Reprinted from SIM Industrial Microbiology NEWS (new formatting)
Vol. 41. No. 6 Nov/Dec 1991. Pages 266-272.
With permission of copyright holder.

FEATURE ARTICLE

CELLULAR AND PLASMA FIBRONECTINS

Clyde R Goodheart and Ralph H. Silverman
ChiFyx, Inc., Northbrook IL 60062

Introduction

The fibronectins (FN) are a family of very closely related cell-adhesive glycoproteins involved in many distinct functions, including cell attachment and spreading, normal cell morphology, cell migration during embryogenesis, opsonization, oncogenic transformation, hemostasis, thrombosis, and wound healing.

There are two major categories of FN. Soluble plasma fibronectin (pFN) is found in the plasma, and insoluble cellular fibronectin (cFN) surrounds cells and anchors them to the substrate. The different FNs are coded by a single gene. The messenger RNA transcribed from the gene is spliced in at least three alternate regions (ED-A, ED-B, and III CS), producing the different forms. The involvement of FN in so many different functions is due to the large size of the molecule (native FN has a molecular weight of about 550,000), and to the presence in the same molecule of binding sites for collagen, fibrin, actin, heparin, *Staphylococcus aureus,* DNA, and cell surfaces (for reviews see: Akiyama and Yamada, 1987; and Hynes, 1985).

Considerable confusion about fibronectin exists in the literature, as discussed by Yamada and Kennedy (1979). This is partly because a common name, fibronectin, is used for similar, but not identical, proteins. Authors often fail to note which type of FN was used for their studies. Further, generalizations based on results from one type of FN are often applied to all types of FN.

In this brief review, which is not intended to be an exhaustive or complete review of the relevant literature, we will discuss some of the similarities and differences between the two major forms of FN, in particular to studies since 1979 that have distinguished between the two forms. It is clear that the two forms of FN are different molecules, with different chemical, physical and biological properties, and with different functions. It is not surprising that they also will have different therapeutic uses.

Biochemical and Biophysical Properties

As indicated by its name, pFN is found in plasma, where it has a concentration of about 300 mg/L. On the other hand, cFN is found as a coating on cell surfaces and in the extracellular matrix, often in a fibrillar form, where it is insoluble. It is secreted into culture media in a soluble form, however.

In addition to the differences in solubility, early work on FNs revealed differences in electrophoretic mobilities. Yamada and Kennedy (1979) compared human and chicken pFNs and cFNs by SDS-PAGE. They found that each species, the pFNs migrate as doublets, while the cFNs migrate more slowly (therefore, having a higher molecular weight) as a single band. cFNs that are secreted into medium co-migrate with cFNs extracted directly from cells. The lower molecular weight of pFNs compared with cFNs is not due to degradation by proteases after harvesting. The doublet found with pFN indicates that the two chains are held together by reduction-sensitive (disulfide) bonds, and that the two chains have different mobilities, and therefore different molecular weights.

FN molecules consist of domains with different binding capacities. The domains are the same in the two chains of pFNs, with some exceptions. In human pFN, the heavy, A chain, has a smaller domain that binds heparin, but also possesses a domain not present in the light, B chain. This domain is called the IIICS (the “III connecting segment,” a variable region connecting the last two type III repeats). The net effects is that the A chain is longer and has a higher molecular weight than the B chain. Neither pFN chain contains the extra domain, present only in cFN, of 90 amino acid residues (Pande, et al., 1987).

Digestion of pFN and cFN by cathespin D yields 3 fragments: 1) a 67 kDa peptide from the N-terminal region containing a fibrin-binding (Fib-1) domain and a gelatin-binding domain; 2) a 130 kDa peptide from the central region containing a cell-attachment domain; and 3) a fragment from the C-terminal region of the molecule which is composed of two nonidentical peptides, one of 60 to 70 kDa in pFN and one of 80 to 100 kDa in cFN. Each of these last two peptides contains a heparin-binding (Hep-2) domain and a fibrin-binding (Fib-2) domain (Tressel, et al., 1988, and Sekiguchi, et al., 1985).

Alternative mRNA splicing of a common transcript is believed to give rise to structural polymorphism between the various forms of FN as well as between different subunits within each type (Schwarzbauer, et al., 1985). Three distinct regions of alternative splicing (ED-A, ED-B, and IIICS) have been identified and may contribute to the generation of up to 20 FN isoforms. Interestingly, two of these three regions that display alternative splicing (ED-A and 111 CS) are clustered around the carboxyterminal heparin-binding (Hep-2) domain, suggesting that this section of the FN molecule is highly variable. It is speculated that the presence of these extra domains alters the physical and biological properties of FNs. For example, cFN is relatively insoluble and strongly binds to cell surfaces and substrates, while pFN is very soluble and probably requires activation to bind to cell surfaces (Tressel, et al., 1988).

