An extracellular matrix culture system for induced pluripotent stem cells derived from human dental pulp cells

Y. CHEN1,2,4, Y.-L. ZHENG1,6, D.-B. QIU3, Y.-P. SUN1, S.-J. KUANG1, Y. XU1, F. HE5, Y.-H. GONG5, Z.-G. ZHANG1,2

1Department of Oral and Maxillofacial Surgery, Guanghua School of Stomatology, Hospital of Stomatology, Sun Yat-sen University; Guangdong Provincial Key Laboratory of Stomatology, Guangzhou, China
2Sun Yat-Sen University, Guangzhou, China
3The Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China
4Guangzhou Development District Hospital, Chinese Association of Medicinal Biotechnology Southern Center of Biology and Therapy, Luogang, Guangzhou, China
5The School of Engineering at Sun Yatsen University, Guangzhou University City, Guangzhou, China
6Guangdong Second Traditional Chinese Medicine Hospital

Yao Chen and Yuliang Zheng contributed equally

Corresponding Author: Zhang Zhiguang, MD; e-mail: drzhangzg@163.com / 434490635@qq.com

Abstract. – OBJECTIVE: Induced pluripotent stem cells (iPSCs) have emerged as a promising tool for treating incurable diseases. The current challenges are to avoid potential xenopathic transmission and immune rejection potentially caused by exposure of iPSCs to animal-derived products. In addition, an efficient feeder cell-free culture condition will be required for minimizing batch-to-batch variation and facilitating scale-up. Therefore, establishing an efficient extracellular matrix (ECM) culture system is considered as a prerequisite for the future clinical application of iPSC-based cell therapies. In this study, we evaluated the feasibility of culturing iPSCs in ECM derived from human dental pulp cells (hDPC).

MATERIALS AND METHODS: iPSCs growing in Matrigel were transferred to ECM or Matrigel and cultured in mTeSRTM1 medium.

RESULTS: The number of adherent cells in the ECM group was higher than that in the Matrigel group after incubation for 8, 12, and 24 h, indicating that the ECM could enhance cell adherence. The adhesion of cells to ECM not only depends on simple physical attachment with ECM, but also mediated by fibronectin in the ECM. The hDPC-iPSCs showed orderly growth in the ECM, suggesting that the ECM could promote the growth and proliferation of hDPC-iPSCs. We also observed that stem cells grewed along to avoid contact inhibition.

CONCLUSIONS: The iPSCs maintained undifferentiated state when cultured in ECM when the iPSCs and ECM are of the same cell origin. ECM and mTeSRTM1, can both be used as new culture medium for iPSCs that facilitates the clinical application of iPSC-based cell therapies in the future.

Key Words: Induced pluripotent stem cells, Extracellular matrix, Dental pulp cells, Cell culture.
free culture for iPSCs is simpler, faster and less expensive. However, most feeder-free systems face problem with unstable cell growth high prone to differentiation.

In this study, dental pulp somatic cell-derived iPSCs were seeded in dental pulp cell-derived ECM and the cell growth was analyzed to support such ECM as an ideal feeder-free culture medium for dental pulp somatic cell-derived iPSCs.

**Materials and Methods**

**Materials and Reagents**

All procedures have been approved by the Ethics Committee of the Guanghua School of Stomatology, Sun Yat-Sen University, and informed consent was obtained before any procedure was practiced. Dental pulp was collected from four wisdom teeth removed from a healthy young male to achieve primary dental pulp cells so as to be reprogrammed into IPS cells. Dental pulp cell-derived ECM was prepared with the help of Dr. Fan He at the School of Engineering at Sun Yat-sen University.

