Collagen matrix as a tool in studying fibroblastic cell behavior

Jiří Kanta*

Department of Medical Biochemistry; Medical Faculty in Hradec Králové; Charles University; Prague, Czech Republic

Keywords: cell culture, cell proliferation, collagen, extracellular matrix, fibroblasts, fibronectin, integrins, metalloproteinases, myofibroblasts, substrate stiffness

Abbreviations: AP-1, activator protein 1; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GT, granulation tissue; HSC, hepatic stellate cells; JNK, c-Jun N-terminal kinase; MFB, myofibroblasts; MKL1, megakaryoblastic leukemia 1; MMP, metalloproteinases; NF-κB, nuclear factor kappa B; PI3K/Akt, phosphatidylinositol 3-kinase/Akt; TIMP, tissue inhibitor of metalloproteinases; TNF-α, tumor necrosis factor α.

Type I collagen is a fibrillar protein, a member of a large family of collagen proteins. It is present in most body tissues, usually in combination with other collagens and other components of extracellular matrix. Its synthesis is increased in various pathological situations, in healing wounds, in fibrotic tissues and in many tumors. After extraction from collagen-rich tissues it is widely used in studies of cell behavior, especially those of fibroblasts and myofibroblasts. Cells cultured in a classical way, on planar plastic dishes, lack the third dimension that is characteristic of body tissues. Collagen I forms gel at neutral pH and may become a basis of a 3D matrix that better mimics conditions in tissue than plastic dishes.

Introduction

Cells in a tissue are surrounded with other cells and with ECM, which is a network containing proteins, glycoproteins, proteoglycans and glycosaminoglycans. ECM provides chemical and mechanical signals whose effects are interdependent. Chemical signals may originate in the chemical structure of ECM components or may be provided by cytokines and growth factors stored in the ECM and released under certain circumstances. ECM is more pliable than hard plastic surface and its mechanical properties contribute to diversity of physiological and pathological situations in the tissue. ECM is degradable and cells can migrate through it.1,2 Cell culture on thin collagen film covering plastic substrate is useful in studies of some aspects of cell behavior, e.g. interaction with integrins,3 but the contact with 3D environment makes the cells in tissue behave differently than the cells in conventional tissue culture on stiff plastic dishes do, as far as their morphology, differentiation, migration, and proliferation is concerned. The cells surrounded with an appropriate scaffold, usually collageneous, acquire tissue-like phenotype not observed in cells in monolayer. Mechanical signals from the ECM to the cells and contractile forces to the ECM are transmitted by protein complexes called focal adhesions. Three-dimensional matrix adhesions differ from adhesions on 2D substrates in protein composition and biological activity.4 Force applied to integrins is transmitted through focal adhesions to the cytoskeleton.5 The cells may integrate global signals coming from the entire surface and sense the spatial organization of activated adhesions.6

Fibroblasts and myofibroblasts are cells involved in the healing of various tissues. Fibroblasts in the tissue surrounding the wound are activated and migrate into the provisional matrix containing fibrin and plasma fibronectin. Fibrin is a major component of the provisional matrix formed during wound healing and enables migration of inflammatory cells and fibroblasts. Collagen production becomes the main fibroblast function and the provisional matrix is gradually replaced with a collagenous ECM.7-9 A part of fibroblasts may differentiate to protomyofibroblasts and further to MFB characterized by prominent stress fibers that contain α-SMA associated with non-muscle myosin. These proteins endow MFB with high contractional force that is combined with synthetic abilities of the MFB.10,11 The wound contracts and the provisional matrix is replaced with GT that is gradually converted to a scar. Most cells then die by apoptosis. However, the reparative process may be dysregulated and result in fibrosis. The most abundant ECM component providing a scaffold that binds other proteins and proteoglycans is collagen I.12,13

Solid tumors contain stroma that resembles GT in many aspects. The stroma is highly vascularized. Fibrin is formed by clotting extravasated fibrinogen and together with other plasma proteins it gives rise to a provisional matrix. Fibroblasts settle in the matrix and produce collagen I and other ECM components. Cancer-associated fibroblasts promote the growth of cancer cells and vice versa they respond to signals from epithelial cells by increased synthesis of collagen and other fibrogenic factors.14,15

Three-dimensional matrices used as a model to study cell behavior in the tissue-like environment in normal and pathological situations are therefore often made of collagen.2,16 Fibrin gels can also be used to provide 3D environment for cells but they
have much smaller influence on cell behavior and their effects may be opposite to those of collagen.\textsuperscript{17,18}

Various models of collagen matrix aiming to mimic in vivo situations will be discussed in this review. Fibroblastic cells transferred from plastic to collagen gel change their morphology and functions and in response they modify their environment. Secretion of MMP by the cells has a particular role in these interactions. The contact of cells with collagen is mediated by specific receptors, integrins. Plasma fibronectin interacts both with collagen and cells in vivo and it is therefore often added to collagen matrix in vitro.