Placenta FN seems to be a variant of cFN, in that it contains the ED-A in one chain rather than in both chains as in cFN (Tressel, et al., 1988).

The amino acid sequence of about 1000 of the residues was deduced from the nucleotide sequence of an over-lapping set of cDNA clones of human cFN. That portion, which includes the hypervariable region, shows a high degree of match with rat cFN, also deduced from cDNA, and with direct amino acid analysis of bovine pFN (Bernard, et al., 1985).

The complete amino acid sequence of bovine pFN was published by Skorstengaard, et al. (1986). The native molecule consists of 2,265 amino acid residues. The calculated molecular weight of the native molecule, not including the carbohydrate, is 486,123 daltons. Comparison of bovine pFN with human cFN (deduced from cDNA), shows 146 differences, or 93% identity. Bovine pFN does not contain the "extra domain," present in human cFN, which may be specific for cFN.

Native pFN is a heterodimer, as originally suggested by Yamada and Kennedy (1979). The two chains differ in amino acid sequence. In bovine pFN, for instance, the A chain has a calculated molecular weight (not including carbohydrate) of 249,655 daltons, and the B chain is 236,468 daltons (Skorstengaard, et al., 1986).

Biological Properties

Found in plasma, pFN has been called cold insoluble globulin (Yamada and Kennedy, 1979), because it precipitates when plasma is stored at cold temperatures. cFN has been called large external transformation-sensitive (LETS) protein, cell surface protein (CSP), or galactoprotein a. It is found on cell surfaces, extracellular matrices, and basement membranes (Yamada and Kennedy, 1979 and Pande, et al., 1987). In cultures, cFNs are on cell surfaces or sloughed or secreted into the culture media (Yamada and Kennedy, 1979). Blood platelets contain about 4,000 molecules of FN per platelet. The FN in platelets resembles cFN more closely than pFN; the minor differences between the FN in platelets and cFN could be due to differences in glycosylation (Paul, et al., 1986).

Hepatocytes synthesize pFN *in vivo* and *in vitro*; however, in culture, hamster hepatocytes also synthesize cFN. The FN secreted by endothelial cells is cFN (Tamkun and Hynes, 1983). In dogs, pFN differs from FN synthesized by chondrocytes. The latter is deposited in cartilage, but it is not cFN, since it lacks the extra domain that defines cFN (Burton-Wurster and Lust, 1989 and Kornblihtt, et al., 1984). Epithelial cells, such as human keratinocytes (Kubo, et al., 1984) produce FN under certain conditions. Presumably this FN is of the cellular type.

The type of FN produced, that is, the splicing pattern of the primary mRNA transcript, is controlled by various materials, such as dexamethasone and transforming growth factor beta (Balza, et al., 1988) and dibutyryl cyclic AMP (Burton-Wurster, et al., 1988). It is also specific for the tissue and differs between embryo and adult in the same tissue (Oyama, et al., 1989). Further, the FNs produced by different tissues or cell types are distinct from each other in the number of types of subunits, as defined by the splicing pattern of the primary mRNA transcript (Sekiguchi and Titani, 1989), or the ratio between the forms of FN (Barone, et al., 1989).

Comparisons of human and chicken pFNs and cFNs showed that cFNs from both species are about 150-fold more active in agglutinating formalin-fixed sheep erythrocytes than the corresponding purified pFNs. Further, cFNs are 50-fold more active in restoring a more normal fibroblastic cell morphology and adhesiveness to various transformed cells (Yamada and Kennedy, 1979). cFN binds cells to the underlying collagen fibers. Fibroblasts embedded in lattices of collagen fibrils induce contraction of the gel under the influence of

cFN but not pFN, demonstrating a functional biological difference between the two (Asaga et al, 1991).