The following reagents were used: mTeSR™1 medium (StemCell, Vancouver, Canada), Matrigel (BD Biosciences, San Jose, CA, USA), high-glucose DMEM, DMEM/F12, knock-out serum replacement (KSR), non-essential amino acid solution, l-glutamine, 2-2-mercaptoethanol, penicillin-streptomycin, trypsin, collagenase IV, Opti-MEM medium (Invitrogen Corporation, Carlsbad, CA, USA), FBS (Gibco, Grand Island, NY, USA), and bFGF (Gibco, Grand Island, NY, USA). Fu-GENE HD (Roche, Basel, Switzerland) was used for transfection. The Reverse Transcription System kit, dNTPs, and Taq DNA polymerase (TaKaRa, Dalian, China) were used for q-PCR. An alkaline phosphatase (AP) detection kit (Millipore, Bedford, MA, USA) was used for immunohistochemistry. The following primary antibodies were used: anti-Oct4, anti-Sox2, anti-Nanog, anti-SSAE-4, anti-TRA-1-60, and anti-TRA-1-81d (Abcam, Cambridge, UK). The following secondary antibodies were used: Alexa FluorR 555R goat anti-rabbit IgG, Alexa FluorR555 goat anti-mouse IgG, and Alexa FluorR555 goat anti-mouse IgM (Invitrogen Corporation, Carlsbad, CA, USA). 1,6-Hexamethylene diamine (Ribobio, Guangzhou, China) was used for amidolysis. Triton X (Invitrogen Corporation, Carlsbad, CA, USA) was used for removal of dental pulp cells to provide access to the ECM. PBS was prepared by the Guanghua School of Stomatology, Sun Yat-Sen University. Total RNA was amplified, labeled and purified by using the GeneChip 3’IVT Express Kit (Cat#901229, Affymetrix, Santa Clara, CA, USA); array hybridization and washes were performed using the GeneChip® Hybridization, Wash and Stain Kit (Cat#900720, Affymetrix) in a Hybridization Oven 645 (Cat#00-0331-220V, Affymetrix) and Fluidics Station 450 (Cat#00-0079, Affymetrix). Slides were scanned by GeneChip® Scanner 3000 (Cat#00-00212, Affymetrix) using Command Console Software 3.1 (Affymetrix) with the default settings.

**Methods**

**Construction of ECM Derived from Human Dental Pulp Cells**

Human dental pulp cells (hDPC) were isolated and cultured. In order to obtain ECM, amidolysis of tissue cells pellets (TCPs) was treated with 10% 1,6-hexamethylene diamine, followed by construction of ECM derived from human dental pulp using ascorbic acid (200 µg/ml). 0.5% Triton X-100 and 20 mM ammonia was used to remove the dental pulp to obtain ECM. The resulting ECM was analyzed by immunofluorescence microscopy.

**iPSC Culture**

DPC-iPSCs (control cells) were seeded in Matrigel-coated six-well plates, cultured in mTeSR™1 and passaged. The experimental group, i.e. the DPC-iPSCs, were seeded in dental pulp cell-derived ECM-coated six-well plates, cultured in mTeSR™1 and passaged. Both the growth of control cells and DPC-iPSCs were monitored and pluripotency evaluated.

**Identification of Pluripotent Stem Cells**

Cell morphology, intracellular structure and nuclear/cytoplasmic ratio were observed by optical microscopy. Parameters of differentiation were examined. Alkaline Phosphatase (AP) staining was performed according to manufacturer’s protocols. Immunocytochemistry was used to detect markers of pluripotency in Hdp-iPSCs. Hdp-iPSCs were seeded in 12-well plates. Immunofluorescence staining was used to detect the expression of Oct4, SSAE-1, SSAE-4, TRA-1-60, and TRA-1-81. Total RNA was extracted from...
Hdpc-iPSCs to detect the expression of hDPC-iPSC-related genes with q-PCR. The primers used for amplification are listed in Table I.

Karyotype analysis of logarithmic-phase iPSCs was performed at the Institute of Genetics at Sun Yat-Sen University using standard procedures and chromosomal analysis using G-banding revealed the karyotype of hDPC-iPSCs.

DNA microarray analysis for hDPC-iPSCs during the logarithmic growth phase was performed according to standard methods. For RNA amplification and labeling, total RNA was amplified, labeled and purified by the GeneChip 3′IVT Express Kit to obtain biotin labeled cRNA. Array hybridization and washing were performed using the GeneChip® Hybridization, Wash and Stain Kit in the Hybridization Oven 645 and Fluidics Station 450. Data acquisition slides were scanned using the GeneChip® Scanner 3000 and Command Console Software 3.1 (Affymetrix, Santa Clara, CA, USA) with default settings. Raw data were normalized with the MAS 5.0 algorithm in Gene Spring Software 11.0 (Agilent Technologies, Santa Clara, CA, USA).

