British Journal of Plastic Surgery (1991), **44**, 302–305

BRITISH JOURNAL OF PLASTIC SURGERY

In vitro foetal wound contraction: the effect of amniotic fluid

D. A. R. Burd*, M. T. Longaker†, T. Rittenberg*, N. S. Adzick†, M. R. Harrison†, H. P. Ehrlich*

**Wound Healing Laboratory, Shriners Burns Institute, Boston, USA, and †Fetal Treatment Program, University of California, San Francisco, USA*

SUMMARY. **This study looks at the remodelling of an *in vitro* system of foetal wound healing. Contraction is confirmed and the stimulatory role of amniotic fluid demonstrated.**

Wound contraction is a dynamic cell-matrix interaction, involving primarily fibroblasts and collagen but being controlled and influenced by a wide range of factors. It is just part of the post-natal healing process, the end result of which, though, is always going to be scarring. Foetal wound healing, however, demonstrates a phenomenal dissimilarity with postnatal healing in the scarless "repair" which can occur when wounds are allowed to heal in utero (Siebert *et al.*, 1990). In the foetus, a rapid deposition of a repair matrix occurs, which is subsequently so completely remodelled that the original tissue matrix architecture is achieved. Matrix remodelling then, would appear to be a fundamentally different process (in outcome) when foetal and post-natal wound healing are compared. This observation has led to the question of whether wound contraction (as an example of matrix remodelling), occurs in the healing of foetal excisional wounds.

In vivo studies suggest that wound contraction and indeed wound healing does not occur in excisional defects in all foetal animals. Somasundaram and Prathap (1972) looked at excisional wounds in foetal rabbits and found that when they were exposed to amniotic fluid no contraction occurred. When the wounds were protected from amniotic fluid by a sheet of Silastic, contraction and re-epithelialization did occur. Confirmation of the lack of contraction of excised rabbit wounds was made by Krummel and colleagues (1987). A further report from Hallock *et al.* (1988) indicated that excisional wounds made in foetal rats, again exposed to amniotic fluid, did not undergo wound contraction or re-epithelialization until after birth. In contrast, excisional wounds in foetal sheep do contract when exposed to amniotic fluid (Longaker *et al.*, 1990). In this paper we report an *in vitro* study of wound contraction using foetal sheep tissues and cells.

Materials and methods

Cells

Fibroblast cultures were established from explants of 3rd (3T) trimester foetal and adult (A) sheep skin. Cultures were maintained in a humidified incubator at 37°C with 5% CO_2 and 95% air. Cells were in their 8–14th passage.

Collagen

Collagen was extracted and purified from 3T and A sheep skin. Briefly, the skin was cut into small cubes and extracted for 24 hours in 0.5 M acetic acid (HAc) and pepsin (0.1 mg/10 mg wet weight of tissue) at 4°C. The extract was then centrifuged at 20,000g for 30 minutes and the pellet discarded. The supernatant was dialysed against three changes of cold 20 mM Na_2HPO_4 and centrifuged. The pellet was saved and taken up in 0.1 M HAc and stirred overnight. The solution was then spun at 20,000g for one hour and the supernatant saved. Collagen was precipitated by the addition of 10% NaCl w/v. After stirring for four hours the collagen was consolidated by spinning for 30 min and the pellet taken up in 0.1 M HAc. The collagen was desalted by dialysing (×4) against 20 volumes of 1 mM HCl. The resulting collagen solution was lyophilized and taken up in sterile 1 mM HCl at 5 mg/ml.

Medium

The medium used for the cell culture and for the manufacture of the lattices was Dulbecco's Modification of Eagle's Medium (DMEM), containing 10% (v/v) foetal bovine serum (FBS) and gentamicin at 15 μg/ml.

Amniotic fluid

Amniotic fluid was collected in sterile fashion and pooled from 80 to 85 day pregnancies (term = 145 days). It was stored at −20°C prior to use.

The model

The *in vitro* model of collagen matrix remodelling used was the fibroblast populated collagen lattice (FPCL) (Bell *et al.*, 1979). In this model, soluble collagen is mixed with cultured fibroblasts and serum rich culture medium. The collagen rapidly polymerizes, trapping

the cells within it. With time, forces produced by the cells organize and condense the collagen matrix producing local alignment of collagen fibrils. The overall effect of this cell generated organization is shrinkage of the lattice referred to as lattice contraction (Fig. 1). The rate of contraction can be assessed by measuring two diameters of the lattice at intervals and calculating the reduction in surface area.

