In vitro Bone Formation from Human Embryonic Stem Cell-derived Osteogenic Cells and Adipose-derived Stromal Cells in Poly(D,L-lactic acid-co-glycolic acid)/hydroxyapatite Composite Scaffolds

Abstract
We explored whether osteogenic cells derived from human embryonic stem cells (OC-hESCs) and adipose-derived stromal cells (ADSCs) would be able to regenerate bone tissues in vivo, after they are seeded on a biodegradable scaffold and implanted on mice. The OC-hESCs and ADSCs were mixed with fibrin gel and then seeded onto the top of scaffolds. Three dimensional porous poly (D,L-lactic-co-glycolic acid)/hydroxyapatite scaffold was used as a cell delivery vehicle with fibrin gel for implantation. The each cell-scaffold constructs were implanted into the subcutaneous space of immune-deficient mice (BALB/c-nu). The implants were retrieved for analysis at 4 and 8 weeks after implantation. In vivo implantation of cells seeded-scaffolds showed a significant new bone formation compared to other control groups (scaffold only, fibroblast-scaffold constructs, BMP-2 loaded scaffold). In addition, presence of BMP-2 in OC-hESCs and ADMSC further enhanced new bone formation. These results suggest the practical feasibility of OC-hESCs and ADMSC as a good alternative cell source for bone regeneration, especially by incorporating BMP-2.

Materials and Method
1) Culture of human embryonic stem cells and osteogenic differentiation: The CHA3-human embryonic stem cells were propagated on STO feeder cells as described previously. Differentiation into osteogenic cells was initiated by embryoid body (EB) formation in suspension culture. Simple EBs were plated on primary bone derived cells (PBDs). After 14 days, Von-kossa stain and alizarin red S stain was used to detect osteo-specific cells in co-cultured hEBs. For immunocytochemistry and FACS analysis, the cells were treated with antibodies against human COL I/OCN then, exposed to secondary antibody solutions as follows: FITC-conjugated IgG. And the expression of osteoblast-specific genes coding for the mineralized tissue was investigated by RT-PCR and measured by Real-time RT-PCR.
2) Cell seeding and transplantation: OC-hESC or ADMSC were seeded on the PLGA/HA scaffolds and cell-scaffold constructs were implanted into the subcutaneous space of immunodeficient mice (BALB/c-nu, 7 weeks old, female). As control groups, human dermal fibroblast-seeded PLGA/HA scaffolds (group 4) were implanted into dorsal, subcutaneous spaces of athymic mice. Green color indicates regenerated bone. B: bone; arrow: scaffold; scale bar: 200 mm. All photographs were taken at the same magnification. (B) Goldner’s trichrome staining of group 2 and 4 implants at a high magnification. This bone contained viable osteoblasts (black arrowheads) and osteocytes (white arrowheads). Arrow: scaffold; scale bar: 20 mm. (C) Bone formation area calculated as (bone area/total area) x 100% (*p<0.01; **p<0.001).

Summary
1. Both human OC-hESC and ADSCs successfully regenerated new bone tissues upon in vivo implantation.
2. The BMP-2–loaded scaffold group showed significantly higher mineralization than the other groups, which demonstrates that BMP-2 played a pivotal role in stimulating mineralization during the bone formation.
3. BMP-2 released from PLGA/HA scaffolds stimulated osteogenic differentiation of ADSCs cultured on the scaffolds, as assessed by expression of the osteogenic marker genes ALP, OPN and OCN.
4. 8 weeks after transplantation into dorsal, subcutaneous spaces of athymic mice, human OC-ESC and ADSCs on BMP-2–loaded PLGA/HA scaffolds exhibited significantly greater bone formation area and higher calcium deposition than control groups on PLGA/HA scaffolds lacking BMP-2.
5. OC-hESCs and ADMSC could be used as a good alternative source of cells for bone regeneration, particularly through their introductions into PLGA/HA scaffolds with BMP-2 growth factor for the repair and regeneration of bone tissue.
6. Such BMP-2–loaded PLGA/HA composite scaffolds would be valuable for in vivo regeneration of bone from differentiated OC-hESC and undifferentiated ADSCs.

References