The formation of embryoid bodies (EBs) was assessed as follows: Vigorously growing (fifth day of P3) hDPC-iPSCs were collected and digested with type IV collagenase and then lightly precipitated before seeding in six-well plates and suspension culture in human embryonic stem cell (hES) culture medium without basic fibroblast growth factor (bFGF). After 7 days, EBs were transferred to dishes coated with 0.1% gelatin and cultured for 12 days before cell differentiation analysis. Expression of germinal layer marker genes was examined by immunofluorescence. The differentiation potential of hDPC-iPSCs was examined after culture in ECM and transplantation (6 × 10⁶) into four severe combined immunodeficiency (SCID) mice.

**Results**

**Construction of ECM Derived from Human Dental Pulp Cells**

Optical microscopy showed spindle-shaped P3 human dental pulp cells of similar morphology with fibroblast cells. Cells arranged in order were aligned along a clear direction and confluent cells showed a swirling and radial pattern. After acellularization, inverted microscope was used to observe the ECM and no complete outline of cells and nuclei was observed this time, but a grid of intact ECM with a wire mesh structure instead (Figure 1a, 1b).

Electron microscope results showed that the human dental pulp cell ECM was mainly a coarse mesh of collagen. A three-dimensional structure formed by fibers of different thickness was observed at high magnification (Figure 1c).

After acellular treatment, immunofluorescence observation showed no evident damage to type I collagen, type III collagen or protein fiber pulp in ECM, all three remained orderly arrangement in the swirling, radial mesh (Figure 1d-f).

**Table I. Oligonucleotide primers used in qPCR.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5′–3′)</th>
<th>Product size (bp)</th>
</tr>
</thead>
</table>
| GAPDH    | Forward: ACCCACTCCTCCAACCTTTG  
Reverse: CTCTTGTGCTCTTGCTGGG | 178 |
| POU5F1   | Forward: CTTGAATCCCGAATGGAAAGGG  
Reverse: GTGTATATCCCAGGGTGATCCTC | 164 |
| SOX2     | Forward: CCCAGCAGACTTCACATGT  
Reverse: CCTCCCATTTCCCTCGTTTT | 151 |
| NANOG    | Forward: TGAACCTCAGCTACAAACAG  
Reverse: TGTTGGTAGGAAGAGTAAAG | 154 |
| ZFP42    | Forward: TCGCTGAGCTGAAACAAATG  
Reverse: CCCTTCTTGAAGGTTTACAC | 170 |
| MYC      | Forward: GTCAAGAGGGCGAACACACACAC  
Reverse: TTGGACGGACAGGATGTATGC | 161 |
| KLF4     | Forward: CGGACATCACAAGCGTAGGAG  | 139 |

Karyotype analysis of logarithmic-phase iPSCs was performed at the Institute of Genetics at Sun Yat-Sen University using standard procedures and chromosomal analysis using G-banding revealed the karyotype of hDPC-iPSCs.
**Cell Morphology**

Normally adherent hDPC-iPSCs grow in Matrigel and in human dental pulp cell-derived ECM with mTeSR™1 medium. Microscopic analysis showed that first two passages of cells grown on human dental pulp cell-derived ECM were of round shape with a spheroid morphology. Cells turned flattened with further passage and after five passages the morphology resembled to that of cells grown in Matrigel (Figure 1g, 1h).

**Cell Identification**

After passaging, hDPC-iPSCs cultured in Matrigel or human dental pulp cell-derived ECM maintained an ES-like clonal and undifferentiat-
ed state. The clones were AP-positive and stained purple, indicating high alkaline phosphatase activity, compared with no staining in Matrigel and ECM (Figure 2a, 2b).

Immunocytochemistry indicated that hDPC-iPSCs cultured in Matrigel or human dental pulp cell-derived ECM expressed characteristic markers of hES pluripotency, including Oct4, SOX2, TRA-1-60, and TRA-1-81, but not SSAE-1 (Figure 2c-2j). q-PCR showed that iPSCs expressed Nanog, Sox2, POU5F, Klf4, ZFP42, and MYC. These findings show that there were no significant difference in the expression of key genes between hDPC-iPSCs cultured in ECM and those cultured in Matrigel ($p > 0.05$; Figure 2k). Bisulfite sequencing analysis of the Nanog and Oct4 promoters revealed almost complete epigenetic reprogramming. The percentages of CpG methylation (%ME) are shown in Figure 2l and 2m. Karyotyping of hDPC-iPSCs in addition to chro-

