1408/B624

Comparison of Osteogenic Cell Differentiation Within 2D and 3D Culture Systems

Wei Liu, Wing Lau, Qing Liu, Wei Sun*

3D Biotek, LLC., 675 US Highway One, North Brunswick, New Jersey 08902

*Department of Mechanical Engineering, Drexel University, Philadelphia, PA 19104

Abstract

The physiology of a variety of different cell types has been shown to be remarkably different within 3-dimentional (3D) environment compared to 2-dimentional (2D) monolayer culture environment. Therefore, various 3D cell culture systems have been developed for conducting 3D cell cultures. Among them, solid 3D scaffolds made from synthetic polymers may offer various structure and mechanical features to match cell/tissue specific applications; therefore, they have drawn tremendous attention, especially in tissue engineering field. There have been few reports comparing osteogenic differentiation of osteoprecursor cell lines within 2D and 3D culture system. The objective of this study was to compare the growth pattern of osteogenic cells within 2D and 3D culture systems. Mouse 7F2 osteoblast cell line and Human mesenchymal stem cells (hMSC) were seeded in separate experiments at the same cell density (cell number/cm^2 surface area) on 2D polystyrene (PS) culture plate and 3D poly-ε-caprolactone (PCL) porous interconnected scaffold (3D Insert™-PCL). Osteogenic differentiation was induced through culture media supplemented with L-ascorbic acid and β-glycerophosphate. Quantitative and qualitative assays of osteogenic differentiation were conducted at multiple time points during 4 weeks of in vitro culture. Osteocalcin secretion and alkaline phosphatase (ALP) activities were detected at significantly higher levels within 3D culture than with the 2D system ($P < 0.05$, t-test). Von Kossa staining showed more extensive mineralization of 7F2 cells on 3D PCL scaffold than in 2D PS monolayer culture. In conclusion, our results demonstrate that the osteogenic differentiation of hMSC and murine osteoblast is enhanced in a 3D culture system compared to a 2D culture environment. This finding demonstrated that 3D cell culture system is a better system in conducting progenitor cell differentiation study and assays.

Introduction

The in vivo environment of a cell in a living organism has a 3-dimensional (3D) architecture where cells interact with each other and with extracellular matrix (ECM). The majority of cell culture studies, however, have been performed on 2-dimensional (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 mimicking the complex 3D environments in vivo. In addition, cells grown on 2D plastic substrates are forced to adapt to an artificial flat, rigid surface, resulting in misrepresentation of findings to some degree with altered metabolism and declined functionality [1-3]. Cells cultured in 2D systems have been shown differ remarkably in their morphology, proliferation, and differentiation from those growing in 3D environments [4, 5].

One of the 3D cell culture matrices, also known as 3D scaffold, was recently introduced to overcome the limitations of 2D cell culture. Extensive studies have shown that growing cells within 3D scaffolds reduces the gap between cell cultures and physiological tissues. Therefore, a 3D cell culture system may prove to be of tremendous advantage over conventional 2D cell culture system.

In development of stem cell based therapies and tissue regeneration products, the differentiation potential of the stem cells has to be assessed to ensure the quality of the isolated or expanded stem cells. These differentiation assays normally include osteogenic, chondrogenic and adipogenic assays. In an effort to develop a standard 3D osteogenic differentiation assay, we have used 3D PCL scaffolds (Figure 1) manufactured using 3D precision micro-fabrication technology. Bone marrow derived mesenchymal stem cells (MSCs) are one type of the adult stem cells that has been extensively studied for tissue regeneration, such as bone and cartilage [7, 8]. In this study, we evaluated the differentiation behavior of both human MSCs and 7F2 osteoblast cell line in both 2D polystyrene (PS) and 3D PCL culture systems.

Materials and methods

Cells

hMSCs were purchased from Lonza Walkersville, Inc (Cat#: PT-2501), and re-suspended in MSCGM Basal Medium (Lonza Walkersville, MD) with 10% fetal bovine serum (FBS), 2mM of L-glutamine and 100IU/ml 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

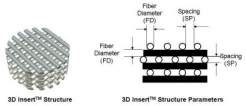

Figure 1. 3D PCL scaffold.

PCL was purchased from Sigma-Aldrich (St. Louis, MO) 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 the spacing are approximately 300μm and 500μm, respectively. Scaffolds were sterilized by soaking in 70% ethanol for 1 hour and air dried in a biosafety cell culture hood. Cells at a density of 4.75 x 103 (1.44x104/cm2) in 200 μl were seeded into 2D 96-well PS tissue culture plate (TCP). Comparable density of cells at 0.1 million in 10 μl were seeded onto scaffold measuring 5.1mm in diameter and 2.1mm in height. The seeded scaffolds were then incubated for 3 h to allow cell attachment. After that the scaffold were flooded with 200 μl of maintenance medium and kept in culture.

Osteogenic Differentiation Induction

24 hour after the cell seeding, the culture 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 by following manufacture's instruction (Cayman Chemical,MI).

