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2008-07-01

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Original Publication Citation

J.C. Dallon, H. Paul Ehrlich: A Review of Fibroblast Populated Collagen Lattices. To appear in Wound Repair and Regeneration.

BYU ScholarsArchive Citation

Dallon, J. C. and Ehrlich, Paul H., "A Review of Fibroblast Populated Collagen Lattices" (2008). *Faculty Publications*. 889.

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A Review of Fibroblast Populated Collagen Lattices

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Running Title: Fibroblast Populated Collagen Lattices

Keywords: collagen, fibroblasts, myofibroblasts, contraction, lattices

Abstract

Bell's introduction of the fibroblast populated collagen lattice (FPCL) (1) has facilitated the study of collagen-cell interactions. As a result of the numerous modifications of the casting of FPCL's, the *in vivo* applications of these *in vitro* findings has been confusing. Here experimental FPCL contraction findings are viewed in regard to three proposed mechanisms responsible for lattice contraction. The cellular mechanisms responsible for generating FPCL contraction are: cell contraction, cell tractional forces related to cell locomotion, and initial cell elongation and spreading.

Introduction

To better study the behavior of cells, a more *in vivo*-like environment was introduced by Ehrmann and Gey (2), who maintained cells on a natural collagen substrate rather than a glass surface. Elsdale and Bard (3) continued the investigations of cultured cells in a more *in vivo*-like environment by plating cultured fibroblasts on a collagen lattice. They reported “activity of motile cells within a lattice makes a disturbance, causing a gradual collapse of the lattice to a dense, opaque body less than one-tenth of the original size.” Differences are found in the physiology and morphology of fibroblasts maintained in a monolayer culture compared to fibroblasts suspended in a 3 dimensional collagen matrix. In 1979, Bell and coworkers (4) introduced the fibroblast populated collagen lattices (FPCL) as a dermal equivalent. It was the dermal component of the “skin equivalent,” a bioengineered skin graft substitute for burn patients (1). The “skin equivalent” was not successful as a skin graft for burn patients, but has had clinical related value as an *in vitro* model for the study of wound healing contraction and cell-matrix interactions. The FPCL contraction model demonstrates the ability of resident cells to reduce the volume of collagen lattices.

Since Bell’s introduction of the FPCL contraction model, many modifications in the manufacture of lattices have been reported. As a consequence of the various methods of casting FPCLs, using the model to understand fibroblast collagen interactions *in vivo* has become confusing and unclear. Here the assumption is made that there are three mechanisms for FPCL contraction: cell contraction, cell tractional forces related to locomotion, and initial cultured cell elongation and spreading. Which of the three cellular mechanisms is responsible for most of FPCL contraction depends on the method used to manufacture the lattice.

The paper is organized in the following manner. First, the differences between the two phenotypes of fibroblasts which are commonly found in FPCLs are reviewed, followed by a description of the main two lattice types which concludes the introduction. Next is a description of the three mechanisms of lattice contraction, followed by a review of the effects of soluble factors, cadherins, and integrins on FPCL contraction. The focus is on contraction in the first 48 hrs after lattices are cast or released. The paper ends with a discussion.

Searching for the mechanism of wound contraction, Majno and coworkers first identified the fibroblast phenotype, myofibroblasts in 1971 (5). Many features of the myofibroblast phenotype distinguish it from the fibroblast phenotype. Myofibroblasts, the cell icon of fibrosis, exhibit characteristics of both fibroblasts and smooth muscle cells. A major morphological characteristic of myofibroblasts is cytoplasmic stress fibers, containing the α -smooth muscle actin (α -SMA) isoform of actin (6). Fibroblast cytoplasmic microfilaments are composed of β -actin, lacking α -SMA. In addition, there are differences in the types of cadherins and integrins expressed. Cadherins are transmembrane proteins involved in cell-cell adhesion, and integrins are involved in cell-matrix adhesion. N-cadherin is expressed in fibroblasts, while OB-cadherin is expressed in myofibroblasts (7). The OB-cadherin is a feature of mesenchymal cells during embryonic development (8). The significance of these differences in cadherins expressed between fibroblasts and myofibroblasts is not clear. Both OB-cadherins (9) and N-cadherins (10)

