Journal of Microbiology and Biotechnology
Vol. 7. No. 4, 267-271, 1997

Contraction Behavior of Collagen Gel and Fibroblats Activity in Dermal Equivalent Model

YANG, EUN-KYUNG, DOO-HOON LEE, SUE-NIE PARK[1], TAE-BOO CHOE[2], AND JUNG-KEUG PARK*

Department of Chemical Engineering, Dongguk University, Seoul 100-715, Korea
[1]Virus/Oncology R.U., Korea Research Institute of Bioscience & Biotechnology, Taejon 305-333, Korea
[2]Department of Microbial Engineering, Konkuk University, Seoul 133-701, Korea

We developed a dermal equivalent (DE) which was engineered using human dermal fibroblasts and a matrix of collagen gel. The *in vitro* construction of the DE was accomplished by casting a porcine collagen type I solution plus concentrated medium with isolated and cultured fibroblasts. These constructs were attached to culture dishes or left floating in culture medium. Contraction of attached gels results in decreased gel thickness without a change in gel diameter, and contraction of floating gels results in decreased gel thickness and diameter. After contraction, there was no increase in cell number in floating gels, but cells in attached gels began to increase after about 4 days of the lag phase in cell growth curve. At this lag phase, addition of fibroblast growth factor (FGF) at a concentration of 0.1 μg/ml promoted cell proliferation in the attached collagen gels, but no effect in floating gels. These results indicate that the method of contraction had an influence on the extracellular matrix (ECM) organization, and this influenced not only cell growth but also fibroblast responsiveness to FGF. This suggests that attached collagen gel is more suitable as a dermal equivalent than the floating gel. And the final contracted area of attached gel is much larger than that of the floating gel since floating gel is contracted in all directions but attached gel is contracted only vertically.

Tissue engineering technology includes tissue culture techniques, in which cells originated from the various tissues of animal or human body are cultured and reconstituted *in vitro* to make either three dimensional artificial tissues or hybrid type artificial organs (5). The field of tissue engineering has grown rapidly over the last several years to encompass a broad range of technologies. One of the primary goals is to form structures and tissues that will allow organ repair or serve as either a temporary or permanent replacement. The applications of tissue engineering include the formation of living tissues for cell biology research, wound repair, test systems for therapeutics, and drug delivery (6). Intensive investigations have resulted in developing technologies for a wide variety of organs and applications. Probably the most advanced development has been achieved for the skin replacement. Several different types of artificial skin tissue have been developed by using tissue engineering and are currently in clinical trials for wound repair and toxicological test systems (3, 8).

Skin is composed of two tissues, a connective tissue or dermis and a covering epidermis. During connective tissue repair, fibroblasts exhibit several different activities. At first, they migrate from adjacent tissues into the wound region and then they proliferate and synthesize a collagen-rich extracellular matrix that effectively fills the wound. The extracellular matrix of the dermis provides the structural and biological support for epidermis. The fibroblasts also contribute to the remodeling of newly synthesized extracellular matrix (16).

In the tissue engineered dermal equivalent (DE), the contraction of the collagen lattice by cells results in the formation of a tissue-like fabric which may be useful for repairing skin wounds or burns (2). This fibroblast-mediated contraction of collagen can be mimicked *in vitro* in the three-dimensional skin equivalent model culture system first described by Bell and colleagues (1, 11). In this DE model, fibroblasts can reach a high degree of differentiation, exhibiting a bipolar morphology, controlled cell division, and regulated synthesis of macromolecules. In the DE model, the fibroblasts reorganize and contract the collagen gels. Reorganization of the col-

*Corresponding author
Phone: 82-2-260-3365. Fax: 82-2-271-3489.
E-mail: jkpark@cakra.dongguk.ac.kr
Key words: tissue engineering, dermal equivalent, artificial skin, fibroblasts, collagen gel

lagen gels by fibroblasts propagated throughout the interconnected collagen fibril network. Previous studies have shown that DNA synthesis and collagen synthesis by fibroblasts decreased after collagen gel contraction (12). Lower synthetic activity was thought to occur as a result of the change in cell shape that accompanied gel contraction. Since cell cycling is blocked soon after cells are incorporated into the collagen matrix, the entire population becomes homogeneous with respect to DNA synthesis and can be compared with cells grown on a monolayer, in which DNA synthesis is blocked because of contact inhibition (9). The effect of fibroblast growth factor (FGF) stimulation on human dermal fibroblasts was evaluated not only in monolayer culture but also in this three dimensional collagen matrix culture system (4).