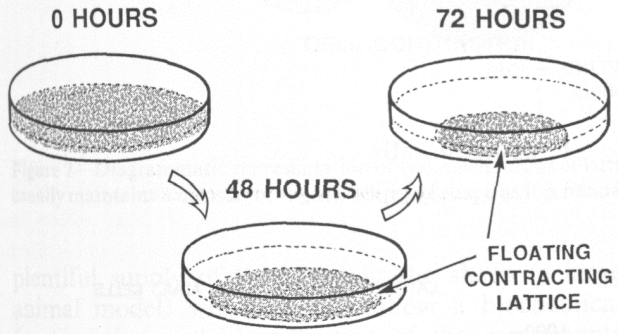


Fig. 1

Figure 1—The contraction of the collagen lattice results in a measurable reduction in surface area of the floating disc of tissue.

Experimental design

The design is shown in Figure 2. FPCL were manufactured with combinations of adult and foetal sheep cells and tissues and the rate of lattice contraction determined. The effect of amniotic fluid on lattice contraction was then studied.

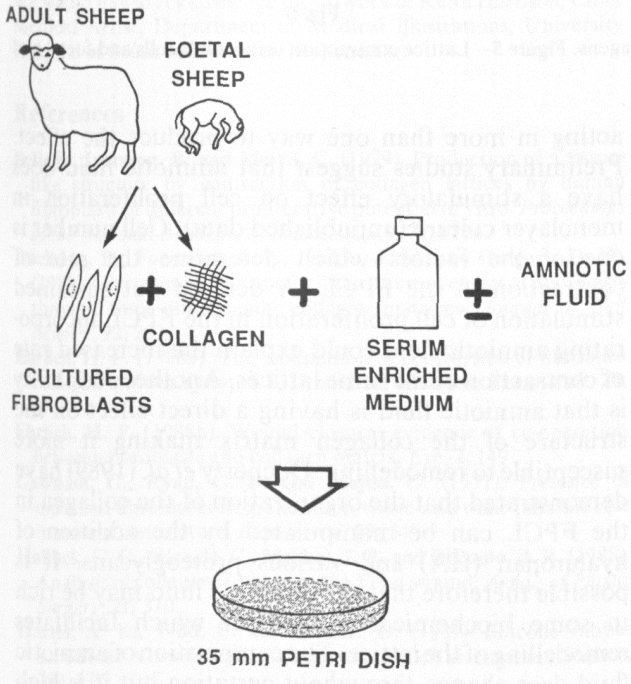


Fig. 2

Figure 2—The experimental design.

Lattice manufacture

Initial lattices were prepared combining 120,000 cells per lattice with serum, medium and collagen solution in the proportions shown in Figure 3A. The mixture was gently vortexed and poured into a sterile 35 mm diameter petri dish. The FPCL were incubated at 37°C and 12 hourly measurements were made to assess lattice contraction.

Subsequent studies with amniotic fluid used a larger total volume and 200,000 cells per lattice. The addition of 1000 µl of inverse proportions of phosphate buffered saline and amniotic fluid and a reduced amount of medium overcame any dilutional effect on the serum when adding large quantities of amniotic fluid to the system (Fig. 3B).

All studies were performed in triplicate and statistical analysis was performed using Paired Student's T-test against control.

Results

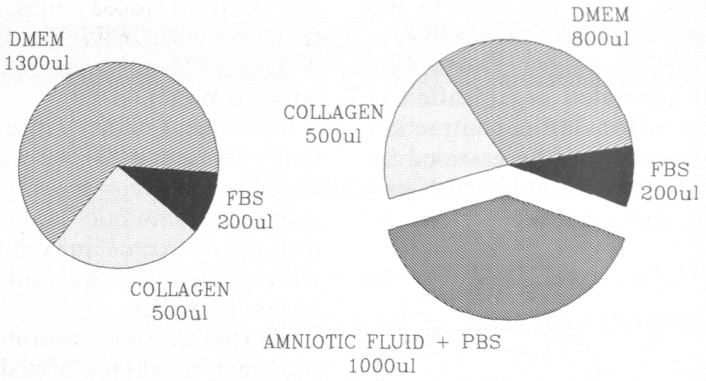
Figures 4 and 5 demonstrate the rate of contraction of lattices made from combination of 3T foetal cells and collagen and adult cells and collagen. These demonstrate that the rate of contraction of lattices made from foetal cells and tissue is similar to those made from adult cells and tissue. The foetal sheep cells, however, were more effective than the adult cells in contracting lattices made from adult collagen, while the foetal collagen was less well organized by adult cells.