are reported to be associated with migrating cells. The major integrin that binds fibroblasts to native collagen is $\alpha_2\beta_1$ integrin. In contrast, myofibroblasts express little $\alpha_2\beta_1$ (11). The $\alpha_2\beta_1$ integrin is reported to be critical for free floating FPCL contraction (11–14). Myofibroblasts, with stress fibers containing α -SMA, exert greater forces than the fibroblast phenotype whose stress fibers do not contain α -SMA (15). Fibroblasts initially plated at low density in monolayer culture convert to myofibroblasts upon reaching confluence (16). Polymerizing a collagen gel over a myofibroblast monolayer causes the conversion of the myofibroblasts into fibroblasts (11).

The method of casting a FPCL determines which mechanism of contraction will cause most of the contraction of the lattice as well as determines the prevalent cell phenotype in the lattice. A free-floating (FF) FPCL, referred to as a relaxed lattice, is mainly populated with fibroblasts. The relaxed FPCL introduced by Bell *et. al.* (4) is cast in Petri dishes and released soon after manufacture. Within the first 4 to 6 hrs, the freshly isolated cultured fibroblasts, having an initial spherical shape, elongate and spread within the collagen matrix. The fibroblasts retain an elongated and spread morphology as the lattice contracts. During the active process of relaxed FPCL contraction, like open wound contraction *in vivo*, there is no observable contraction of fibroblasts. In contrast, Tomasek and coworkers introduced the attached-delayed-released (ADR)-FPCL, a stressed FPCL (17). These FPCLs are cast in tissue culture dishes, where they remain attached to the underlying surface. The attached FPCLs are maintained in this attached state for 4 days. During those 4 days, the fibroblasts develop tension and differentiate into myofibroblasts. When the stressed FPCLs are released on day 4, the released FPCLs contract very rapidly, with most of the contraction being completed within 10 min. The mechanism for ADR lattice contraction, like the released of splinted open wounds in rats, is by the contraction of cells, mainly myofibroblasts (18). The stressed FPCL contains myofibroblasts like granulation tissue, but unlike the contracting full excisional open wound, the released, stressed FPCL is populated with contracted cells. This is not surprising since in contracting wounds there is never a sudden release of tension allowing the cells under tension to suddenly contract.

Mechanisms for FPCL Contraction

The main mechanism responsible for generating FPCL contraction is dependent upon the method used to cast FPCLs. All three mechanisms involve the elimination of water from between collagen fibrils, leading to the compaction of the collagen fibrils. The three mechanisms are cell contraction, cell tractional forces related to cell locomotion, and initial cultured cell elongation and spreading. Although fibroblasts are associated with all three mechanisms, because they are the initial and more motile cell type they are commonly responsible for the last two mechanisms of cell contraction. The mechanism of lattice contraction via cell contraction is more commonly associated with the myofibroblast phenotype.

Cell Contraction Mechanism

One mechanism for FPCL contraction is cell contraction. In this mechanism, a FPCL is contracted as cells, which are under tension, contract their cell bodies and thus contract the lattice immediately surrounding the cells. Although both fibroblasts and myofibroblasts can contract lattices in this manner, exposing fibroblasts to tension is one method of converting them to the myofibroblast phenotype. Thus this mechanism is typically associated with myofibroblasts.

Myofibroblasts maintain the tension along their bodies by sustained myosin ATPase activity. Myosin ATPase activity causes the sliding of actin-myosin filaments, which generates tension and cell contraction (19). Myosin ATPase activity is optimized by the phosphorylation of the serine-19 of the myosin regulatory peptide, myosin light chain (MLC). The phosphorylation of MLC serine-19 is primarily by the calmodulin dependent myosin light chain kinase (MLCK). MLC serine-19 phosphate turns over by the action of MLC phosphatase. Rho kinase phosphorylation of MLC phosphatase inhibits its phosphatase activity, maintaining MLC in a chronic or sustained phosphorylated state (20). Rho kinase has limited MLC serine-19 kinase activity and also phosphorylates MLC at serine 19. The Rho kinase activity is intracellular “location dependent”, acting in the center of the cell and absent from the cell’s periphery (20). Myofibroblast generated cell contractile forces are by sustained myosin ATPase activity. The Rho kinase activity in myofibroblasts shifts the MLC to a chronic phosphorylated state, resulting in habitual tension. The inhibition of serine-19 MLC phosphate turnover is the hallmark of sustained myosin-ATPase activity, a characteristic of myofibroblasts (21).