Contraction of these floating gels results in decreased gel thickness and diameter. But contraction of gels attached to the underlying culture dishes results in decreased gel thickness without any change in gel diameter. During this culture period, fibroblasts in floating gels inferior to fibroblasts in attached gels although the cells are remaining in the floating gels. Since attached gels are contracted only vertically, collagen fibrils are aligned in the plane of cell spreading then collagen gels are well organized and the fibroblasts have bipolar morphology. Floating gels, however, are contracted in all directions, collagen fibrils are arranged randomly and then the gels are unorganized and the fibroblasts have changed to stellate morphology (4, 7).

In this study we made the two DE model systems, the attached and the floating collagen gels. We analyzed the consequences of long-term collagen gel contraction on initial cell density and the extent of cell growth in these two systems. To compare the cell activity in the two DE model systems, we analyzed the fibroblasts responsiveness to fibroblast growth factor (FGF).

MATERIALS AND METHODS

Primary Culture of Fibroblasts

Human dermal fibroblasts were aseptically isolated from a circumcised neonatal foreskin as before (15). The epidermis and dermis came loose by incubation in 0.9 units/ml dispase in culture medium without serum for 16 hours at 4°C. After the epidermis stripped off mechanically and dermis minced and attached on the surface of tissue culture flask and fed with Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) for about 1 week. The dermal fibroblasts spreading as radial outgrowth from attached pieces of dermis grew out and were cultured in DMEM with 10% fetal bovine serum.

Construction of Dermal Equivalent

The *in vitro* production of a unit of dermal equivalent begins by casting a porcine collagen type I (Cell Matrix, Nitta gelatin, Japan) plus concentrated medium with isolated and cultured fibroblasts. Seven volumes of acid soluble collagen solution (3.0 mg/ml) were mixed with 2 volumes of 5× concentrated DMEM and 1 volume of 0.05 N NaOH containing 2.2% sodium bicarbonate and 200 mM Hepes buffer solution. This neutralized collagen solution was kept on ice to prevent immediate gelation and the centrifuged fibroblasts pellet was resuspended in the collagen solution. Aliquots of the cell/collagen mixtures were placed on culture plate.

Collagen gels were polymerized by raising the temperature to 37°C and incubating. After polymerization, the fibroblasts were dispersed throughout the gels and became the attached gels. To obtain cultures of floating collagen gels, the attached gels were gently lifted off the bottom of the wells with a spatula. Human dermal fibroblasts contracted the collagen gel into a fibrillar connective tissue-like dermal lattice after incubation (at 37°C, 5% CO_2 in air) in the culture medium DMEM containing 10% FBS (10, 13, 14).

Fibroblast Growth Factor (FGF) Responsiveness

Fibroblasts pellet was resuspended to neutralized collagen solutions, which made the final cell density 5×10^5 cells per 1.0 ml. Aliquots of the cell/collagen mixtures were placed in Nunc 12-well culture plates. Cells in collagen gels were cultured for 2 days in 10% serum-containing medium and then transferred to medium containing 2% fetal bovine serum (FBS) instead of 10% FBS and fibroblast growth factor (FGF) at a concentration of 0.1 μg/ml (9, 12). During the contraction, there was no increase in cell number in floating gels, but cells in attached gels began to increase after about 4 days of the lag phase in cell growth curve. FGF was added in the cell/collagen mixture during this lag phase.

Counting of Cells in Dermal Equivalent

At the end of the incubation, the gels were solubilized by treatment for 2 h at 37°C with 3 mg/ml collagenase (type I, 380 units/mg solid, Sigma Chemical Co. MO, U.S.A.) dissolved in buffer containing 130 mM NaCl, 10 mM Ca acetate, and 20 mM Hepes at pH 7.2. Single cell preparations were obtained by incubating the samples for an additional 20 min at 37°C with 0.25% trypsin. Aliquots of the samples were mixed with trypan blue and viable cell number was measured with a hemocytometer.

