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Biodegradable Honeycomb Collagen Scaffold for Dermal Tissue Engineering

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Abstract

Tissue engineering requires a mechanically stable, biocompatible, and biodegradable scaffold that permits cell adherence and proliferation, allows preservation of cell-specific properties, and suitable for surgical implantations. In this study, honeycomb collagen sheet was used for three-dimensional (3D) cultures of human skin fibroblasts and characterized as an effective and suitable scaffold for dermal tissue engineering. About 1-mm-thick honeycomb collagen sheets, prepared from bovine dermal atelocollagen, cross-linked by UV-irradiation, and sterilized by heat, were placed on the proliferating fibroblasts on day 3 of the culture. The cells attached quickly to the collagen scaffold, proliferated inside the honeycomb pores, and formed a structure similar to dermis within 60 days. On day 60, total cellular DNA content of the 3D cultures was 12-fold higher when compared with the 2D control cultures without the scaffold. Measurement of procollagen type I in the media demonstrated a 20-fold increase. Scanning electron microscopy of the 3D cultures showed a well-formed structure similar to dermis and biodegradation of the honeycomb collagen scaffold. Our study proved that honeycomb collagen sheet is a mechanically stable, biocompatible and biodegradable scaffold for dermal tissue engineering, and also potentially useful for other cell based therapies and tissue engineering applications.

Introduction

Tissue engineering is an emerging field with the prospect to provide functional replacement of impaired tissues or organs to patients. It is an interdisciplinary field that brings together the principles of the life sciences and medicine with those of engineering. This technology involves the implantation of an engineered biological substitute, which is either functional at the time of implantation or has the potential to integrate and form the expected functional tissue at a later stage. There has been substantial progress recently in the development of increasingly complex tissue engineered structures. Continued progress in tissue engineering will depend on the further development and integration of several classes of enabling tools that will allow not only precise and reproducible fabrication of scaffolds, but also quantitative characterization of the biological integration of the tissue engineered constructs. Furthermore, advances in *in-vivo* imaging, such as positron emission tomography (PET), make it possible to provide a non-invasive monitoring of the development and incorporation of the engineered tissues.

It has been reported that honeycomb collagen sponge prepared from bovine dermal atelocollagen is a suitable scaffold for three dimensional cell cultures, which has enormous potential in the field of various tissue engineering applications. The biodegradable honeycomb collagen sheet can be cut into suitable thickness and various sizes depending on the application. The aim of our present investigation was to employ the honeycomb collagen sheet for proliferation and multiplication of human skin fibroblasts into a dermal-like structure and to characterize the scaffold for dermal tissue engineering. Our study was also aimed to evaluate the use of honeycomb collagen scaffold for biomedical engineering and cell based therapy.

Figure 1

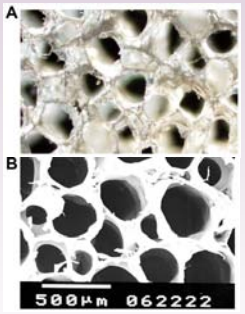

Figure 1. **(A)** Phase contrast micrograph of 1 mm thick honeycomb collagen sheet prepared from bovine atelocollagen solution (x 40). **(B)** Scanning electron micrograph of 1 mm thick honeycomb collagen sheet (x 50). The average pore size of the honeycomb collagen scaffold was around 300 µm.

Figure 2

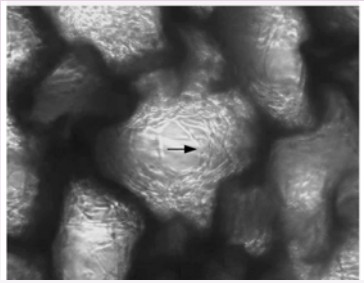

Figure 2. Phase contrast micrograph of 14 day old culture of human skin fibroblasts on honeycomb collagen sheet (x 100). All honeycomb pores are partially or fully filled with the proliferating fibroblasts. The fibroblasts proliferated inside the honeycomb well in a circular manner from the wall toward the center of the well. The arrow indicates the proliferating fibroblasts filling the honeycomb pore in a circular manner. The black area represents the walls of honeycomb scaffold.

Figure 3

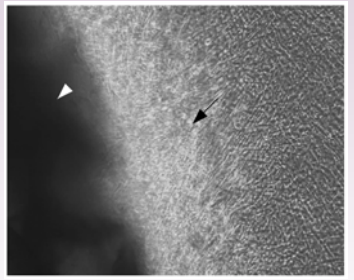

Figure 3. Phase contrast micrograph of 30 day old culture of human skin fibroblasts on honeycomb collagen sheet (x 100). The fibroblasts completely filled the whole scaffold and proliferated out from interior and top of the scaffold. The arrow indicates the prominent 3-dimensional growth of fibroblasts surrounding the scaffold. The black area indicates edge of the honeycomb scaffold (white arrowhead).