Simple calculations can determine if this mechanism is feasible. For instance, if one assumes that myofibroblasts contract a specific amount ($100\ \mu\text{m}$) in a one time cell contraction, a comparison of the predicted contraction and the experimental data should be of the same magnitude. Taking into consideration the cell density of each experiment but neglecting the different concentration of collagen, a predicted contraction would be 56 percent compared to the 45-50 percent observed contraction (7), 50 percent compared to 44 percent, 62 percent compared to 64 percent, 79 percent compared to 70 percent (17), and a predicted 37 percent compared to the observed contraction of 60-71 percent (22). The theoretical predictions are reasonably close to the observed values in most cases. Here the predicted change is a volume change, and the observed change is a change in area. The comparison is made without a $2/3$ power correction since in the ADR-FPCL manufacture of a lattice the resulting lattice is very thin. When the lattice is released the thickness seems to stay the same or increase.

Mechanism of Cell Locomotion caused Tractional Forces

Another mechanism for contracting FPCLs is through the tractional forces exerted on a lattice by the fibroblast’s locomotion forces. Rapid myosin ATPase activity is associated with generating cell locomotion (22). The rapid phosphorylation and dephosphorylation of MLC serine-19 requires MLCK and MLC phosphatase activities. An illustration of tractional force is the wrinkling of the surface of an elastic-silicone-thin-film (23). The mechanism for fibroblast induced

Figure 1: Schematic of the phosphorylation of the myosin light chain (20). The Rho pathways are active in myofibroblasts shifting the system to a state where there is less dephosphorylation of the myosin light chain resulting in sustained tension.

relaxed-FPCL contraction is through the compacting of collagen fibers by bundling thin collagen fibrils into thicker collagen fibers. Fibroblasts via its cell surface $\alpha_2\beta_1$ integrin attach to collagen fibrils. The integrin-collagen fibril complex is attached to the cytoplasmic microfilaments, which under myosin ATPase activity contract, pulling the integrin-collagen complex over the cell's plasma membrane. Microfilament contraction causes the translocation of collagen fibrils to become parallel with the fibroblast's membrane. Hence the organization of the collagen fibers mimics the orientation of the fibroblasts. The process promotes the compaction of collagen fibrils into long thicker collagen fibers. Polarized light optics at the periphery of the fibroblast shows the collagen fiber birefringence pattern is parallel to the cell.

Cell Elongation Mechanism

The final mechanism is that of cell elongation. The cell elongation and spreading mechanism of FPCL contraction occurs within the first 4 hrs after casting a FPCL. The monolayer tissue cultured fibroblast released by trypsin is initially a spherical cell. When it attaches and elongates within a newly polymerized collagen lattice, it generates forces that translocate the collagen

fibrils. This occurs in both moderate and high cell density lattices. In moderate cell density lattices, the forces are not great enough to continue contracting the lattice and the cell locomotion mechanism takes over. The forces generated from a high density of cells by cell elongation and spreading is great enough to cause a decrease in lattice area as well as thickness. The elongating fibroblasts pull collagen fibrils toward them, which leads to the compaction of the collagen, where water is squeezed from between the fibrils. There is neither organization of collagen nor is there any cell contraction associated with this mechanism of lattice contraction.