**Collagen Matrix Models**

Rodent tail tendons contain almost pure collagen I that can be extracted with diluted acids.\textsuperscript{19–21} Collagen forms gel when its solution is neutralized. The concentration of 1 to 2 mg collagen/ml is frequently used to form matrix. Collagen contained in bovine skin is more crosslinked and its extraction requires the use of pepsin. This enzyme cleaves off telopeptides, the nonhelical amino acid sequences at the C- and N-ends of the collagen molecule. The lack of telopeptides may interfere with gel formation and change the properties of the gel. The pores in gel made of acid-extracted rat tail collagen are 1–2 \( \mu \text{m} \) in diameter. The pores in the gel from pepsin-digested collagen are larger and allow easier migration of the cells.\textsuperscript{16} Cells can be seeded on top of the gel or be suspended in collagen solution. The cells on collagen may be covered with a second layer of collagen gel to add the cells the third dimension. Fibroblasts placed between 2 collagen layers migrate into them.\textsuperscript{22,23}

Fibroblasts cultured for a few weeks in the presence of L-ascorbic acid 2-phosphate form a multilayered structure surrounded by hydroxyproline-rich ECM.\textsuperscript{24} The self-produced dermis-like structure formed in the presence of ascorbic acid in a long term culture contains collagens I and VI.\textsuperscript{25}

Fibroblasts embedded in collagen gel cause its contraction. When collagen gel formed in tissue culture dishes remains attached to the walls of the dish, fibroblasts can contract it only in the vertical direction. When the gel is detached from the dish immediately after gelation, it floats in the culture medium and contracts in all directions. The resulting matrix is called floating. The gel may be maintained under tension for 1 or more days before it is detached. This type of matrix is called stressed or stress-released. Contraction of floating collagen matrices gives rise to mechanically relaxed tissue resembling dermis, attached matrices resemble granulation tissue.\textsuperscript{26–28} The shape of liver MFB growing on hard plastic and on collagen gels is shown in Figures 1–3. The cells respond to changing stiffness and tension of the substrate.

Collagen properties can be modified by crosslinking collagen molecules and fibrils. Collagen I is a substrate of transglutaminase that introduces \( e-(\gamma- \text{glutamyl}) \) lysine cross-links into its molecule at 37\(^\circ\)C.\textsuperscript{29,30} Fibroblast attachment, spreading and proliferation is enhanced on collagen polymerized as a result of transglutaminase treatment.\textsuperscript{31} Collagen can also be crosslinked by 0.2% glutaraldehyde and used as a matrix for cell culture. No toxicity to fibroblasts is observed when collagen is treated with this glutaraldehyde concentration.\textsuperscript{32}

Mechanical properties of the matrix play a significant role in determining cell behavior. The stiffness of isotropic material is characterized by Young’s modulus (elastic modulus). Its unit is Pascal (Pa). The stiffness of soft tissues is low; the stiffness of liver is 0.1 to 1 kPa, dermis 1–5 kPa and fibrotic tissues 20 to 100 kPa. Young’s modulus of the provisional matrix in healing wounds (0.01 to 0.1 kPa) is comparable to that of collagen gel.\textsuperscript{33} Collagen fibrils in 0.2–2.0\% gel are similar to those in tumor ECM.\textsuperscript{34}

Collagen stiffness can be increased when a portion of liquid is removed by ultracentrifugation,\textsuperscript{35} gel compression\textsuperscript{35} or by evaporating the solvent.\textsuperscript{36} Fibroblasts seeded in matrix containing 20 or 40 mg collagen/ml are viable, migrate and proliferate. They reach a density similar to that found in human dermis.\textsuperscript{36,37}

The differences between cell growth on hard plastic surface and on soft collagen gel suggest that the rigidity of the substrate plays a crucial role. Cells can be placed between 2 sheets of collagen-coated polyacrylamide gel. The stiffness of polyacrylamide gel can be controlled by changing the percentage of the crosslinking compound, bis-acrylamide, in the reaction mixture and can be adjusted to correspond to the physiological stiffness of tissues. This treatment makes fibroblasts that are well spread in 2D culture change their morphology into bipolar or stellate
characteristic of fibroblasts in vivo. Increased rigidity affects not only stress fibers formation but also integrin expression on the cell surface. Polyethylene glycol (PEG) can be covalently bonded to collagenous matrix extracted from porcine heart. PEG gels retain fibrillar structure, are more resistant to enzymatic degradation and do not inhibit metabolic activity of incorporated fibroblasts.

