LGR6⁺ Epithelial Stem Cell Augmentation of Fracture Healing

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Abstract

Currently, atrophic and hypertrophic non-unions are a challenging complications in fracture care. We propose an alternative approach to the current standard of care in fracture healing by introducing a theory based on the isolation of the patient's own autologous epithelial stem cells (ESC) which reside in an easily accessible portion of the skin. We hypothesize that LGR6+ ESCs will aid in fracture healing and prevention of fracture non-unions.

LGR6+ ESC were isolated from the hair follicle's follicular bulge of green fluorescent expressing (GFP) expressing Sprague Dawley (SD) rats, SD-Tg (UBC-EGFP) 2BalRrcc, using a FACSAria II flow cytometer with LGR6+, CD45- and CD90+ as markers. The multipotency of the isolated stem cells were determined by inducing them towards osteoblasts, chondrocytes and adipocytes. The induction was confirmed using Alizarin Red, Alcian Blue and Oil Red O stain respectively. The isolated cells were seeded on control tissue culture polystyrene (TCPS) and collagen coated coverslips (CCS) and divided into two groups (uninduced and osteo-induced) for further analysis. Confocal microscopy and Scanning Electron Microscopy (SEM) was used to determine cell morphology, adhesion and growth. A Vi-Cell cell counter was used to measure cell viability (or survival). A FluoReporter Blue Fluorometrics dsDNA quantitation kit was used to determine proliferation rate. Finally, a qPCR assay will be performed to determine the osteogenic gene markers expression.

Methods/Results

Isolation of SD GFP-LGR6 from SD-Tg (UBC-GFP) 2BalRrrc Rats

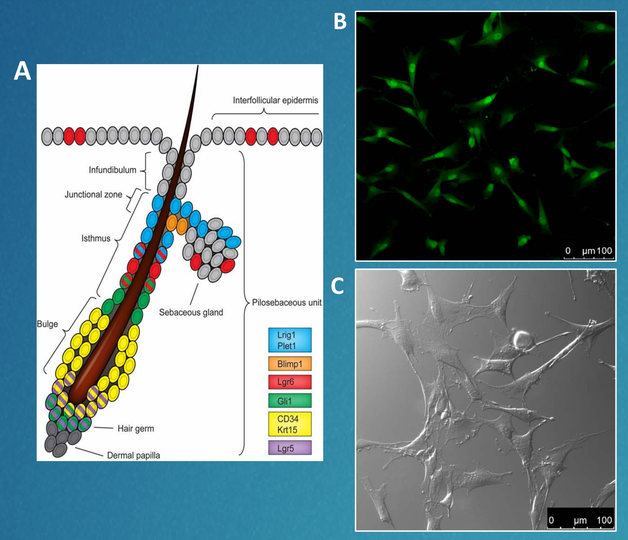


Fig 1: Isolation rat LGR6⁺ stem cells from the hair follicle's follicular bulge. (A) Schematic of hair follicular bulge cellular units (B) GFP expression in isolated LGR6 (Magnification: 10X with 3 zoom) (C) DIC image of isolated LGR6 (Magnification: 20X with 2 zoom)

Induction of Isolated rat LGR6+ Epithelial Stem Cells

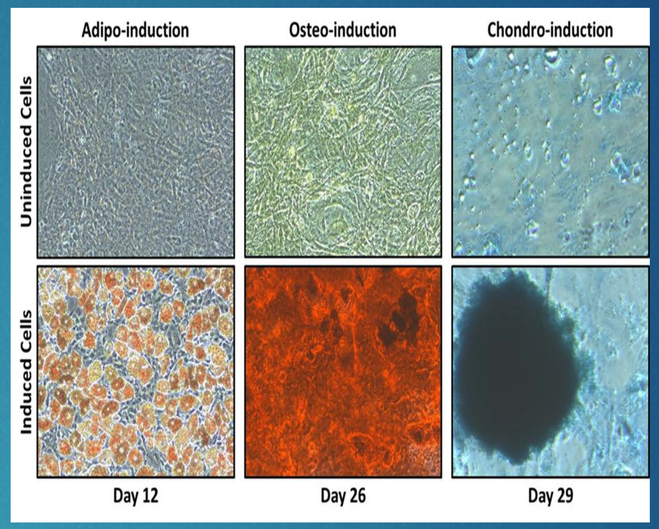


Fig 2: Induction of isolated rat LGR6+ epithelial stem cells into adipo-, osteo- and chondro- lineage to confirm stemness. Oil red O stain used for adipo-induction. Alzarin red stain used for osteo-induction. Alcian blue stain used for chondro-induction.