In the cell elongation mechanism, the published data indicates that for a 0.5 ml lattice with a density of 0.5×10^5 cells per ml the area of the lattice is reduced to 90 percent at 6 hrs and 70 percent at 8 hrs. Similarly for a lattice with 5×10^5 cells per ml the area is reduced to about 33 percent of the original lattice size in 6 to 8 hrs (24). If one considers a dimensional argument, in the high density case each cell roughly reduces a volume of $(100 \mu\text{m})^3$ to an volume of $(58 \mu\text{m})^3$. Assuming that during cell elongation cells reduce lattice volume by the same amount, the lower density case should have an area (here the $2/3$ power correction is used since volume and area reductions are being compared) of 80 percent the original in 6 to 8 hrs. This is consistent with the data. One would expect the cell elongation to take 4 to 8 hrs which is again consistent with the data. In experiments by Stopack and Harris, fibroblasts wrinkled silicon substrates after elongating but not in the process of elongation (23). This would suggest that the forces involved in cell elongation are weaker than those in cell locomotion and less effective in contracting lattices. Since the cells would be reaching out in all directions and drawing the collagen inward, in cell elongation, the collagen would not be organized, which is the case.

Effects of Soluble Factors

A healing wound contains a plethora of cytokines and other soluble factors, which influence fibroblast behavior. Affecting wound contraction via the introduction of soluble agents is an approach for elucidating the mechanism for wound contraction. An agent that inhibits FPCL contraction is expected to inhibit or promote wound contraction *in vivo*. A summary of agents that affect FPCL contraction are listed in Table 1.

The disruption of cytoskeletal structures and/or cell locomotion affects FPCL contraction. Cytochalasin B disrupts microfilaments (25) and colchicine disrupts microtubules. Both these agents inhibit relaxed-FPCL contraction (4). Cytochalasin B blocks lattice contraction with high cell density lattices, which is dependent upon a mechanism of cell elongation and spreading (24, 26). Colchicine disruption of microtubules inhibits directional fibroblast locomotion (27). Unlike cytochalasin, colchicine does not affect the high-density, cell elongation mechanism of lattice contraction (28). High-density FPCL contraction that requires only cell elongation as the mechanism for lattice contraction is susceptible to the disruption of microfilaments, but not the disruption of microtubules.

Agents that alter the phosphorylation state of MLC at serine-19 will influence myosin AT-

Pase activity, which is required for lattice contraction via cell locomotion as well as the cell contraction mechanism. Increasing intracellular concentrations of cyclic adenosine monophosphate (cAMP) promote Protein Kinase A that phosphorylates MLCK. The phosphorylation of MLCK inhibits its kinase activity which prevents the phosphorylation of MLC at serine-19 (29). Phosphorylated MLCK can not bind to calcium-saturated-calmodulin, which is required for optimizing MLCK activity. In the absence of MLCK activity there is reduced myosin ATPase activity, which retards cell locomotion, but not sustained myosin ATPase activity or cell elongation (22, 24). Prostaglandin E2 (PGE2), forskolin, cholera toxin, and pentoxifylline (PTX) increase the intracellular concentration of cAMP, which affects cell motility and lattice contraction. PGE2 as well as forskolin and cholera toxin inhibits relaxed-FPCL contraction (30–32). Addition of dibutyryl cAMP, which is a form of cAMP that can pass through cell plasma membranes and accumulate within the cell, raises intracellular cAMP levels, leading to inhibited relaxed-FPCL contraction (31, 33). PTX, a nonspecific phosphodiesterase inhibitor, increases intracellular cAMP levels and inhibits relaxed-FPCL contraction (34–37). Elevated cytoplasmic levels of cAMP that inhibit MLCK phosphorylation and reduce myosin ATPase activity can be overcome by directly introducing, by electroporation, calcium-calmodulin-independent-MLCK protein into fibroblasts prior to their incorporation into relaxed-FPCL. Such relaxed-FPCL do not require calcium-saturated calmodulin binding for generating myosin ATPase activity and demonstrate normal lattice contraction (32).

