In vitro Micro-Mineralized Tissue Formation by the Combinatory Condition of Adipose-Derived Stem Cells, Macroporous PLGA Microspheres and a Bioreactor

Min-Seo Jung¹, Han Byul Jang², Soo-Eon Lee², Jae-Hong Park², and Yu-Shik Hwang*,¹

¹Department of Maxillofacial Biomedical Engineering and Institute of Oral Biology, Kyung Hee University, Seoul 130-701, Korea
²Department of Pediatric Dentistry, School of Dentistry, Kyung Hee University, Seoul 130-701, Korea

Received May 28, 2013; Revised July 16, 2013; Accepted July 25, 2013

Abstract: For the final purpose of accelerating the restoration of defected bone tissue, researches for developing in vitro three dimensional (3D) mineralized tissue using various stem cells, scaffolds and culture systems have been extensively done. In this research, an integrated bioprocess to generate stem cell-based 3D mineralized construct was developed using adipose-derived stem cell (ADSC), highly porous biodegradable poly(D,L-lactide-co-glycolide) (PLGA) microspheres and a high-aspect ratio vessel (HARV) bioreactor system. First, ADSCs adhered uniformly on poly-L-ornithine-coated macroporous PLGA microspheres, and the ADSC/microsphere composites were cultured in the presence of osteogenic supplements in a HARV bioreactor. Alkaline phosphatase (ALPase) activity assay, immunocytochemical staining and quantitative real-time polymerase chain reaction (qPCR) analysis showed temporal increase of ALPase activity, molecular expressions of type I collagen, osteocalcin and runx2 and upregulation of runx2, Sp7, type I collagen and bone sialoprotein mRNA during 3D osteogenic culture of ADSC/microsphere composites. Finally, 3D dynamic osteogenic culture generated highly mineralized micro tissues as validated by alizarin red-S staining and SEM-EDS. The results demonstrated that cell-based 3D micro-mineralized tissue could be generated by integrated bioprocess, and potentially utilized for bone tissue regeneration. The integrated bioprocess in this study may provide an efficient and scalable culture system for application in bone tissue engineering.

Keywords: adipose-derived stem cell (ADSC), microsphere, bioreactor, osteogenic, mineralization, micro-tissue.

Introduction

Recently, various somatic stem cells and biomaterials have been developed and clinically applied to restore bone defects, and many efforts to develop in vitro three-dimensional (3D) bone tissue-like constructs prior to transplantation. Recently, in the field of bone tissue engineering, bone tissue-like construct has been fabricated by combining stem cell including bone marrow-derived stromal cell (BMSC) and adipose tissue-derived stem cell (ADSC) with various 3D scaffolds which are made of natural or synthetic biomaterials in bench top scale.¹² For in vitro 3D tissue formation, various scaffolds with porous structure have been designed to support 3D cell growth and differentiation of stem cells, and proper culture system is also essential with suitably designed scaffold. Thus, 2D static culture condition does not provide suitable culture environment for homogenous 3D tissue formation, and cell growth and tissue formation are usually confined on top of surface of scaffold.³⁴ It was reported that higher concentration of nutrient at outer surface of scaffold resulted in the migration of cells which were initially seeded at internal part of the 3D porous scaffold towards that surface via chemotaxis, and the remaining cells inside of scaffold showed reduced expression of osteogenic specific genes.⁵ It has been also reported that transport of nutrient through 3D scaffold via diffusion might not satisfy the metabolic requirement of cells which reside at the center point of 3D scaffold for long culture period.⁶ Therefore, for efficient in vitro 3D tissue formation, bioreactors have been utilized to facilitate mass transport of nutrient and differentiation-inducing supplements with respect to the supply of oxygen and medium components. In recent years, among various types of bioreactors, rotating wall vessel (RWV) bioreactors have been widely used in cell-based 3D bone tissue formation in vitro.⁷⁸ The RWV bioreactor was designed to optimize to produce laminar flow and minimize the shear stresses and turbulence on cells in 3D culture, providing adequate nutrition and oxygenation, ideal for mammalian cell culture that supports 3D tissue growth.¹⁰ The suspension culture environment of microcarrier in RWV bioreactor is established by the balance between the free falling of the microcarrier inside the bioreactor as a result of gravity and the centrifugal forces due to the rotation of vessels.¹⁰¹¹ Accompanied with bioreactor system, another important
factor in 3D culture system is the selection of microcarrier for cell growth and 3D tissue formation. In bone tissue engineering, various biomaterials such as natural or synthetic polymers and ceramics have been used as supporting template for 3D cell growth and osteogenic differentiation of stem cells. For instance, porous beta tricalcium phosphate (β-TCP) ceramic scaffold was reported to provide spatial growth of bone marrow-derived osteoblasts in perfusion culture system, and the subsequent implantation of the subcultured cell/β-TCP composites aided in forming bone tissue through pores within the scaffold. Such 3D scaffold material could provide a template for spatial growth of cells to achieve the desired form of 3D tissue in vitro and in vivo. However, even though scaffold materials have highly porous structure for 3D tissue formation, the shape of scaffold might be one of factors which should be considered due to the motion mechanism of scaffold within bioreactor. Studies about simulation and experimental validation of such particle motion in bioreactors to optimize suspension culture have been done. Non-homogenous shape like cylindrical or disc type may give rise to very complex motion in rotating bioreactors, which could be very difficult to model and evaluate the influence of mechanical stress to the scaffolds, resulting in poor consistency in data. In contrast, porous scaffold with spherical shape could make it easier relatively to expect and evaluate their motion and the effect of environmental condition in bioreactors. Therefore, the selection of porous 3D scaffolds with adequate shape and bioreactor culture system and their integration are essential to generate in vitro 3D cell based tissue engineered construct.

In this study, bioprocess to develop stem cell-based 3D mineralized tissue was designed by integrating ADSCs, porous PLGA microspheres and a high-aspect ratio vessel (HARV) bioreactor as illustrated in Figure 1. First, porous PLGA microspheres were fabricated by a water-in-oil-in-water (W1/O/W2) double emulsion method, and surface of microspheres were treated with various molecules to improve initial cell adhesion. After ADSC adhesion in PLGA microspheres, the ADSC/PLGA microspheres were transferred to vessels of a HARV bioreactor, and finally develop in vitro 3D bone tissue-like mineralized construct under 3D dynamic osteogenic culture condition, resulting in efficient and reproducible culture system for bone tissue engineering applications.

Experimental

Materials. 50:50 poly(D,L-lactide-co-glycolide) (PLGA) was purchased from the Lakeshore Biomaterials™ (USA). Polyvinyl alcohol (PVA, 87-89% hydrolyzed, Mw 31,000-50,000) was purchased from Sigma (USA). Human Adipose-Derived Stem Cell (ADSC), MesenPRO RS™ Medium supplemented with MesenPRO RS™ Growth Supplement, alpha-minimum essential medium (α-MEM), fetal bovine serum (FBS), phosphate-buffered saline (PBS), trypsin-EDTA and penicillin-streptomycin (PS) were purchased from Invitrogen (USA). All cell culture plastics were purchased from Thermo Finnigan (USA). All other chemicals and regents were of analytical grade.

Preparation of Macroporous PLGA Microsphere and Scanning Electron Microscopic Observation. Macroporous Microspheres were fabricated by a W1/O/W2 double-emulsion method, as described in with slight adaptation. A