### Interaction of Fibroblasts with Collagen Gel

The cells can be plated on the surface of collagen gel or incorporated into it to form a tissue-like structure. Fibroblasts are rounded when they are embedded into collagen gel; they adopt stellate morphology within a few hours and they are spindle-shaped later. Fibroblast and MFB morphology in 3D matrix is comparable to that in their original tissue but much different from polygonal appearance they adopt on a planar substratum. Fibroblasts on collagen gel aggregate; this tendency decreases with increasing collagen concentration.

Fibroblasts embedded in collagen remodel surrounding matrix. They have few cell adhesions on their surface but they produce dendritic extensions that interact with collagen fibrils. Fibroblasts align the flexible collagen meshwork around themselves and hold collagen fibrils in place. The fibrils are then stabilized by noncovalent interactions that do not require cell presence. Fibroblasts in attached matrices develop isometric tension. The forces they generate do not depend on the stiffness of the substrate. The forces produced by fibroblasts not expressing α-SMA increase rapidly within the first 6 hours after embedding the cells into collagen.

When collagen matrix is attached to the walls of the culture dish, distinct actin stress fibers that develop in fibroblasts can be visualized by phalloidin staining. When fibroblasts are embedded in collagen layer cast on polyacrylamide gels, actin fibers staining with rhodamine phalloidin appear if Young’s modulus is adjusted to 1.6 to 3.6 kPa. Stress fibers formation is facilitated by cell-cell contact. Direct linkage of the cytoskeleton stress fibers mediated by cell surface cadherins maintains tension between neighboring cells.
cells reaches a critical level, α-SMA that is at first diffusely distributed in the cytosol is incorporated to preexisting β-actin-containing stress fibers. Transcription factor MKL1 attached to globular actin (G-actin) is released after G-actin polymerization and translocated to the nucleus. It binds to the α-SMA gene promoter and initiates α-SMA expression. Matrix stiffening also activates the small GTPase RhoA and Rho kinase (ROCK) that control the balance between polymerized and depolymerized actin. These changes correspond to the increasing tension in the forming GT in healing wounds. Cytoplasmic actin microfilament system containing α-SMA characterizes MFB.

Profibrogenic cytokine TGF-β1 and smad signaling are involved in gel contraction by fibroblasts derived from normal skin or hypertrophic scars. TGF-β-pretreated fibroblasts cause significantly more rapid gel contraction. The degree of substrate stiffness determined by underlying polyacrylamide gel modulates TGF-β-induced transdifferentiation of fibroblasts. Both substrate stiffness and the presence of TGF-β are required for the differentiation of liver portal fibroblasts to MFB.

Generation of the threshold tension necessary for α-SMA incorporation requires formation of large, „supernature“ adhesion sites. Contractility of both muscles and non-muscle cells is dependent on the interaction of actin and myosin. Non-muscle myosin II is closely associated with actin stress fibers in cochlear fibrocytes and the contraction of collagen matrix can be prevented by an inhibitor of myosin II function. Aging dermal fibroblasts lose the ability of force generation in collagen gel which may be caused by decreased expression of myosin light chain kinase and Rho kinase.

Increasing collagen concentration supports cell proliferation and suppresses apoptosis. Fibroblasts migrate along collagen concentration gradient to the stiffer regions of a collagen construct. This effect is called durotaxis. α-SMA-positive MFB appear in wound GT when Young’s modulus is about 20 kPa. The stiffness of collagen matrices containing 1–2 mg collagen is about 50 Pa and the stiffness of plastic used in tissue culture is about 1 GPa.

Fibroblasts, MFB and other cells involved in wound healing are affected by intrinsic forces produced by the ECM and extracellular fluid. The final outcome of ECM remodeling is determined both by tissue stiffness and by mechanical loading of the tissue, i.e. the force applied to tissue. Mechanical forces influence both cell proliferation and gene expression. Prolonged mechanical loading may result in higher tissue stiffness.

Most cells grow only if their surfaces are attached to the ECM. The attachment of cells to ECM molecules is mediated by integrins. These receptors consisting of subunits α and β link ECM with the actin cytoskeleton and transmit signals from the outside to the cell and vice versa. Integrins α1β1, α2β1, α10β1 and α11β1 are collagen receptors. The engagement of integrins leads to the activation of signaling cascades, focal adhesion kinase (FAK), extracellular signal-regulated protein kinase (ERK) and Rho GTPases. Dscoidin domain receptors (DDR) 1 and 2 represent another family of cell-surface receptors. They are activated by collagen and regulate cell proliferation and ECM synthesis. They are expressed on fibroblasts in healing wounds and in tumors.