Levels of tyrosine phosphorylation affect FPCL contraction. Genistein, a tyrosine kinase inhibitor (38), inhibits relaxed-FPCL contraction but does not stressed-FPCL contraction (22). Genistein is also effective at inhibiting relaxed high cell density FPCL contraction (39) which suggests that tyrosine phosphorylation is important for cell spreading and elongation. Tyrosine phosphorylation affects smooth muscle contraction (40, 41), the formation of cytoplasmic stress fibers, and focal adhesions (42). Vanadate blocks protein tyrosine phosphatases, leading to the retention of phosphorylated tyrosine in proteins (43). Vanadate inhibits stressed-FPCL contraction, but does not inhibit relaxed-FPCL or high cell density FPCL contraction (39). In fibroblast cultures vanadate prevents the formation of stress fibers, the expression of α -SMA and the transformation of fibroblasts into myofibroblasts. Vanadate treated stressed-FPCL do not contain myofibroblasts, which explains their failure to undergo lattice contraction through a cell contraction mechanism. The relaxed-FPCL, which contracts through fibroblast generated tractional forces contain cells that lack stress fibers with α -SMA, will undergo contraction in the presence of vanadate. Rats topically or systemically treated with vanadate show normal wound contraction in the absence of myofibroblasts (44, 45). The granulation tissue fibroblasts from vanadate treated open wounds do not have stress fibers or express α -SMA (44).

Soluble agents that block gap junction intercellular communications (GJIC) like heptanol, octanol and endosulfan inhibit relaxed-FPCL contraction (24, 28). Endosulfan inhibits GJIC, possibly by disrupting the normal phosphorylation activity associated with gap junction formation (46). These GJIC uncouplers have minimal effect upon cell elongation and do not change high cell density FPCL contraction (24). Fibroblasts derived from mice, who fail to express connexin 43, the protein that makes up the gap junction structure, show a limited capacity of

contracting relaxed-FPCL (28).

TGF β_1 and TGF β_2 promote relaxed-FPCL contraction (47, 48). Tamoxifen, a synthetic non steroidal anti-estrogen agent, which inhibits TGF β activity, inhibits relaxed-FPCL contraction (49). Peroxynitrite increases the synthesis of TGF β by fibroblasts. When included in the casting of relaxed-FPCLs, there was enhanced lattice contraction (50). The mechanism for TGF β enhancement of lattice contraction is not clear with relaxed-FPCL since they undergo lattice contraction in the absence of myofibroblasts or α -SMA expression (6).

Bell and coworker showed that cell proliferation was not linked to enhanced relaxed-FPCL contraction (4) in the first 48 hrs. Cytosine arabinoside (Ara-C) inhibits DNA synthesis, stopping cell proliferation and promoting cell entrance into apoptosis (51). Low concentrations of Ara-C do not affect relaxed-FPCL contraction (4).

The growth factor PDGF enhances the contraction of high cell density FPCLs, which supports the notion that PDGF promotes cell elongation. PDGF does not affect the contraction of relaxed-FPCLs with moderate cell density levels (24, 52).

Agents that affect cell metabolism have been examined in lattice contraction studies (53). Neither antimycin A or dinitrophenol, which block aerobic metabolism show any affect on relaxed-FPCL contraction, when glucose was available. Both of these inhibit aerobic metabolism of glucose. The energy needed for lattice contraction was made available by anaerobic metabolism. If culture medium had pyruvate substituted for glucose, antimycin A was effective at blocking relaxed-FPCL contraction. Likewise, iodoacetamide, a substance that blocks anaerobic metabolism, inhibits relaxed-FPCL contraction. This suggests that aerobic metabolism is not necessary for lattice contraction, where relaxed-FPCL contraction proceeds by the energy provided by anaerobic metabolism.

Modulation of calmodulin dependent reactions will alter relaxed-FPCL contraction. Calcium regulates the myosin ATPase activity via saturating calmodulin, which promotes optimal MLCK phosphorylation of MLC. When relaxed-FPCLs are treated with verapamil or nifedipine, calcium ion channel blockers (54), lattice contraction is inhibited (53). Similarly W-7, a specific inhibitor of calmodulin-dependent reactions (55), blocks relaxed-FPCL contraction (53). MLCK dependent myosin ATPase activity is crucial for relaxed-FPCL contraction.

It is reported that at seven days, matrix metalloproteinases affect lattice contraction (56). There is no information about these proteinases affecting lattice contraction at 48 hrs.