Function of Fibronectins

FNs serve many functions, some of which are only beginning to be understood. For instance, in culture, fetal bovine serum is known to enhance the attachment of cells to the surface of the culture vessel. This was thought to be due to the pFN contained in the serum. Recent studies, however, have suggested that another protein, vitronectin, in fetal bovine serum accounts for the spreading effect (Lydon and Foulger, 1988). Unlike FN, vitronectin is not found in basement membranes. It is, however, found in reactive and fibrotic tissue, as is FN, suggesting that it may be involved in inflammation and repair (Reilly and Nash, 1988).

pFNs opsonize various foreign materials. pFN molecules bind to certain bacteria and to nectrotic debris at a wound site, activating the foreign material for elimination by the reticuloendothelial system (Blumenstock and Saba, 1982; Martin, et al., 1988; and Doran, et al., 1986).

Both types of FN are involved in wound healing, but in different ways. A wound initiates a complex set of reactions that controls bleeding, produces immediate but temporary repair, and eventually rebuilds the wounded tissue into its former structure. Although many cell types and many molecular species are involved in this lengthy process, FNs play important roles.

ffrench-Constant, et al. (1989) reviewed the events in wound healing and studied the FN mRNAs in cells surrounding wounds. Immediately after wounding, a clot forms at the site, aided by pFN. Platelets rupture in the clot, releasing their contents of growth factors and FN. pFN is deposited as part of the clot. Within hours, mRNA is detectable in cells surrounding the wounded area. The mRNA is specific for cFN, in a form that is closely related to that found in developing embryos. This cFN directs the migration of various cells into the wounded area, in the same way that FNs direct cell migration during embryogenesis. These workers pointed out that a number of clinical trials have used pFN throughout the course of the healing process. Instead, they say it is more appropriate to follow the pFN with cFN after the first few days to provide more efficient acceleration of wound healing. (Also see Colvin, 1989, for an extensive review of FN in wound healing.)

In view of its involvement with cell migration and differentiation during embryogenesis and wound healing, it is not surprising that FN is involved also with expression of the malignant properties of dedifferentiation and metastasis. It was observed as long ago as 1973 that malignant transformation by viruses results in loss of the pericellular matrix (the subject has been reviewed many times; see Vaheri, et al., 1989 for a recent review). Also, malignant cells shed less cFN than do normal cells. For instance, none of 13 different human colon adenocarcinomas produced detectable amounts of FN or showed detectable cell-surface staining with anti-FN antibody (Varani et al., 1991).

Rutka, et al. (1987), studied the growth and other properties of a human glioma cell line grown on an ECM (extracellular matrix) laid down by leptomeningeal cells in culture. The glioma cells usually grow as cuboidal cells, with tight packing and piling. On ECM, however, they were more differentiated and resembled normal astrocytes in culture, with production of thin cytoplasmic processes similar to axons and dendrites. Their growth was much slower on the ECM than on the bare plastic surface, or on ECM that had been treated with trypsin. Growth and differentiation of the cell line was not affected by purified pFN; this is not surprising, however, since only the cellular form (which, of course, was not available to the researchers) would be expected to be active. When grown on ECM, the anaplastic cell line contained about 20 times as much glial fibrillary acidic protein than when grown on plastic, indicating that the ECM promoted differentiation, since manufacture of this protein occurs in the differentiated cell. Also, the glioma cell line produced a proteolytic enzyme when grown on plastic that was not detectable when it was grown on ECM, because the enzyme is made only by the malignant, dedifferentiated cell. (This enzyme may be related to the malignant cells' ability to migrate through the basement membrane.)

Diagnostic Uses

Reports of either pFN or cFN being used in diagnosis are extremely limited. Because FNs are adhesive proteins that bind to certain specific sites, such as fibrin and collagen, Uehara, et al. (1988) suggested that FN could be radiolabeled and used as a tracer to locate atherosclerotic lesions and thrombi. As FNs become more readily available commercially, it may be expected that more diagnostic uses will be developed.

Therapeutic Uses

Neither pFN nor cFN, from any species, is available for therapeutic use. pFNs have been used extensively, however, in various clinical trials. The pFNs often were prepared by the investigators from the plasma of the patient to be treated. cFNs should be more efficacious for many treatment

purposes. cFNs are involved extensively in later stages of wound healing, and in control of migration and morphology of malignant cells. For these purposes, it may even prove to be best to have cFNs from specific tissues or stages of development. cFNs have not been used because they have not been available commercially, even for research and testing purposes, and it is too difficult and costly for individual investigators to prepare them for trial purposes.

Severe sepsis.

In severe sepsis, pFN and other plasma protein levels are much lower than normal. Many workers have attempted to treat sepsis and septic shock with infusions of pFN or with cryoprecipitate, based on the non-immune opsonization property of pFN. Results generally have been disappointing (for example, see Doran, et al., 1986), although Holman, et al. (1988) found that less replacement fluid was necessary in experimental sepsis in sheep when cryoprecipitate that was rich in pFN was also infused. cFN has not been used, nor would it seem appropriate, since it is pFN that normally performs the opsonin function.