“Synthetic” Phenotype of Fibroblasts in Mechanically Stressed Collagen Matrices

Attached matrix

Physiological levels of tissue stiffness function as a brake on fibroblast proliferation and collagen I synthesis. Fibrotic diseases are accompanied by tissue stiffening which is no longer regarded as a mere consequence of the disease; it has become clear that it may drive the whole process. Fibroblasts switch between proliferative and quiescence phenotypes. Fibroblasts in attached gels assume a “synthetic” phenotype. They proliferate and synthesize collagen. The number of cells in attached gels increases rapidly while the culture in floating gels regresses. However, the cells remaining in the floating gels are viable and divide at the same rate when they return to standard culture conditions. Fibroblasts in attached gel are bipolar, fibroblasts in floating gel are stellate. DNA synthesis measured by 3H-thymidine incorporation into DNA is almost one order of magnitude higher in the cells on plastic than in the cells in attached matrix and about 2 orders higher than in the cells in floating matrix. Fibroblast proliferation is proportional to collagen concentration in the matrix when the gel is compressed and its Young’s modulus increased. DNA synthesis in attached gel is dependent on the ERK pathway. This signaling pathway is disrupted when the gels are released. The cytoskeleton is then disorganized and DNA synthesis is inhibited. However, these 2 events are independent because only DNA synthesis in adhering gel is affected by an ERK inhibitor.

The release of attached matrix from the walls of the culture dish 24 hours after casting, (stress-released matrix), induces secretion of cytokines IL-6 and IL-8 by the embedded fibroblasts. The response of cells to TGF-β1 and TNF-α changes, growth inhibition is less severe in stress-released matrix than in the attached one. The signaling networks that include these cytokines are modified.

Collagen synthesis is downregulated on both RNA and protein levels when fibroblasts are transferred from plastic to 3D collagen matrix. Both total protein synthesis and collagen synthesis measured by 3H-proline incorporation is several times higher in attached gels than in floating gels. The expression of fibrillar collagen I and III in liver portal fibroblasts increases on a stiff substrate in parallel with α-SMA, while the expression of net-forming collagen IV decreases. Tensile strength also controls the expression of collagen type XIII that is associated with collagen fibrils. Collagen α1(I) mRNA synthesis and steady-state level is decreased in fibroblasts transferred from plastic into collagen gel. No change is observed in the expression of fibronectin mRNA. Total protein synthesis and collagen synthesis are high in the cells on plastic and in attached fibrin gel but low in floating collagen and fibrin gels. Mechanical forces seem to play a dominant role in this case.
Floating matrix

Contraction of freely-floating matrix by cells is dependent on \( \alpha \)-SMA expression.\(^{86,87}\) Fibroblasts in relaxed collagen gel lose stress fibers and focal adhesions and do not proliferate. They form dendritic extensions that have microtubule cores and actin-rich-tips.\(^{86,87}\) The extent of contraction is dependent on initial collagen concentration; lower density gels contract more rapidly. The release of mechanical tension triggers fibroblast apoptosis.\(^{90,91}\) This effect is specific of collagen, apoptosis is not observed in contractile fibrin gels.\(^{92}\) Signal transduction from the ECM is disturbed.\(^{93}\) rRNA content is lower in collagen matrices than in the cells in monolayer.\(^{94}\) mRNA expression of TGF-\( \beta \)1 increases in the order of plastic, attached matrix, stress-relaxed matrix and floating matrix. The expression of collagenase mRNA is higher in collagen matrix than in the cells on plastic.\(^{95}\) The release of stressed matrix is followed by a burst of c-fos expression and ERK 1/2 kinase activation.\(^{96}\)

Cell survival, collagen synthesis and degradation are regulated by integrins. Antibodies to \( \alpha 2 \beta 1 \) integrins prevent the contraction and reduce apoptosis.\(^{97-99}\) Integrin \( \alpha 11 \) mRNA and protein are up-regulated in attached collagen gel and down-regulated in fibroblasts grown in floating gel.\(^{100}\) Rat liver MFB utilize \( \alpha 1 \beta 1 \) integrin for collagen matrix contraction as \( \alpha 2 \) subunit is not expressed in HSC, their precursors in vivo.\(^{101}\) The expression of \( \alpha 2 \) subunit in fibroblasts cultured in collagen gel is dependent on NF-\( \kappa B \) activity that is induced by contact of the cells with collagen.\(^{102}\) The expression of the 2 receptors is regulated differentially and their functions are not identical. \( \alpha 1 \beta 1 \) mediates downregulation of collagen gene expression and \( \alpha 2 \beta 1 \) mediates induction of collagenase.\(^{103}\) Both of them are able to mediate gel contraction but their expression in vitro depends on the environment and in vivo on the physiological state of the tissue.\(^{101}\) Matrix contraction may be enhanced by collagen V that binds integrin \( \alpha 2 \beta 3.\(^{104}\)