Effects of Cadherins and Integrins

Experimental data show integrins are critical for lattice contraction, where relaxed-FPCLs treated with a monoclonal antibody to $\alpha_2\beta_1$ integrin show inhibited lattice contraction. However, with high cell density populated FPCLs the $\alpha_2\beta_1$ integrin does not play a roll in lattice contraction (14, 24). The presence of the $\alpha_2\beta_1$ integrin is important for generation of tractional forces but does not play a role in the elongation of newly introduced fibroblasts into a collagen lattice. Antibodies to $\alpha_1\beta_1$, $\alpha_5\beta_1$, and $\alpha_3\beta_1$ integrins do not affect relaxed-FPCL contraction. An

antibody to the β_1 integrin protein retards relaxed-FPCL contraction, which shows the β_1 integrin protein requires a specific α integrin protein partner. The $\alpha_2\beta_1$ integrin contains both α_2 and β_1 integrin proteins, which is the critical combination for relaxed-FPCL contraction, while other α integrin proteins do not partner with the β integrin protein to promote relaxed-FPCL contraction.

Cell-cell adhesions typically involve calcium dependent cadherin-cadherin interactions. The N-cadherin is the major cadherin isoform in fibroblasts, while OB cadherin is the prevalent isoform in myofibroblasts. Stressed-FPCL treated with an antibody direct to OB-cadherin show impaired lattice contraction, while N-cadherin treated antibody stressed-FPCLs contract normally (7). These experiments show cell-cell contacts are important for lattices contracted by the cell contraction mechanism, but cadherins are not critical for lattices, involving a tractional force mechanism.

Discussion

The evidence indicates that FPCL contraction has multiple mechanisms, which may or may not be related to open wound contraction. The mechanism for lattice contraction seems to be dependent upon the method used in the manufacture of the lattice. This paper reviews some of the experimental data that demonstrate the 3 mechanisms responsible for lattice contraction: cell contraction, cell locomotion and cell elongation. Stressed-FPCLs contract through a cell contraction mechanism. Relaxed-FPCLs having a moderate cell density contract through a tractional force mechanism that is related to cell locomotion. Relaxed-FPCLs with a high cell density contract through a cell elongation and spreading mechanism. Fibroblast spreading and elongation is involved in all FPCL contraction studies. However, the forces generated by cell elongation are weak compared to the forces of cell contraction and cell locomotion. Because cells are initially rounded when cast into a collagen lattice, it is necessary for them to elongate and spread out within the collagen matrix. High concentrations of fibroblasts undergoing cell elongation are necessary to generate enough force to cause lattice contraction. The forces of cell contraction or cell locomotion by tractional forces are great enough that fewer cells are needed to produce lattice contraction. In stressed-FPCL contraction, the change in lattice area is rapid completed in minutes. Likewise, the contraction of densely populated FPCL is rapid completed within hours. The contraction of relaxed-FPCL with a moderate cell density is slow and completed in days. Wound contraction of a full thickness wound is a slow process, usually completed in weeks.

One must be careful in applying the results of the findings from *in vitro* experiments to *in vivo* clinical problems. Examples are the findings that PDGF promoted wound contraction and vanadate will inhibit wound contraction. PDGF promotes open wound closure by advancing reepithelialization, not through wound contraction (57). The closure of full thickness wounds in vanadate treated rats is identical to wound contraction in untreated rats. Vanadate inhibits stressed-FPCL contraction but does not interfere with relaxed-FPCL contraction. In the closure