Further studies (using lattice composition in Fig. 3B) were performed using 3T fibroblasts and collagen and a range of amniotic fluid from 4 to 40%. Stimulation of contraction of experimental lattices was observed. The effect was not immediate, but did become statistically significant at 96 hours (Fig. 6).

Discussion

The FPCL is a well established *in vitro* model for studying the biology of remodelling matrices. Several factors determine the rate of contraction; cell number, serum concentration, collagen concentration and collagen type (Ehrlich, 1988a). The finding that the foetal collagen was less easily remodelled than the adult collagen by both the foetal and adult cells is somewhat surprising. Foetal tissue is rich in type III collagen; FPCL made from Type III enriched adult tissue, found in uterine leiomyoma, contract faster than those made from Type I collagen. Further work is underway to investigate this finding.

There has been some controversy over the mechanism of lattice contraction. There are two major hypotheses diagrammatically represented in Figure 7. The proponents of the cell contraction hypothesis refer to the myofibroblast, a morphologically distinct tissue fibroblast originally identified by Gabbiani and colleagues in granulation tissue (1971). It is thought that these specially adapted cells, acting either independently or in unison contract whilst holding onto the collagen matrix, thereby causing the matrix to condense (Skalli and Gabbiani, 1988). The alternative hypothesis suggests that lattice contraction is brought about by tractional forces produced by fibroblasts as they move through the matrix (Ehrlich, 1988b). These



a. 120,000 cells b. 200,000 cells

Fig. 3

Figure 3—Composition of lattices.

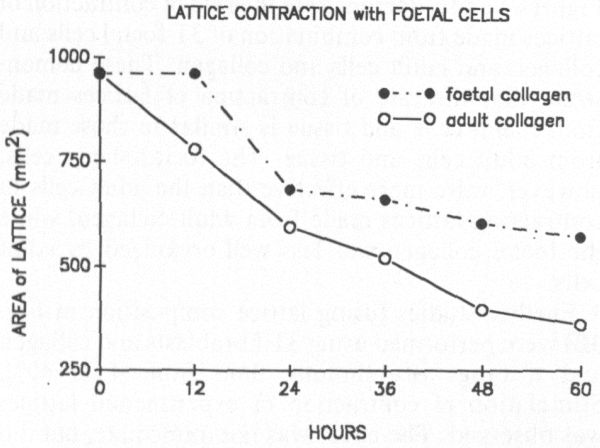


Fig. 4

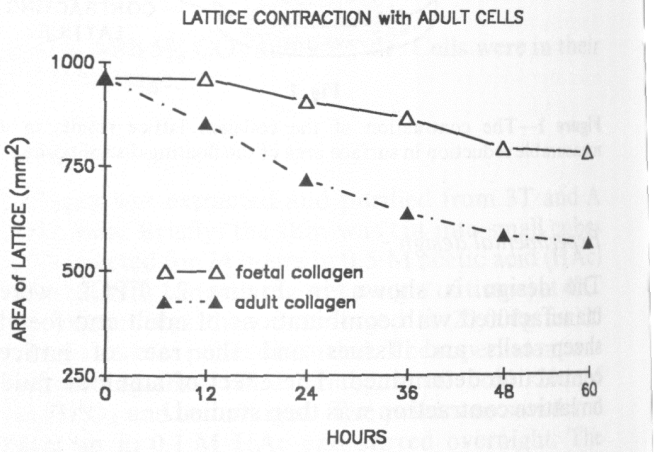


Fig. 5

Figure 4—Lattice contraction using foetal cells and foetal and adult collagens. **Figure 5**—Lattice contraction using adult cells and foetal and adult collagens.