RESULTS AND DISCUSSION

The morphology of human dermal fibroblasts as an outgrowth from attached pieces of dermis is shown in Fig. 1. This photograph shows the tissues and cells of 10 days culture after primary explantation. The dense area is a tissue fragment and cells are seen migrating radially from the explant.

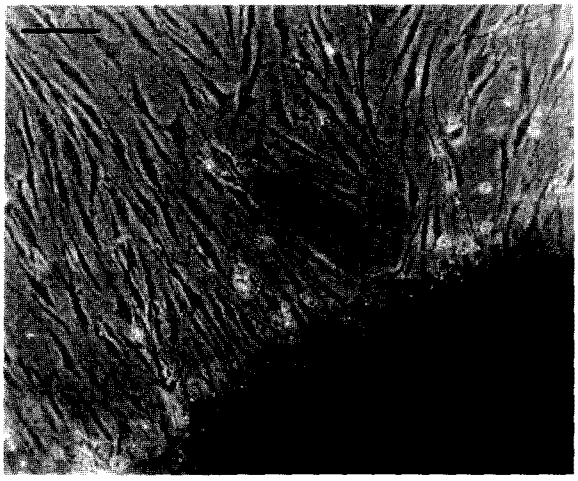

Fig. 1. Primary explant from human neonatal foreskin (scale bar=100 μm).
The black dense area is tissue fragment and the fibroblasts grew out and cultured for 10 days.

When fibroblasts are incorporated into hydrated collagen gel, the gel is contracted and water squeezed out. In the absence of cells, the gel undergoes no change in radius. The contracted gel with cells resembles skin or dermis in texture, has reasonable consistency and can be readily handled. When the lattice was first made up with cells, it was almost transparent, but it gradually became opaque as water was excluded and the diameter is reduced as shown in Fig. 2 and Fig. 3. We interpret the mechanism of collagen gel contraction as the organization of collagen fibrils in the extracellular matrix, that is, fibroblasts are capable of interacting with collagen fibrils to produce a denser arrangement of gels, with water being excluded in the process.

The contraction of attached or floating collagen gels was measured by changes in diameter and volume (Fig. 2). Attached collagen gels have no change in radius but have about 25% reduction in thickness. In case of the floating gels, the diameter was reduced to about 50% and the final volume of the gel was about 20% of its original value.

Fig. 3 shows that the rate and degree of contraction for floating gels is dependent on the number of cells initially incorporated into the gel. In general, the effect of initial cell density on contraction becomes less significant as a function of time, i.e., the final gel diameter

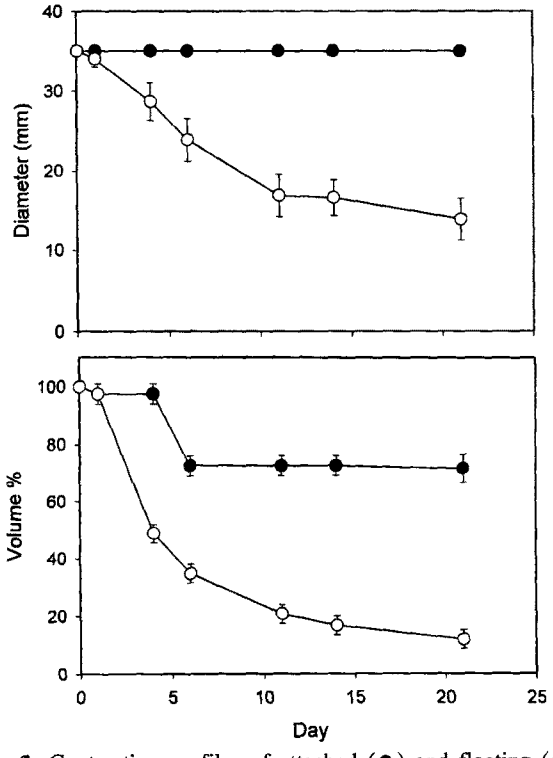

Fig. 2. Contraction profiles of attached (●) and floating (○) collagen gels.
The gels were incubated for 3 weeks and diameter and volumes of gels were measured at various times. The initial cell density was 3×10^4 cells/ml.

