was specifically used for hMSC osteogenic induction. Throughout the 4 weeks of in vitro culture, the induction media were changed every 2-3 days.

Cell Proliferation Assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed according to manufacturer's instructions (Cayman Chemical, MI).

Alkaline Phosphatase Activity Assay

Cell lysate was prepared using M-PER Mammalian Protein Extraction Reagent (Pierce, IL) according to the manufacturer’s instructions. The concentration in culture medium supernatant was determined by using intact Human Osteocalcin EIA kit or mouse osteocalcin EIA kit (Bioscience Technologies Inc, MA).

Osteocalcin Secretion Assay

Three days prior to culture medium supernatant collection, hMSC culture medium was replaced with osteogenic induction factor-supplemented Lonza Bovineline (Lonza, Walkersville, MD) containing 5% FBS and 2mM L-glutamine. The osteocalcin concentration in culture medium supernatant was determined by using intact Human Osteocalcin EIA kit or mouse osteocalcin EIA kit (Bioscience Technologies Inc, MA).

Von Kossa Staining

The PCL scaffold and 2D TCP were fixed with 10% phosphate-buffered formalin for 30 minutes. After washing with DI-H2O, the scaffolds and TCPs were exposed to bright light for 15 min. The cultures were then dehydrated with 100% ethanol for 1 min followed by air drying.

Results

Cell proliferation assay showed that hMSCs proliferated in both 2D and 3D conditions. In 2D PCL scaffolds, cell number reached its peak at week 1, 7 days earlier than 2D PS-TCPs; however, cell number was highest in 2D than in 3D. After 2 weeks of osteogenic induction, cell number from 2D PS culture quickly decreased to a level that was comparable to 3D PCL scaffolds, indicating reduced cell viability due to over-confluency of cultured cells. In contrast, cell numbers in 3D PCL scaffolds remained at their highest level until 4 weeks of mineralization (Figure 2). These findings suggest that hMSCs proliferate faster in 3D than in 2D.

Assay for Alkaline Phosphatase (ALP) and Osteocalcin Activity

ALP assays were performed from cell lysates to assess the osteogenic differentiation of both hMSCs and osteoblasts in both 2D and 3D cultures. Data showed that ALP activity, a early stage marker of osteogenesis, was doubled in cells cultured in the 3D Immers™-PCL scaffolds as compared to 2D cultures at week 1 (Figure 3). In 3D culture, ALP activity reached its peak at week 2 while it took 3 weeks for ALP to reach peak in 2D culture. In addition, production of osteocalcin, a late stage marker of osteogenesis, was dramatically enhanced in the supernatant of cells grown on 3D Immers™-PCL at week 3 (Figure 4). Only a slight increase in osteocalcin secretion was observed in week 4 2D culture.

Conclusions

Our results demonstrate that 3D PCL scaffolds provide a better 3D culture environment for the in vitro osteogenic differentiation of hMSCs and osteoblast cells than conventional 2D polystyrene TCPs, as shown by enhanced osteogenic marker production in 3D cultured cells. This finding suggests that using 3D scaffolds is a better way for assessing the osteogenic potential of progenitor cells. Furthermore, this research study will contribute to the design of practical, applicable synthetic 3D scaffolds for bone engineering and related research.

References


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