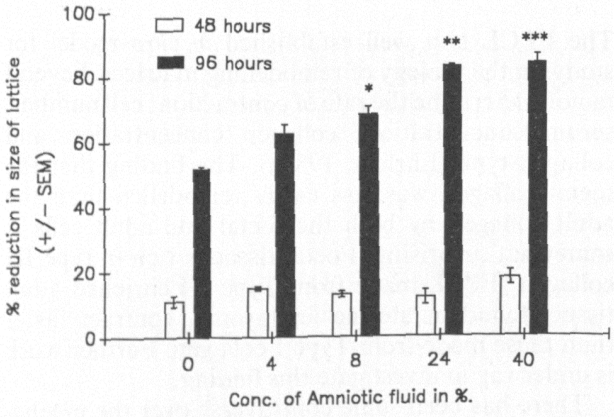


Fig. 6

Figure 6—The effect of increasing concentrations of amniotic fluid on the contraction of lattices made from 3T fibroblasts and 3T collagen (*p=0.0094, **p=0.0001, ***p=0.0025).

tractional forces have been demonstrated by Harris causing distortion of silastic films over which the cells move (Harris *et al.*, 1981; Stopack and Harris, 1982).

Whatever the case, this study demonstrates that using foetal sheep cells and tissues, matrix remodelling occurs *in vitro* and that amniotic fluid stimulates the process. The mechanism of action of the amniotic fluid as yet remains unclear and it is possible that it is acting in more than one way to produce the effect. Preliminary studies suggest that amniotic fluid does have a stimulatory effect on cell proliferation in monolayer culture (unpublished data). Cell number is one of the factors which determine the rate of contraction in the FPCL. A delayed but sustained stimulation of cell proliferation in the FPCL incorporating amniotic fluid could explain the increased rate of contraction of the same lattices. Another possibility is that amniotic fluid is having a direct effect on the structure of the collagen matrix making it more susceptible to remodelling. Docherty *et al.* (1989) have demonstrated that the organization of the collagen in the FPCL can be manipulated by the addition of hyaluronan (HA) and various proteoglycans. It is possible therefore that the amniotic fluid may be rich in some biochemical constituent which facilitates remodelling of the lattice. The composition of amniotic fluid does change throughout gestation but it is high in HA. When HA is added to FPCL an increased rate of lattice contraction has been observed possibly related to the "lubricating" effect of the HA on the collagen fibres.

Amniotic fluid has a complex composition which varies both within and between species. This variation may well determine some of the observed interspecies differences in foetal excisional wound healing. The

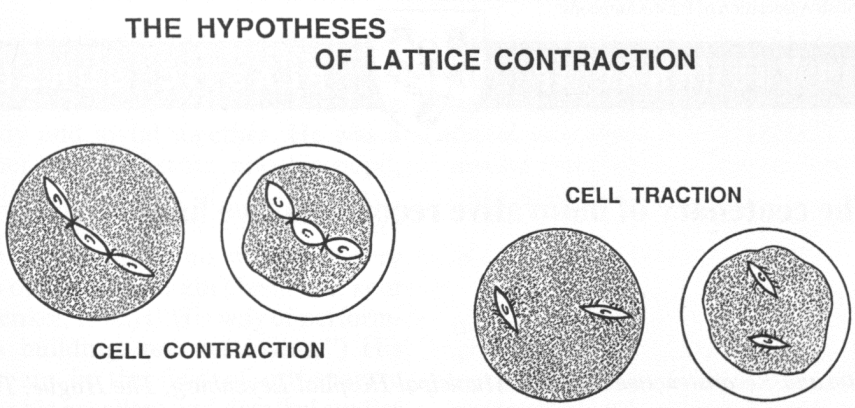


Fig. 7

Figure 7—Diagrammatic representation of two mechanisms of lattice contraction. The contracting collagen lattice floats in the medium and usually maintains a circular or slightly elliptical shape as it is remodelled by the cells.

plentiful supply of this fluid in the sheep (a large animal model) will hopefully allow a biochemical fractionation and identification of the components affecting the remodelling matrix.