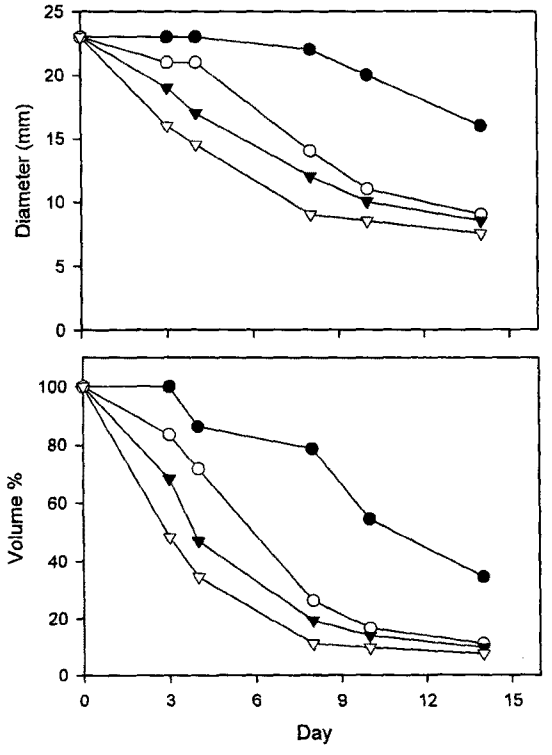

Fig. 3. Contraction profiles of floating collagen gels at various cell seeding density of 1×10^4 cells/ml (●), 5×10^4 cells/ml (○), 1×10^5 cells/ml (▼), and 5×10^5 cells/ml (▽).

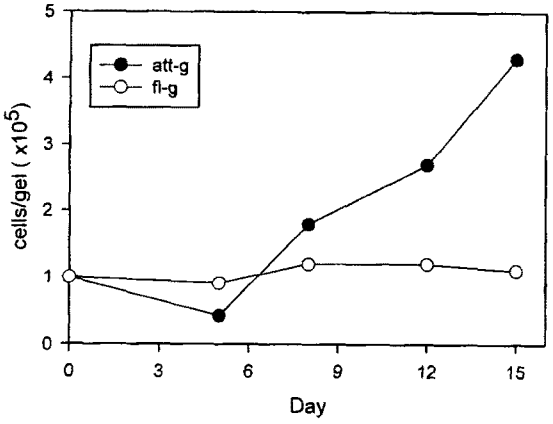

Fig. 4. Cell growth curves of contracted gels.
Collagen gels containing fibroblasts were attached (att-g) to culture dishes or floating (fl-g) in culture medium. Initial cell seeding density was 1×10^5 cells/ml.

and the volume approach a common values at concentrations above 5×10^4 cells/ml of initial cell density. But dermal equivalents (DE) with 1×10^4 cells/ml as initial cell density were contracted incompletely. During the first week of culture, the rate and degree of contraction increased as the initial cell density increased. But there was no significant increase during the second week of culture.

The cell growth curves in the attached gel or floating gel have shown in Fig. 4 at the initial cell density of 1×10^5 cells/ml. The lag period of the cell growth in the attached gel was about five or six days. This lag phase thought to be an adaptation period to new gel matrix environment. In case of the floating gel, there was no increase in cell number after the lag period. But the cell number increased up to about 4×10^5 cells/ml in case of the attached gel.

Although initial cell density was raised to 5×10^5 cells/ml, after four days of contraction, there was no increase in cell number in floating gels but cells in attached gels were increased as we can see in Fig. 5. Fibroblast growth factor has been known to modulate DNA and collagen synthesis by fibroblasts in cell culture (7). Addition of FGF promoted cell proliferation and reduced the lag period in attached collagen gels but no effect in floating gels. Accordingly the fibroblasts in the attached gel only showed the responsiveness to FGF.

Our results indicate that the method of extracellular matrix organization influenced not only cell growth but also fibroblast responsiveness to the growth factor, FGF. After contraction, collagen fibrils interacted with the cells, and then the attached collagen gels were well organized and fibroblasts had elongated to form bipolar morphology. Floating gels, however, were unorganized; the cells had deformed to show stellate morphology, and collagen fibrils were arranged randomly.