Increased synthesis of collagen is observed in the skin of \( \alpha 1 \)-null mice. Col1(I) mRNA levels in both granulation tissue and fetal fibroblasts are higher in the cells isolated from \( \alpha 1 \)-null animals and embedded in collagen gel than in wild-type cells. Integrins \( \alpha 1 \beta 1 \) provide a feedback inhibition of collagen synthesis.\(^{105}\) Blocking of \( \beta 1 \) subunit by a monoclonal antibody, which abrogates phosphorylation of Akt/protein kinase B, protects cells from contraction-induced apoptosis. Phosphatidyl-inositol 3-kinase (PI3K)/Akt signaling is a regulator of cell survival. Downregulation of PI3/Akt survival signals results in apoptosis. Constitutive expression of phosphatidylinositol 3-kinase (PI3K) protects fibroblasts from both apoptosis and anoikis.\(^{106}\)

Secretion of metalloproteinases

MMP are a family of zinc-dependent proteinases that are secreted to the extracellular space or localized to the cell surface. They are collectively able to cleave all components of ECM and they can modify other biologically active molecules. A subgroup of collagenases comprises MMP-1, -8, -13 and -14 that degrade fibrillar collagens. Gelatinases MMP-2 and -9 cleave denatured collagen and collagen type IV.\(^{107}\)

Both mechanical forces and the chemical nature of collagen play an important role in regulating MMP expression. Collagenase mRNA expression and activity are higher in fibroblasts cultured on type I collagen gel when compared to cells on plastic.\(^{108}\) Releasing stress by treating fibroblasts on plastic with cytochalasin D that disrupts cell cytoskeleton enhances expression and secretion of MMP-1, -2, -3, -13 and membrane-bound MMP-14. The active form of MMP is more strongly expressed in cells cultured in floating matrices than in cells in monolayer.\(^{109}\) Increased expression of MMP-2 and its inhibitor TIMP-2 mRNAs is observed in fibroblasts when collagen gel with cultured cells is prestrained.\(^{110}\) Contact of human MFB with collagen I gel results in the activation of proMMP-2 that is not observed in the cells grown on plastic or plastic coated with a thin layer of collagen I or IV, laminin or Matrigel. The induction of MMP-2 by accumulating collagen I may contribute to the remodeling of ECM in fibrotic liver.\(^{111,112}\) Increased expression of the active form of MMP-2 on collagen gel is accompanied by up-regulation of MT1-MMP protein. Metalloproteinase MT1-MMP (MMP-14) is known to activate MMP-2.\(^{113}\)

Collagen degradation is more rapid in floating matrices than in attached gels.\(^{114}\) Active forms of MMP-1 and MMP-2 can be detected around human HSC cultured on collagen gel by in situ zymography and in the culture medium, respectively.\(^{115}\) The expression of MMP-13 increases in rat liver MFB when the cells are embedded in attached collagen gel. However, the observed collagen degradation is a result of a joint action of a few proteinases.\(^{18}\)

Collagen contraction is enhanced by MMP activity and impaired by MMP inhibition. MMP activity is stimulated in floating gel.\(^{116-118}\) Gel contraction by fibroblasts is greatly accelerated when the matrix is treated with plasmin that may activate MMP-1 secreted by the cells. Fibroblasts in healing wounds are in close proximity to keratinocytes that produce plasminogen activator in response to cytokines. Plasmin may play a role in provisional matrix remodeling.\(^{119}\) Contraction of floating collagen matrix gives rise to a mechanically relaxed tissue resembling dermis. The cells in floating matrix show low capacity to synthesize DNA and proliferate, decreased responsiveness to growth factors and decreased ability to synthesize collagen.\(^{26}\) Three-dimensional matrix, especially at higher stiffness, impedes cell proliferation and migration. The cells may secrete proteinases and degrade adjacent matrix to create space for these activities. Interconnected multicellular networks are formed in gels with low Young’s modulus.\(^{120}\)

Integrins \( \alpha 1 \beta 1 \) and \( \alpha 2 \beta 1 \) have different functions in the regulation of MMP expression. MMP-1 mRNA level in fibroblasts cultured in retracting collagen gel is higher than that in cells on plastic. The difference can be eliminated when \( \alpha 2 \beta 1 \) integrin is blocked by a specific antibody. In contrast, the difference is much larger when \( \alpha 1 \) subunit is blocked. Changes in MMP expression are paralleled by changed expression of Ets-1 transcription factor.\(^{103,121}\) The induction of MMP-13 in periodontal ligament fibroblasts is dependent on integrin \( \alpha 1 \beta 1 \).\(^{122}\) The induction of MMP in collagen gel may be affected by signaling
pathways downstream of integrin ligand binding. Contact of human skin fibroblasts with 3D collagen results in simultaneous activation of 3 groups of mitogen-activated protein kinases, ERK 1/2, JNK and p38. Tyrosine kinase inhibition suppresses MMP-13 expression, ERK1/2 inhibition enhances the expression. Collagen activates a member of protein kinase C family PKCζ and NFκB DNA binding. The expression of MMP-3, -9, -13, and -14 mRNA as well as the activation of MMP-9 is enhanced in activated rat HSC embedded in collagen I gel. The stimulation of MMP-9 expression requires NF-κB and AP-1 activities.