This paper has focused on a highly experimental system to study a fundamental biological process. By investigating the underlying mechanisms involved in wound contraction it is hoped that new insights will be gained into manipulating the clinical problems related to contracting matrices, in particular, wound contraction, scar contracture and capsular contracture.

Acknowledgement

We would like to acknowledge the artwork of Keith Harrison, Chief Medical Artist, Department of Medical Illustrations, University Hospital of South Manchester, Manchester.

References

Bell, E., Ivarsson, B. and Merril, C. (1979). Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. *Proceedings of the National Academy of Science, USA*, **76**, 1274.

Docherty, R., Forrester, J. V., Lackie, J. M. and Gregory, D. W. (1989). Glycosaminoglycans facilitate the movement of fibroblasts through three-dimensional collagen matrices. *Journal of Cell Science*, **92**, 263.

Ehrlich, H. P. (1988a). The modulation of contraction of fibroblast collagen lattices by types I, II, and III collagen. *Tissue-Cell*, **20**, 47.

Ehrlich, H. P. (1988b). Wound closure: evidence of co-operation between fibroblasts and collagen matrix. *Eye*, **2**, 149.

Gabbiani, G., Ryan, G. B. and Majno, G. (1971). Presence of modified fibroblasts in granulation tissue and their possible role in wound contracture. *Experientia*, **27**, 549.

Hallock, G. G., Rice, D. C., Merkel, J. R. and DiPaolo, B. R. (1988). Analysis of collagen Content in the Fetal wound. *Annals of Plastic Surgery*, **21**, 310.

Harris, A. K., Wild, P. and Stopak, D. (1981). Silicone rubber substrata: A new wrinkle in the study of cell locomotion. *Science*, **208**, 177.

Krummel, T. M., Nelson, J. M., Dieglemann, R. F., Lindblad, W. J., Salzberg, A. M., Greenfield, L. J. and Cohen, I. K. (1987). Fetal response to injury in the rabbit. *Journal of Pediatric Surgery*, **22**, 640.

Longaker, M. T., Burd, D. A. R., Yen, T. S. B., Gown, A. M., Jennings, R. W., Duncan, B. W., Siebert, J. W., Harrison, M. R. and Adzick, N. S. (1990). Midgestion Excisional Wounds Contract in Utero. *Proceedings of Plastic Surgery Research Council*, **35**, 101.

Siebert, J. W., Burd, D. A. R., McCarthy, J. G., Weinzweig, J. and Ehrlich, H. P. (1990). Fetal wound healing: a Biochemical study of scarless healing. *Plastic and Reconstructive Surgery*, **85**, 495.

Skalli, O. and Gabbiani, G. (1988). The biology of the myofibroblast relationship to wound contraction and fibroconnective diseases. In Clark, R. A. F. and Henson, P. M. (Eds) *The molecular and cellular biology of wound repair*. New York: Plenum Press.

Somasundaram, K. and Prathap, K. (1972). The effect of the exclusion of amniotic fluid on intra-uterine healing of skin wounds in rabbit foetuses. *Journal of Pathology*, **107**, 127.

Stopak, D. and Harris, A. K. (1982). Connective tissue morphogenesis by fibroblast traction. *Developmental Biology*, **90**, 383.

The Authors

D. Andrew R. Burd FRCSEd, Senior Registrar, Department of Plastic Surgery, Withington Hospital, Manchester, M20 8LR, UK. Formerly, Research Fellow, Wound Healing Laboratory, Shriners Burns Institute, Boston, Massachusetts 02114, USA.

Michael T. Longaker, MD, Research Fellow, Fetal Treatment Program, University of California, San Francisco, California 94143, USA.

Toni Rittenberg MD, Research Fellow, Wound Healing Laboratory, Shriners Burns Institute.

N. Scott Adzick MD, Assistant Professor of Pediatric Surgery and Co-Director, Fetal Treatment Program, University of California.

Michael R. Harrison MD, Professor of Pediatric Surgery and Co-Director, Fetal Treatment Program, University of California.

H. Paul Ehrlich PhD, Director, Wound Healing Laboratory, Shriners Burns Institute.

Requests for reprints to Mr Burd.

Paper received 7 October 1990.
Accepted 14 December 1990.

Presented at the Summer Meeting of the British Association of Plastic Surgeons in Belfast, July 1990.