Table 1: Effect of Drugs on Fibroblast Lattice Contraction

Drug	Details	Contraction	Effect on Cells	Mechanism	Reference
Cytochalasin B	Relaxed	none	blocks actin filaments growth	reduces motility	(4,24,26)
Vinblastine	Relaxed	none	blocks actin filament growth	reduces motility	(53)
Colchicine	Relaxed	less	disrupts microtubule formation	reduces motility	(28)
Colcemid	Relaxed	less	disrupts microtubule formation	reduces motility	(4,26)
Prostaglandin E2 (PGE2)	Relaxed	less	increases cAMP	reduces motility	(30)
Forskolin	Relaxed	less	activates adenylate cyclase	reduces motility	(30–32)
dibutyryl cAMP	Relaxed	less	increases cAMP	reduces motility	(31,33)
Cholera	Relaxed	less	activates adenylate cyclase	reduces motility	(31,32)
Pentoxifylline (PTX)	⁺ Relaxed-Burn Relaxed	less less	inhibits phosphodiesterase	reduces motility	(34,36) (35)
Iodoacetamide	Relaxed	none	blocks anaerobic metabolism		(53)
Verapimal	Relaxed	less	blocks calcium ion channel	reduces motility	(53)
Nifedipine	Relaxed	less	blocks calcium ion channel	reduces motility	(53)
W-7	Relaxed	none	inhibits calmodulin	reduces motility	(53)
Endosulfan	Relaxed	less	disrupts GJIC		(24)
Heptonal	Relaxed	less	disrupt GJIC		(28)
Octonal	Relaxed	less	disrupt GJIC		(28)

⁺ 300,000 cells per ml or more; ⁻ 30,000 cells per ml or less; no marking other cell densities.

Table 2: Effect of Drugs on Fibroblast Lattice Contraction Cont.

Drug	Details	Contraction	Effect on Cells	Mechanism	Reference
Vanadate	Relaxed Stressed	same less	inhibits tyrosine phosphatase	inhibits myofibroblasts formation	(22)
Genistein	Relaxed +Relaxed Stressed	less less same	inhibits tyrosine kinases		(22, 24) (39) (22)
TGF β_2	Relaxed-Carpal	more	various	increases myofibroblasts	(48)
	Relaxed- Dupuytren's	more	various		
Tamoxifen	+Relaxed-Carpal	less	various, decreases TGF β	decreases myofibroblasts	(49)
	+Relaxed- Dupuytren's	less	various		
peroxynitrite	+Relaxed	more	various, increases TGF β_1	increases myofibroblasts	(50)
Ara-C	Relaxed	same	inhibits prolifera- tion		(4)
PDGF	+Relaxed Relaxed	more none			(28, 52) (28)
Anti-CD49b	+Relaxed Relaxed	same less			(28)
Anti-OB-cadherin	Stressed without TGF β (fibroblasts)	same	binds to OB-cadherin		(7)
	Stressed with TGF β (myofibroblasts)	less			
Anti-N-cadherin	Stressed without TGF β (fibroblasts)	same	binds to N-cadherin		(7)
	Stressed with TGF β (myofibroblasts)	same			

⁺ 300,000 cells per ml or more; ⁻ 30,000 cells per ml or less; no marking other cell densities.

of an open wound by wound contraction, tension within granulation tissue is a result of the resistance of the skin musculature surrounding the defect pulling the wound open. The tension causes the generation of myofibroblasts to resist those forces. The myofibroblasts stabilize the forces pulling the wound open. There are several plausible scenarios for what happens next. Once the forces are neutralized, the remaining resident fibroblasts could remodel the extracellular matrix as they move, causing the wound contraction. Or as more fibroblasts become myofibroblasts the forces produced could cause a gradual contraction of the wound via partial cell contraction. Either of these proposed scenarios could occur without myofibroblasts and the experiments with vanadate treated rats indicate that the extra force associated with myofibroblasts is not necessary for wound contraction.

The stressed-FPCL contraction model where myofibroblasts are required for lattice contraction is not representative of open wound contraction where there is never a sudden release of tension. However, the stressed-FPCL contraction model is representative of the contraction of the released splinted wound, where the sudden release of tension allows the contraction of the cell body (58). More insight into the mechanisms for FPCL contraction and how it relates to open wound closure will lead to a better understanding of wound contraction.

Acknowledgments

This work was supported in part by NSF grant DMS-0622971.

Abbreviations

α -SMA – α smooth muscle actin

ADR-FPCL – attached delayed release fibroblast populated collagen lattice

cAMP – cyclic adenosine monophosphate

FF-FPCL – free floating fibroblast populated collagen lattice

FPLC – Fibroblast populated collagen lattice

GJIC – gap junction intercellular communications

MLC – myosin light chain

MLCK – myosin light chain kinase

PDGF – platelet derived growth factor

PGE2 – prostaglandin E2

PTX – pentoxifylline

TGF β – transforming growth factor β

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