Evaluation of Honeycomb Scaffold Combined with KUSA/A1 Cells for Tissue Engineering

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Abstract: We evaluated the efficacy of honeycomb scaffold combined with KUSA/A1 cells in vivo. The transplants were subjected to radiographical, histological and immunohistochemical examinations after 2 and 4 weeks of implantation. KUSA/A1 cells alone showed small nests of bone formation. Whereas, KUSA/A1-Atelocollagen revealed abundant new bone formation. We also determined the immunolocalization of type I collagen, CD34, Osteocalcin, and PCNA in this newly formed bone. Our results indicated that collagen scaffold plays an important role allowing vessel formation and cell anchorage especially through the proliferation and differentiation process in a confined space. This study can possible enhances existing therapeutic applications.

Keywords: tissue engineering, KUSA/A1 cells, atelocollagen, honeycomb scaffold, bone formation.

Introduction

In order to stimulate new bone formation, stem cells together with Honeycomb Collagen Scaffold could be a good candidate for bone regeneration therapy. Collagen scaffold has a high affinity for cells, and this scaffold has Atelocollagen Type I, which is reabsorbable biomaterial.[1] KUSA-A1 cells are marrow stromal stem cell line, which are capable to form bone in vivo.[2] We evaluated the mechanism of bone induction by KUSA/A1 cells combined with honeycomb atelocollagen scaffold.

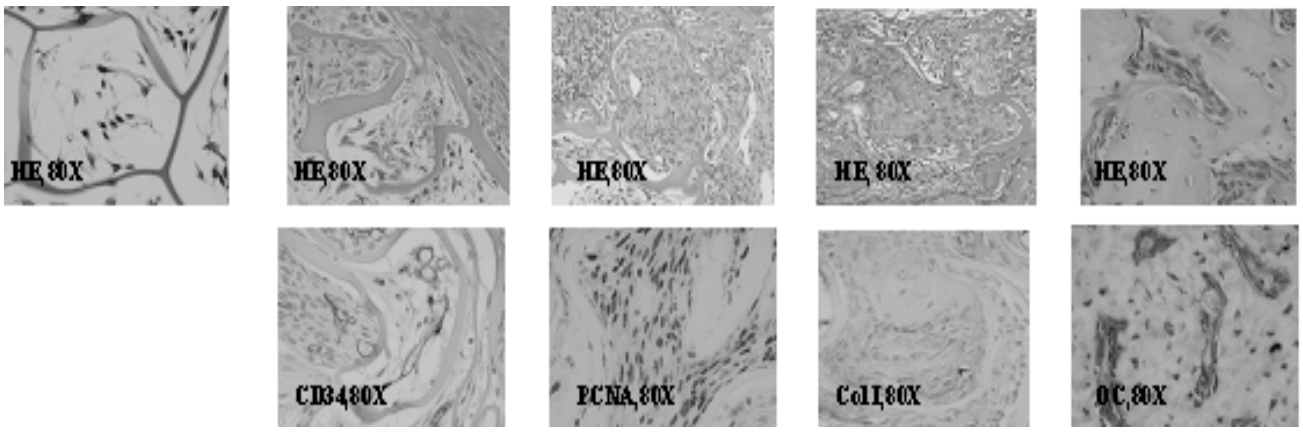
Materials and Methods

In this study were used 4-week-old male Severe Combined Immunodeficient (SCID) mice, KUSA-A1 cell line (kindly provided by Dr.Umezawa, Center of Human Development) and honeycomb atelocollagen scaffold (3x3x2 mm cube, Koken). $5x10^6$ of KUSA/A1 cells alone and $1x10^6$ of KUSA/A1 cells with atelocollagen were implanted in the subcutaneous pockets of mice. The transplants were subjected to histomorphometry (NIH image), radiographical, histological and immunohistochemical examinations after 2 and 4 weeks of implantation.

Results and Discussion

To understand the process of bone induction, CD34 for vessel formation, PCNA for cell proliferation and Col I and OC for matrix were stained. The mechanism of bone induction by KUSA/A1 cells within a pore of the scaffold was the follow: 1- Excessive vessel formation within the pore of the scaffold, which was immunostaining for CD-34 localized in developing vessels

Fig.1 Mechanism of bone formation by KUSA/A1 cells within a pore of the scaffold



1) KUSA/A1 cells in a pore 2) Vessel formation stage 3) cell proliferation stage 4) Matrix formation stage 5) bone formation stage

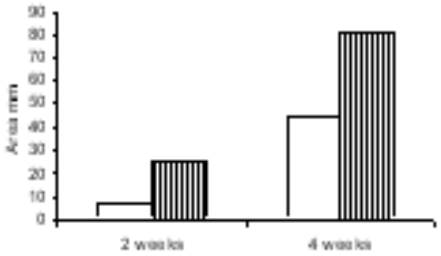


Fig. 2 Histomorphometry of new bone

(Fig.1.2). 2- Cellular proliferation forming micromass strongly positive for PCNA (Fig.1.3). 3- Induction of bone matrix from the center of the micromass, and also from the collagen membrane, which was immunostaining for type I collagen (Fig.1.4). The new bone formation within a pore of the scaffold starts from the center of the micromass and also from the collagen membrane of the scaffold. 4- Osteoblast-like cells and osteocyte-like cells of woven bone were positive for OC (Fig.1.5).

The area of new bone was measure using NIH image. Interestingly, using fewer numbers of cells combined with scaffold, there was more bone formation compared with KUSA alone at 2 and 4 weeks (Fig.2).

In conclusion, less amount of stem cells are capable to induce the desirable amount of new bone in tissue engineering compared to KUSA/A1 cells alone**.** This study supports that atelocollagen honeycomb scaffold plays an important role in cellular anchorage and in vessel invasion, giving the precise shape and size for the new bone formation.

References

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