Cellular Morphology/Adhesion, Growth/Survival and Proliferation of the isolated rat Lgr6⁺ ESCs on a Decellularized Collagen Matrix

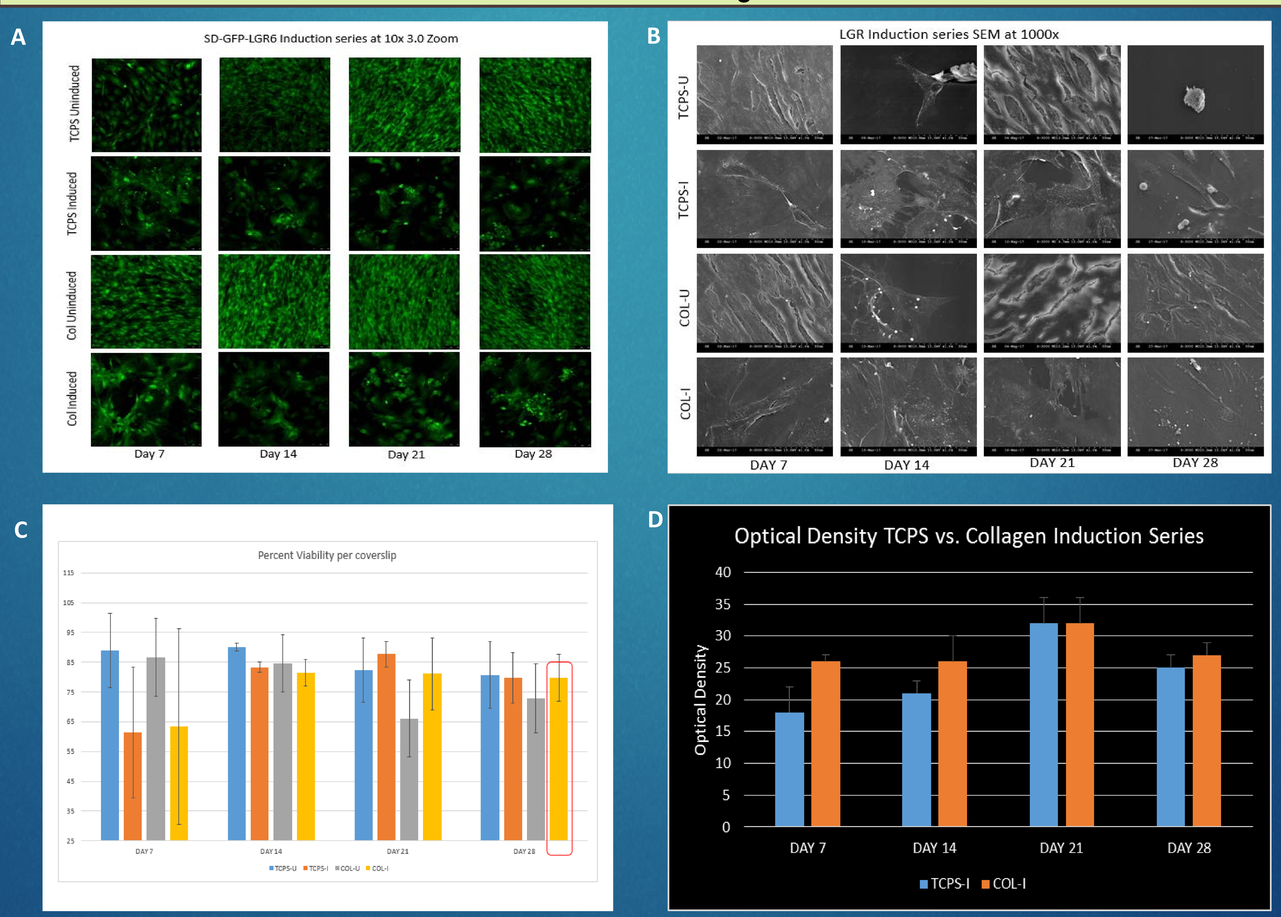
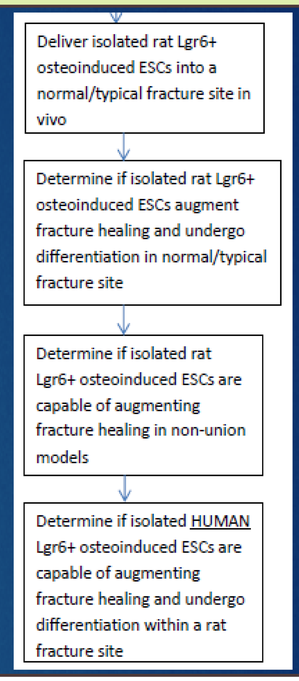


Fig 3: (A) Cell adhesion and growth on collagen matrix via immunofluorescence (B) additional imaging with SEM (C) Vi-Cell count for cell viability (D). Cell Proliferation assay using FluoReporter Blue Fluorometrics dsDNA quantitation kit

Future Studies

In-vivo rat fracture model



Conclusions

1. We have successfully isolated LGR6+ ESC and optimized our protocol to ensure repeatability.

2. We have shown the multipotency of the isolated ESC.

3. We have shown via Immunofluorescence staining with confocal imaging and SEM that osteo-induced lineage of LGR6+ ESC show adhesion and growth as well as cell viability on collagen matrix.
   - Proliferation assay with interesting results. Minimal proliferation of induction strain due to differentiation phase with low mitotic turnover.
   - qPCR assays are in process for complete analysis of osteogenic differentiation.

References

1. Tseng S, Reddi H et al. Non-unions and the potential of stem cells in fracture healing. *J Bone Joint Surg Am*. 2008;90(suppl 1):92-98.

2. Nath M, Seissler J et al. Isolation of in vitro expansion of Lrg6 positive multipoint hair follicle stem cells. *Cell Tissue Res*. 2011 Jun. 344 (3): 435-44.