Alkaline Phosphatase Assay

Cell lysate was prepared using M-PER® Mammalian Protein Extraction Reagent (Pierce, IL) followed by centrifuge at 14,000 rpm for 5 minutes. The lysate in supernatant was collected and analyzed using p-Nitrophenyl Phosphate Liquid Substrate System (pNPP) and 4-nitrophenol solution (PNP) (Sigma,MO) according to manufacturer's instructions.

Osteocalcin Secretion Assay

Three days prior to culture medium supernatant collection, the hMSC culture medium of the wells to be tested was replaced with osteogenic induction factor-supplemented Lonza BioWhittaker* General-Purpose Serum-Free Media (Lonza, MD). The culture medium supernatant osteocalcin concentration was measured using Intact Human Osteocalcin EIA kit or mouse osteocalcin EIA kit (Biomedical 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-H_2O, the culture was stained with 2% silver nitrate in DI-H_2O for 10 min in dark. After washing with DI-H_2O, the scaffolds and TCP were exposed to bright light for 15 min. The cultures were washed and then dehydrated with 100% ethanol for 1 min followed by air dry.

Results

Cell Proliferation by MTT

Cell proliferation assay showed that hMSCs proliferated in both 2D and 3D conditions (Figure 2). In 3D PCL scaffolds, cell number reached peak at week one which was one week earlier 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.

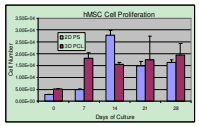

Figure 2: Cell proliferation of hMSC from the culture of 2D PS TCPs and 3D PCL scaffolds was examined using MTT proliferation assay kit (Cayman Chemical).

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™-PCL scaffolds as compared to 2D culture at week 1 (Figure 3). In 3D culture, ALP activity reached peak at week 2 while it took 3 weeks for ALP to reach peak in 2D culture. In addition, production of osteocalcin, another marker of osteogenesis at late stage, was dramatically enhanced in the supernatant of cells grown on 3D Insert™-PCL at week 3 (Figure 4). On the other hand, a slight increase in osteocalcin secretion was only observed at week 4 in 2D culture.

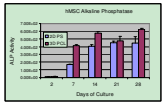

Figure 3: ALP activity of hMSC was measured using the p-Nitrophenyl Phosphate Liquid Substrate System (*Sigma*). The result is expressed as the 4-nitrophenol release.

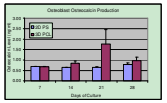

Figure 4: Osteocalcin production of 7F2 osteoblast over culture course was measured using the mouse osteocalcin EIA kit (*Biomedical technologies*).

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 on 2D polystyrene TCP (Figure 5).

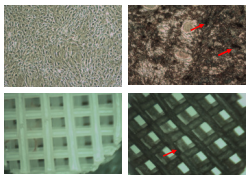

Figure 5. Von Kossa staining (Sigma) of hMSC in 2D PS plate (upper) and 3D PCL scaffold (lower) on day 7 and day 28 of culture. The dark brown-black staining (indicated by red arrows) shows positive staining of mineralization spots on day 28. No apparent positive staining was observed on day 7.

Conclusions

Our results demonstrated that 3D PCL scaffolds provide a better 3D culturing environment for *in vitro* osteogenic differentiation of hMSCs and osteoblasts than conventional 2D polystyrene TCP with greatly enhanced osteogenic marker production. This finding suggested that using 3D scaffold is a better way in assessing the osteogenic potential of progenitor cells. This will contribute to the design of practical applicable synthetic 3D scaffolds in bone engineering and related research.

References

1. Zhang, S., Beyond the Petri dish. Nat Biotechnol, 2004. 22(2): p. 151-2.
2. Abbott, A., Cell culture: biology's new dimension. Nature, 2003. 424(6951): p. 870-2.
3. Cukierman, E., et al., Taking cell-matrix adhesions to the third dimension. Science, 2001. 294(5547): p. 1708-12.
4. Birgersdotter, A., R. Sandberg, and I. Ernberg, Gene expression perturbation in vitro--a growing case for three-dimensional (3D) culture systems. Semin Cancer Biol, 2005. 15(5): p. 405-12.
5. Weaver, V.M., et al., Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. J Cell Biol, 1997. 131(1): p. 231-45.
6. Agrawal, C.M. and R.B. Ray, Biodegradable polymeric scaffolds for musculoskeletal tissue engineering. J Biomed Mater Res, 2001. 55(2): p. 141-50.
7. Pittenger, M.F., et al., Multilineage potential of adult human mesenchymal stem cells. Science, 1999. 284(5411): p. 143-7.
8. Caplan, A.I., Mesenchymal stem cells. J Orthop Res, 1991. 9(5): p. 641-50.

Acknowledgments

We thank Eda Yildirim for generous gift of cell line and reagents. We also thank Jack Wang for technical advice.

For further information

Please contact techsupport@3dbiotek.com. More information on this and related projects can be obtained at *www.3dbiotek.com*.

3D Biotek