The Influence of Fibronectin on Collagen Properties

Type I collagen is a major component of connective tissues but its action can be modified by other components of the ECM as well as by cytokines and agents used for treating the cells. Fibronectin contained in blood plasma and to a smaller extent in fetal bovine serum used in cell culture influences the events in collagen matrix substantially.

Medium containing fetal bovine serum is procontractile. Collagen gel contraction by human dermal fibroblasts is inhibited when serum used in culture medium is depleted of fibronectin by affinity chromatography. The inhibition can be abolished by adding plasma fibronectin or vitronectin to culture medium containing fibronectin-depleted fetal bovine serum. Fibronectin is much more efficient. The stimulated gel contractility is inhibited by peptides containing arg-gly-asp (RGD) sequences. Fibronectin fibrils are associated with stress fibers formed in the cells in attached collagen gel. Fibroblasts in floating gel do not form stress fibers or form fibronectin fibrils. Fibronectin added to collagen sponges accommodating chick fibroblasts enhances DNA synthesis in the cells. Fibronectin-coated sponges enhance wound healing in vivo.

F-actin and α5 integrin are induced by fibronectin in trabecular meshwork cells cultured in collagen gel. Fibronectin promotes gel contracture by human corneal fibroblasts. It stimulates the formation of stress fibers in the cells and increases the amounts of integrin subunits α5 and β1 and of Paxillin, a component of focal adhesions. Fibroblasts cultured on collagen matrix form clusters that are stabilized by fibronectin fibrils. Cell clustering requires α5β1 integrins and can be prevented by blocking Rho kinase or myosin II activity. A succession of events taking place in the first hours of matrix contraction has been proposed. The earliest stage involving fibronectin and the integrin subunit α5 is followed by vitronectin-mediated cell attachment and the sequence is completed by the appearance of α2 integrin subunit and its interaction with collagen. Fibronectin produced by the fibroblasts themselves may enhance matrix contraction. Fibronectin is present on cell surfaces and associated with collagen in attached matrices. It disappears from the cell surface after the matrix is released. Protein and DNA synthesis decrease. Fibronectin-null mouse embryonic fibroblast adhere to collagen gel in the absence of serum but do not spread or proliferate. Addition of plasma fibronectin results in increase in cell number and formation of multicellular structures. Inhibition of fibronectin polymerization prevents cell proliferation.

Conclusion and Perspectives

The number of studies of cell behavior utilizing various 3D tissue models is increasing. Three-dimensional structure produces cells that differ in many aspects from their counterparts cultured on flat plastic dishes. Both morphology and metabolism of the cells are changed. Type I collagen as the most abundant protein found in tissues is the basis of many 3D models. Its chemical properties, the ability to interact with cells and to bind other ECM components, contribute a distinct specificity to the contact with cells. Collagen can be further modified by crosslinking or by casting on a stiffer substrate to better mimic matrix development in healing wounds or in other tissues under physiological or pathological situations. Future collagen matrix models may come even closer to situations in vivo by better controlling physico-chemical properties of the matrix, by incorporating into it other ECM proteins, glycoproteins and proteoglycans and by allowing fibroblastic cell interaction with inflammatory cells, epithelial and endothelial cells that also participate in the events going on in tissues.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

This research was supported by PRVOUK P37/1.
11. Kraman R, DiRocco DP, Humphreys BD. Understanding the origin, activation and regulation of matrix-producing myofibroblasts for treatment of fibrotic disease. J Pathol 2013; 231:273-89; PMID:23567628.

12. Bienkowski RS, Gotkin MG. Control of collagen deposition in mammalian lung. Proc Soc Exp Biol Med 1995; 209:118-40; PMID:7770462; http://dx.doi.org/10.3181/00379727-209-43886a.

14. Dvorak HF: Tumors: wounds that do not heal. New England J Med 1986; 315:1659-60; PMID:3715230; http://dx.doi.org/10.1056/NEJM198612253152606.

17. Han Y-P, Zhou L, Wang J, Xiong S, Garner WL, Yannas IV. Abrogation of myofibroblasts: role in connective tissue remodeling. Am J Pathol 2002; 160:1403-12; PMID:12168956; http://dx.doi.org/10.1016/S0002-9440(10)64793-5.

18. Jiroutov.

25. Hazeki N, Yamato M, Imamura Y, Sasaki T, Nakamura M. Morphological and biochemical analyses on three-dimensional collagen matrices. J Cell Biol 1997; 138:273-84; PMID:9207695; http://dx.doi.org/10.1083/jcb.138.2.273.

26. Grinnell F. Fibroblasts, myofibroblasts, and wound contraction. J Cell Biol 1994; 124:401-4; PMID:8106541; http://dx.doi.org/10.1083/jcb.124.4.401.