Figure 2. Identification of iPSCs with AKP, q-PCR, Immunocytochemistry and bisulfite sequencing analysis. (a, b) The clones were AP-positive and stained purple, indicating high alkaline phosphatase activity, compared with no staining in Matrigel and ECM. (c-j) hDPC-iPSCs cultured in Matrigel or human dental pulp cell-derived ECM expressed markers characteristic of hES pluripotency. (k) q-PCR from DPC-iPSCs cultured both in matrigel and ECM for markers: Nanog, Sox2, POU5F, Klf4, ZFP42, and MYC. Bar represents mean ± SD. *$p > 0.05$. (l) Bisulfite sequencing analysis of the Nanog and Oct4 promoters revealed almost complete epigenetic reprogramming. The percentages of CpG methylation (%ME) are shown.
mosomal analysis using G-band analysis revealed a normal karyotype for the hDPC-iPSCs from the donor. Karyotypes of iPSCs from both culture conditions were identical to those of the pulp cells (Figure 3a, 3b).

For identification of iPSCs by gene expression profiling, we used iPSCs cultured in Matrigel as positive control and iPSCs cultured in ECM as the experimental group. Expression of the U133 gene was evaluated using the Affymetrix microarray platform to analyze the expression of the iP genome and the expression of genes of the three germ layers was compared. DNA microarray analysis showed no significant differences in the expression of key genes, indicated by the thermal map and scatter diagram for hDPC-iPSCs cultured in both ECM and Matrigel (p > 0.05; Figure 3c, 3d).

hDPC-iPSCs cultured in Matrigel or human dental pulp cell-derived ECM both differentiated into cystic EBs in vitro (Figure 4a, b). The EBs exhibited adherent growth and differentiated into the three germinal layers, i.e., the endoderm, mesoderm, and ectoderm, as indicated by specific labeling for α-SMA, ALB, β-tubulin, GFAP (Figure 4c-4j).

Differentiation of Matrigel-cultivated hDPC-iPSCs was examined during 2 weeks since transplantation into the backs of four SCID mice and tumor formation was observed in all animals. By the 4th week, the size of tumors was approximately 1.5 × 1.5 × 1.2 cm³; and the animals were sacrificed to collect tumors were collected. The sampled tissues were sectioned for HE staining and it was found that tissue was originated from three germinal layers, including keratinized squamous epithelium and neural tissue from the ectoderm, muscle tissue and fat cells from the mesoderm, and intestinal epithelium from the endoderm, which indicated teratoma formation.

The differentiation capacity of hDPC-iPSCs cultured in human dental pulp cell-derived ECM was examined after transplantation into the backs of SCID mice; five mice exhibited tumor formation. The tumors grew to a size of 2.0 × 2.0 × 1. × 5 cm³ after 4 weeks before the animals were sacrificed. The tumors were collected and sectioned for HE staining. Tissues originating from three germinal layers were observed, including keratinized squamous epithelium, neural tissue (ectoderm), muscle tissue, fat cells (mesoderm) and intestinal epithelium (endoderm), which indicated teratoma formation. One mouse survived without tumor formation (Figure 4k-4m).

## Discussion

iPSCs was considered more suitable for future clinical use compared with ES cells due to the absence of immune rejection and lack of ethical concerns. In 2007, Yamanaka and Thomson provided a new direction for stem cell research and regenerative medicine by reprogramming human skin fibroblasts into iPSCs. iPSCs shared similar properties with ES cells that feeder cells are needed for culture during reprogramming and passaging, iPSC culture is typically performed as described by Thomson and Odorico and Xu et al with mouse embryo fibroblast cells (MEF) as feeder cells to obtain stable iPSCs; however, the clinical utility of iPSCs was limited by use of feeder layer and animal-derived ingredients.

Feeder-free culture systems are modified based on the Thomson and Odorico system. Due to specific expression of genes and membrane proteins, ES and iPSC cells cannot grow in adherent culture as somatic cells do. Matrigel is a soluble basement membrane matrix composed of laminin, collagen IV, FGF, tissue plasminogen activator and other factors. At room temperature, Matrigel aggregates and becomes bioactive and can serve the act basement membranes of mammalian cells by resembling endogenous environment and improving the adherent growth of iPSCs. However, cells growing in Matrigel are unstable and prone to differentiate, which may be attributed to the microenvironment of the 2D culture system.