Figure 4

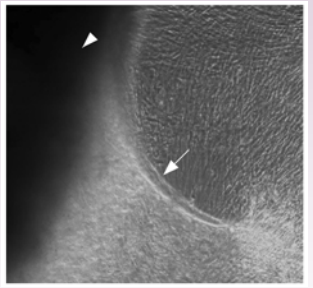

Figure 4. Phase contrast micrograph of about 45 day old culture of human skin fibroblasts on honeycomb collagen sheet (x 100). A portion of the culture surrounding the scaffold was cut and removed to show the thickness (1 mm) of the culture and also to demonstrate the marked 3-dimensional growth of fibroblasts around the honeycomb collagen scaffold (arrow). The black area indicates the honeycomb scaffold (arrowhead).

Figure 5

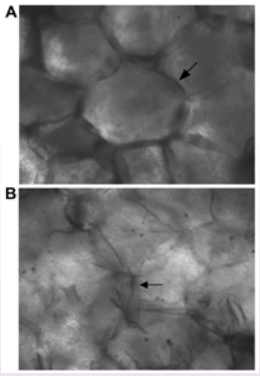

Figure 5. **(A)** Phase contrast micrograph of around 50 day old culture of human skin fibroblasts on honeycomb collagen sheet (x 100). The honeycomb collagen scaffold pores are totally filled with the fibroblasts. In addition, the fibroblasts formed layers covering the entire scaffold including the honeycomb walls (arrow). The honeycomb structure was still intact.

(B) Phase contrast micrograph of about 60 day old culture of human skin fibroblasts on honeycomb collagen sheet (x 100). The honeycomb scaffold is completely filled and covered with fibroblasts. The entire structure is almost distorted and has started degrading. The arrow indicates biodegradation of the scaffold walls. The cultures were processed for scanning electron microscopy at this stage.

Figure 6

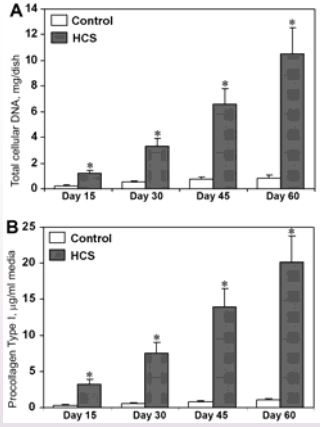

Figure 6. **(A)** Total cellular DNA content of control cultures and cultures with honeycomb collagen scaffold on days 15, 30, 45 and 60 after placing the honeycomb sheet. The DNA content was more than 12-fold higher when compared to the corresponding control cultures on day 60 *(*P < 0.001, n=5).*

(B) Procollagen Type I C-peptide present in the media of control cultures and cultures with honeycomb collagen scaffold on days 15, 30, 45 and 60 after placing the honeycomb collagen sheet. On day 60, the procollagen type I content in the serum free media was 20-fold higher compared to the corresponding control cultures without the scaffold *(*P < 0.001, n=5). HCS-Honeycomb collagen scaffold.*

Figure 7

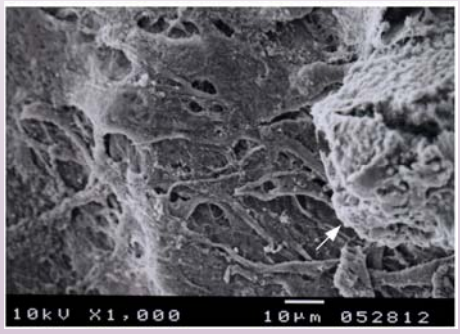

Figure 7. Scanning electron micrograph of about 60 days old culture of human skin fibroblasts on honeycomb collagen sheet (x 1000). The fibroblasts formed a network similar to dermis on honeycomb collagen scaffold. A part of the degrading scaffold is also visible (arrow).

Conclusions

- ***Honeycomb collagen scaffold is a good substratum for multiplication and proliferation of human skin fibroblasts.***
- ***Proliferation of human skin fibroblasts on honeycomb collagen sheets produced 3-dimensional cultures of more than 1-mm thickness.***
- ***3-dimensional cultures of human skin fibroblasts on honeycomb collagen scaffold produced a structure similar to dermis within 2 months.***
- ***Honeycomb collagen sheet is a mechanically stable, biocompatible and biodegradable scaffold for dermal tissue engineering.***
- ***Honeycomb collagen scaffold is potentially useful for cell-based therapies and tissue engineering applications.***

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