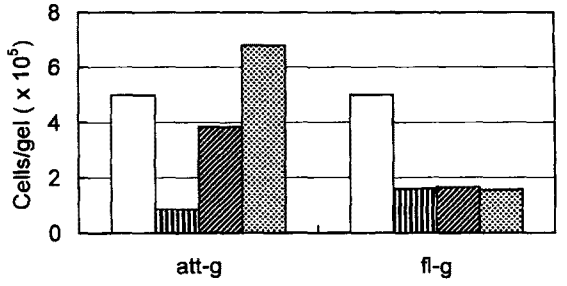

Fig. 5. Effect of FGF on cell growth in contracted collagen gels.
Fibroblasts were cultured in attached collagen gels (att-g) or floating collagen gels (fl-g) for 4 days. After two days of culture, the media were switched to medium containing 2% FBS and FGF. The initial cell density was 5×10^5 cells/ml. □, 0 day; ■, 2 day; ▣, 4 day; ▣, 4 day (+FGF).

These results suggests that attached collagen gel is more suitable as a dermal equivalent than floating gel. Another advantage of attached gel is that it gains a larger area as compared with contracted floating gel since floating gel is contracted in all directions but attached gel is contracted only vertically.

Acknowledgement

This study was supported by a grant from the Korea Science & Engineering Foundation, 1996, Project No. 961-1105-035-2.

REFERENCES

1. Bell, E. 1983. Structural integration of skin equivalents grafted to Lewis and Sprague-Dawley rats. *J. Investigative Dermatol.* **81**: 429-436.
2. Boyce, S. T. 1988. Structure of a collagen-GAG dermal skin substitute optimized for cultured human epidermal keratinocytes. *J. Biomed. Materials Res.* **22**: 939-957.
3. Brunt, J. V. 1991. Artificial organs from culture. *Bio/Technology* **9**: 136-140.
4. Dubertret, L. 1991. Activities of human acidic fibroblast growth factor in an in vitro dermal equivalent model. *J. Investigative Dermatol.* **97**: 793-798.
5. Edington, S. M. 1994. A new force in biotech.: tissue engineering. *Bio/Technology* **12**: 361-364.
6. Langer, R. and J. P. Vacanti. 1993. Tissue engineering. *Science* **260**: 920-924.
7. Nakagawa, S. and F. Ginnell. 1989. Long-term culture of fibroblasts in contracted collagen gels: Effects on cell growth and biosynthetic activity. *J. Investigative Dermatol.* **93**: 792-798.
8. Navsaria, H. A., S. R. Myers, I. M. Leigh, and I. A. Mckay. 1995. Culturing skin in vitro or wound therapy. *Trends Biotechnol.* **13**: 91-100.

9. Nusgens B., C. Merrill, C. Lapiere, and E. Bell. 1984. Biosynthesis of collagen by cells grown as a monolayer or in dermal equivalent tissues *in vitro. Coll. Rel. Res.* **4**: 351-363.
10. Parenteau, N. L. 1992. The organotypic culture of human skin keratinocytes and fibroblasts to achieve form and function. *Cytotechnology* **9**: 163-171.
11. Saiag, P. and E. Bell. 1985. Psoriatic fibroblasts induce hyperproliferation of normal keratinocytes in a skin equivalent model *in vitro. Science* **230**: 669-672.
12. Sarber, R., C. Merrill, T. Soranno, and E. Bell. 1981. Regulation of proliferation of fibroblasts of low and high population doubling levels grown in collagen lattice. *Mech Ageing Dev.* **17**: 107-117.
13. Summerlin, W. T. and M. Karasek. 1970. Transplantation of organ cultures of adult human skin. *J. Investigative Dermatol.* **55**: 310-316.
14. Wilkins, L. M. 1994. Development of a bilayered living skin construct for clinical applications. *Biotechnol. Bioeng.* **43**: 747-756.
15. Yang, E. K., J. H. Lee, T. B. Choe, and J. K. Park. 1993. Development of dermal equivalent using mouse fibroblasts. *Kor. J. Appl. Microbiol. Biotechnol.* **4**: 381-391.
16. Yuspa, S. H. 1980. Optimized conditions for the growth of human epidermal cells in culture. *J. Investigative Dermatol.* **75**: 176-182.

(Received March 18, 1997)