27. Rhee S, Grinnell F. Fibroblast mechanics in 3D collagen matrices. Adv Drug Deliv Rev 2007; 59:1299-305; PMID:17825456; http://dx.doi.org/10.1016/j.addr.2007.08.006.

28. Carlson MA, Longaker MT. The fibroblast-populated collagen matrix as a model of wound healing: a review of the evidence. Wound Repair Regen 2004; 12:134-47; PMID:15086764; http://dx.doi.org/10.1111/j.1799-9693.2004.002280.x.

33. Hinz B. Tissue stiffness, latent TGF-β1 activation, and mechanical signal transduction: implications for the pathogenesis and treatment of fibrosis. Curr Rheumatol Rep 2009; 11:120-6; PMID:1926884; http://dx.doi.org/10.1007/s11926-009-0017-7.

38. Morrisey EV, Lederer DJ, Greenhorn CR, Marincola FM, Salgia R, Liao C, Morgan DO, Moore K. Alloproteinases expression. Biomaterials 2005; 26:4131-40; PMID:1601722.

41. Xu X, Wang J, Yang Y, Surzycki V, Papaioannou V, Wu Y, Liao J, Deshmukh M. c-Jun-NH2-terminal kinase 2 mediates mechanical anchorage of fibroblasts in three-dimensional collagen matrices. Mol Biol Cell 2005; 16:5070-6; PMID:16107563; http://dx.doi.org/10.1091/mbc.E05-01-0007.

44. Yeung T, Georges PC, Flanagan LA, Marg B, Ortiz R, Boundy R, Hinz B. Fibroblast-collagen-matrix contraction: the cellular response to transglutaminase-crosslinked collagen. Biomaterials 2005; 26:6518-29; PMID:15927250; http://dx.doi.org/10.1016/j.biomaterials.2004.04.015.

46. Grinnell F. Contraction of hydrated collagen gels by fibroblasts: evidence for two mechanisms by which collagen fibrils are stabilized. Collagen Rel Res 1986; 6:515-29; PMID:3702949a.

47. Harris AK, Stropak D, Wild P. Fibroblast traction as a mechanism for collagen morphogenesis. Nature 1981; 290:249-51; PMID:7207616; http://dx.doi.org/10.1016/0022-3628(80)90410-7.

48. Guidry C, Grinnell F. Production of ordered collagen gels by fibroblasts: type VI collagen is a major component. J Biol Chem 2004; 279:4820-8; PMID:14617627; http://dx.doi.org/10.1074/jbc.C103041200.

49. Freyman TM, Yau K, Yokko R, Gibson LJ, Fibroblast contractile force is independent of the stiffness which resists the contraction. Exp Cell Res 2002; 272:153-62; PMID:11777340; http://dx.doi.org/10.1006/excr.2001.7409.

51. Grinnell F. Fibroblast-collagen-matrix contraction: growth-factor signalling and mechanical loading. Trends Cell Biol 2000; 10,362-5; PMID:10939209; http://dx.doi.org/10.1016/S0962-8924(00)01802X.

52. Kessler D, Dethlefsen S, Haase I, Plomann M, Hirche H. Mechanical anchorage of fibroblasts in three-dimensional collagen matrices. Mol Biol Cell 2005; 16:5070-6; PMID:16107563; http://dx.doi.org/10.1091/mbc.E05-01-0007.

53. Erwanto Y, Kawahara S, Katayama K, Takenoyama S, Kana M. Morphological and biochemical analyses on three-dimensional collagen matrices. Curr Protoc Cell Biol 2011; 91:394-9; PMID:21530503; http://dx.doi.org/10.1002/9780471166166.43e3; JCB-ADC4953e3.00.2H.

54. Grinnell F. Fibroblast-collagen-matrix contraction: growth-factor signalling and mechanical loading. Trends Cell Biol 2000; 10,362-5; PMID:10939209; http://dx.doi.org/10.1016/S0962-8924(00)01802X.

55. Huang X, Yang N, Fiore VF, Barker TH, Sun Y, Morris SW, Ding Q, Thannickal VJ, Zhou Y. Matrix stiffness-induced myofibroblast differentiation is mediated by intrinsic mechanotransduction. Am J Respir Cell Mol Biol 2012; 47:340-8; PMID:22461426; http://dx.doi.org/10.1165/rcmb.2012-00934R.

56. Hinz B, Gabbiani G. Cell-matrix and cell-cell contacts of myofibroblasts: role in connective tissue remodeling. Thromb Haemost 2003; 90:993-1002; PMID:14652602.

57. Kopp J, Preis E, Said H, Hafemann B, Wickert L, Grosen AM, Pallau N, Dooley A. Abrogation of...
transforming growth factor-β signaling by SMAD7 inhibits collagen gel contraction of human dermal fibroblasts. J Biol Chem 2005; 280:21570-6; PMID:15788410; http://dx.doi.org/10.1074/jbc.M505327200.