The microenvironment in this case refers to the space and interrelationships on which the cells rely for survival. It contains extracellular matrix components and certain cell-derived bioactive molecules and provides support, connections, nutrients and protection, which is critical for cell proliferation, differentiation and migration, especially for stem cell attachment, survival, migration, proliferation, differentiation and matrix remodeling. Stem cells are usually affected by specific microenvironment in which the migration, self-renewal and committed differentiation are mediated by complex extracellular stress and chemical signals. Cell-derived decellularized ECM has been used to study the interaction between cells and ECM. In 2001 Cukierman et al first proposed the use of fibroblast-secreted ECM, which combines the matrix components such as type I and type III collagen, fibronectin, laminin, elastin and large proteoglycans, as well as the 3D structure and flexibility observed in the
Figure 3. Identification of iPSCs by chromosomal analysis and DNA microarray analysis. (a, b) Karyotyping result of dental pulp-induced pluripotent stem (DPC-iPS) cells and chromosomal analysis using G-banding. (c, d) The expression of the U133 gene was evaluated using the Affymetrix microarray platform to analyze the expression of the iPS genome, and the primary genes of the three germ layers were compared ($p > 0.05$).
Figure 4. Identification of iPSCs by in vitro and in vivo differentiation. (a-j) hDPC-iPSCs cultured in Matrigel or human dental pulp cell-derived ECM differentiated into cystic EBs in vitro. EBs exhibited adherent growth and differentiated into three germinal layers (endoderm, mesoderm and ectoderm) as indicated by specific labeling with SMA, ALB, $\beta$-tubulin and GFAP, respectively. (k) hDPC-iPSCs cultured in Matrigel or human dental pulp cell-derived ECM differentiated into teratoma formation in vivo. (l, m) HE staining showed that the three germ layers of the organization contained squamous epithelial, neural tissue keratosis (ectoderm), muscle, fat cells (mesoderm), and intestinal gland epithelial (endoderm) tissues (magnification 100x).
In vivo microenvironment, in studying the interaction between cells and the matrix. The acellular matrix that remains after decellularization provides adequate space for the growth of MSCs. Similar to the in vivo reaction, fibroblasts in the 3D ECM microenvironment rapidly synthesize integrin α5β1 and αVβ3, which enhance the contact with the ECM, making the attachment process faster than in a 2D culture environment or Matrigel.

Cell-secreted ECM can rebuild and mimic the internal microenvironment, regulate stem cell behavior and influence the self-renewal and differentiation potential of stem cells. Chen et al.25 prepared ECM from mouse bone marrow cells for in vitro culture and found that bone marrow-derived ECM improved proliferation of bone marrow MSCs and osteogenic differentiation. Hoshiba et al.30-41 induced human bone marrow MSCs to secrete ECM with bone marrow- or adipose tissue-specific properties to regulate stem cell differentiation. Pei et al.24 prepared human bone marrow cell-derived decellularized ECM, which was used for in vitro culture of bone marrow MSCs and found to promote proliferation and osteogenic differentiation, which supported that ECM can be used for large-scale MSC culture and maintaining stem cell properties. Li et al.36 found that ECM derived from human fetal synovium-derived stem cells (hSDSCs) in hypoxic culture improved cell expansion and cartilage differentiation. It was also reported by He et al.26 that the expansion of synovium stem cells and cartilage differentiation can be improved when synovium stem cell-derived ECM was used for culture. Kim et al.27-29 showed that ECM culture systems may facilitate future clinical application of pluripotent stem cell-based therapies. Previous studies by Hoshiba et al.23,30-41 resulted that a matrix mimicking that of osteoblasts affected the differentiation of mesenchymal stem cells into osteoblasts, and ECM facilitates mesenchymal stem cell culture and amplification, the effect of which on cell function was cell source-dependent; moreover, the culture of mesenchymal stem cells by such ECM promoted their differentiation into chondrocytes.

As shown above, increasing evidences suggest that ECM mimics the in vivo microenvironment better than 2D culture systems and contains matrix proteins such as fibrin, type I collagen and laminin that allow stem cells to grow in a physiologically relevant environment by improving attachment, proliferation and migration. Primary cell-derived ECM not only provides 3D structure but also contains complex components that can influence signal transduction. Establishment of a functional in vitro microenvironment and recovery of the full potential of stem cells are critical for developing therapeutic stem cell applications and study of microenvironment-associated signal transduction.