6. Liu XD, Umino T, Erdl R, Veyts T, Skold CM, Takiya H. Persistent of TGF-β1 induction of increased fibroblast contractility. In Vitro Cell Dev Biol Anim 2001; 37:193-201; PMID:11370814; http://dx.doi.org/10.1209/1021-7600(2001)37<193:PPOT-1G>2.0.CO;2.

7. Meyer-ter-Vehn T, Han H, Grehn F, Schlunck G. Extracellular matrix elasticity modulates TGF-β-induced p38 activation and myofibroblast transdifferentiation in human tenon fibroblasts. Invest Ophthalmol Vis Sci 2011; 52:9149-55; PMID:22058331; http://dx.doi.org/10.1167/iovs.10-6679.

8. Assoian RK, Klein EA. Growth control by intracellular tension and extracellular stiffness. Trends Cell Biol 2008; 18:297-305; PMID:18087523; http://dx.doi.org/10.1016/j.tcb.2008.05.002.

9. Liu XD, Umino T, Liu XD, Wang HJ, Romberger DJ, Spurzem JR, Rennard SI. Contraction of fibroblast-containing collagen gels: effects on cell growth and biosynthetic activity. J Invest Dermatol 1995; 93:792-8; PMID:258476; http://dx.doi.org/10.1111/j.1523-1747.1995.tb22442.x.

10. Nakagawa S, Pawelek J, Grinnell F. Long-term culture of fibroblasts in contracted collagen gels: effects on cell growth and biosynthetic activity. J Invest Dermatol 1995; 100:693-706; PMID:20733059; http://dx.doi.org/10.1038/jid.20100482.

11. White DJ, Puranen S, Johnson MS, Heino J. The collagen matrix metalloproteinase 2 gene in young patients with cancer progression. Cancer Metastasis Rev 2012; 31:295-321; PMID:22356781; http://dx.doi.org/10.1007/s10555-012-9346-z.

12. Liu F, Mih JD, Shea BS, Kho AT, Sharif AS, Tager AM, Tischkowitz DJ. Freepoint amputation of fibrosis through matrix stiffening and COX-2 suppression. J Cell Biol 2010; 190:693-706; PMID:20733059; http://dx.doi.org/10.1038/jid.20100482.

13. Lambert CA, Soentam EP, Nusgens BV, Lapiere CM. Pretranslational regulation of extracellular matrix macromolecules and collagenase expression in fibroblasts by mechanical forces. Lab Invest 1992; 66:444-51; PMID:199722.

14. Hadjipanayi E, Madera V, Brown RA. Close dependence of fibroblast proliferation on collagen scaffold matrix stiffness. J Tissue Eng Regen Med 2009; 3:77-84; PMID:19051218; http://dx.doi.org/10.1002/term.138.

15. Sollberg S. Normal human primary fibroblasts undergo apoptosis in three-dimensional collagen cultures. J Invest Dermatol 1998; 110:153-7; PMID:9457911; http://dx.doi.org/10.1046/j.1523-1747.1998.00095.x.

16. Fringer J, Grinnell F. Fibroblast quiescence in floating collagen matrices. Decrease in serum activation of MEK and Raf but not Ras. J Biol Chem 2003; 278:20612-7; PMID:12663662; http://dx.doi.org/10.1074/jbc.M212365200.

17. Vareidi M, Tredget EE, Glaha H, Scott PG. Stress-relaxation and contraction of a collagen matrix induces expression of TGF-β and triggers apoptosis in dermal fibroblasts. Biochem Cell Biol 2000; 78:427-36; PMID:11102081; http://dx.doi.org/10.1139/o00-014.

18. Rosenfeldt H, Lee DJ, Grinnell F. Increased c-fos expression by fibroblasts contracting stressed collagen matrices. Mol Cell Biol 1998; 18:2659-67.

19. Schiro JA, Chan BMC, Roswit WT, Kasner PD, Penrod AP, Hemler ME, Eisen AZ. Kupper T1 integrates mechanical stress to the tension state of cell-matrix interactions. PNAS 2007; 104:5425-30; PMID:17369366; http://dx.doi.org/10.1073/pnas.0608301040.

20. Carracedo S, Lu N, Popova SN, Jonsson R, Eckes B, Sollberg S. Contractation-dependent apoptosis of normal dermal fibroblasts. J Invest Dermatol 2001; 116:686-92; PMID:11348456; http://dx.doi.org/10.1046/j.1523-1747.2001.01342.x.

21. Racine-Sanson L, Rockey DC, Bessel DM. The role of α1β1 integrin in wound contraction. A quantitative analysis of liver myofibroblasts in vitro and in primary culture. J Biol Chem 1997; 272:30911-7; PMID:9388237; http://dx.doi.org/10.1074/jbc.M2049311.