ECM is used as a biomaterial scaffold for tissue or organ regeneration after acellularization to remove the cells and cell fragments completely, thereby not only avoiding the host immune response and minimizing adverse effects on tissue and organ reconstruction, but also maintaining a complete mesh and biological activity. Commonly used acellularization methods included (a) physical methods, such as repeated freeze-thaw cycling and pressure treatment; (b) treatment with chemical reagents such as SDS, CHAPS or Triton X-100; (c) enzymatic digestion, e.g., with trypsin, neutral protease or nuclease. The main purpose of the physical methods is to destroy the cell membrane for cell lysis; however, cell debris cannot be separated from the cell extracellular matrix. Thus, physical methods must be combined with chemical reagents to remove cell debris. The main function of the chemical reagents is to destroy the connections between nucleic acids, proteins and lipids, and to dissolve the cell membrane. Biological enzymes are mainly used to promote the separation of cell debris and extracellular matrix. At present, the method of manufacturing ECM combines detergent and enzyme treatments are commonly used to remove residual cell debris thoroughly. However, such treatments may impact the three-dimensional structure of the resulting matrix, which makes it necessary to choose the acellularization method based on the specific tissue and cell type. Our experiment used dental pulp cells as biological materials, and chemical reagents (Triton X-100) treatment combined with physical washing as acellularization method to avoid enzymatic destruction of the ECM and to ensure the protein content and integrity of the ECM. Triton X-100, a type of non-ionic detergent that can destroy the connections between proteins and lipids, lipids and lipids, and nucleic acids and proteins, provides mild effect to remove cells from thin tissue without breaking the protein-protein linkage. Use of Triton X-100 is conducive for protecting the structural integrity of the ECM considering the delicate ECM network structure. Our experiments showed that the reticular membrane in-
Cell survival and development require three basic elements: nutrition, energy and information. Our experiment showed that iPSCs cannot survive in synovium stem cell-derived ECM or in human dental pulp cell-derived ECM with KSR culture medium.

Conclusions

We concluded that the specific microenvironment of cell-derived ECM and culture media influenced the survival of different types of stem cells. Moreover, different types of cell-derived ECM provide 3D structure that has a complex influence on cell signaling, which in turn affects cell survival and development.

A pluripotent stem cell culture system should first provide permanent proliferation of undifferentiated stem cells to ensure the desired cell numbers as well as the cellular function. Selecting the optimal cell culture system for pluripotent stem cells is therefore critical for stem cell research and clinical applications. Knockout serum replacement (KSR) medium is a serum replacement composed of bovine serum albumin, transferrin, insulin and other components, however, was confirmed inappropriate for the growth of pluripotent stem cells. mTeSR\textsuperscript{12}, a complete, chemically defined serum-free culture medium based on DMEM/12F and supplemented with albumin, vitamins, amino acids, trace elements, lipids and certain specific factors such as zbFGF and LiCl, turned out to be a better choice for our objective, by providing microenvironment appropriate for pluripotent cell growth combined with human dental pulp cell-derived ECM. Our finding may provide a foundation for a new iPSC culture system.

Acknowledgements

This study was supported by grants from the Medical Ethics Committee of Affiliated Stomatological Hospital of Sun Yat-Sen University. We would like to acknowledge Dr. Dongbo Qiu, Dr Zhangqi (Vaccine Institute of the Third Affiliated Hospital, Sun Yat-Sen University), Professor Fang He, and Professor Hongmin Gong (The School of Engineering at Sun Yat-sen University) for technical guidance. We would like to acknowledge Dr. Zhengyu Liang, Dr Xu Yue, Dr Zheng Youhua, and Dr Kuang Shijun (Department of Oral and Maxillofacial Surgery, Guanghua School of Stomatology, Hospital of Stomatology, Sun Yat-sen University; Guangdong Provincial Key Laboratory of Stomatolog) for their excellent technical support. We would like to acknowledge the financial support of the National Science Foundation of China (grant number 81271115) to Professor Zhangzhi Guang for this study. This study was supported by grant from the National Science Foundation of China (81271115).

Conflict of Interest

The Authors declare that there are no conflicts of interest.
References


23) HOSHIBA T, KAWAZOE N, CHEN G. The balance of osteogenic and adipogenic differentiation in human mesenchymal stem cells by matrices that mimic stepwise tissue development. Biomaterials 2012; 33: 2025-2031.