Periodontal disease.

FN has been used extensively in experimental treatment of periodontal disease. Dogs (Smith, et al., 1987a and b; Wikesjo, etal., 1988), cats (Ryan, et al., 1987), monkeys (Caffesse, et al., 1987), and humans (Thompson, et al., 1987) have been treated. In some studies, the FN was of bovine origin, while in others, the FN was from the species being treated. In the human studies, the FN was prepared from the patients' own plasma.

Usually, the root was cleaned (scaled) and citric acid was applied to the root prior to adding the pFN. This exposes the collagen fibers in the surface of the root, so that the FN can attach. In all the studies, treatment with citric acid and pFN was found to be effective in encouraging reattachment of the periodontal ligament to the root, eliminating the pocket. In addition to the FN, there may be other attachment proteins in the tooth cementum (Somerman, et al., 1991).

In all cases, pFN was used; because it is not available, cFN has not been studied in this application. Based on knowledge about the differences in functions between pFN and cFN, cFN should be much more effective than pFN in treating periodontal disease. It is cFN that serves to attach cells to underlying collagen, and therefore it would be appropriate for reattaching the periodontal ligament to the roots. Further, cFN is a normal component of the periodontal ligament (Lukinmaa et al., 1991). Many research periodontists are looking forward to having cFN available for use in perfecting this treatment for periodontal disease in humans.

Wound healing.

Under usual conditions, healing of a wound is a complex series of well-orchestrated events involving many different cell types. Both pFN and cFN play important roles in the process. As pointed out by Colvin (1989), evolutionary pressures have favored quick, efficient wound closure and healing. Perhaps optimum amounts of FNs, growth factors, and other controlling proteins are already present in a healing wound. Chronic, non-healing wounds are most likely to benefit from intervention, such as by application of exogenous FN - conditions must not have been optimal, or the wound would have healed spontaneously.

Work with FNs and wound healing falls generally into several categories: 1) surgical incisions, 2) chronic skin ulcers, 3) burns, and 4) corneal lesions. Results of effects of FN in enhancing the healing process are dependent upon the type of wound studied. In some instances, investigators have included collagen in their experimental protocol, to more closely duplicate natural conditions.

Animal models of wounds may be a simple surgical incision through the skin, a punch biopsy with a diameter of one or a few millimeters, or removal of a larger piece of skin. A surgical incision is generally clean, in the sense that extravasated blood is sponged away, leaving only a small clot on the incision. The blood is not removed from the bed of a punch biopsy or skin excision, and the bed of the wound will be filled with a large clot. The clot, of course, contains pFN, growth factors released from platelets, fibrin, and other components important in the healing process.

Falcone, et al. (1984) studied the effect of pFN on surgical incisions in rat skin. They found that application of pFN to the edges of the incised skin before suturing resulted in significantly higher breaking strengths of the wound in the first 11 days post surgery than in incisions that did not receive pFN. Similar results were found by Litvinov, et al. (1987), especially in the first few days after wounding. Excision lesions also healed faster in experiments reported by Cheng, et al. (1988). Doillon and Silver (1986) used collagen sponges containing pFN in rats and found favorable effects on wound healing.

Chronic skin ulcers provide a different kind of experimental model. Conditions for healing must not have been optimal, or the wound would have healed. Wysocki and Grinnell (1990) found that while FN was intact in acute wounds (blister fluid),

it was degraded in chronic diabetic and stasis ulcers. Several groups of investigators (Grinned et al., 1988; Doillon, etal., 1988; and Vasil'ev, et al., 1987) have applied pFN to such wounds, either as a wash, or in a collagen sponge. In some patients, wounds previously had been treated by various means for up to five years, without healing. All the investigators found that a significant number of the wounds healed with the fibronectin treatment.

Nagelschmidt, et al. (1987a and b) have reported the use of pFN in small, standardized burns in rats. They found that addition of pFN did not enhance healing unless they also administered large amounts of gelatin, which deplete the endogenous pFN. To generalize, it seems reasonable that following a large-area burn, the amount of necrotic material released may overwhelm both the endogenous pFN that opsonizes the debris, and the reticulo-endothelial system's ability to clear it from the system. Replacement with exogenous pFN during the debridement stage of burn healing, and cFN during the reconstruction stage, seems reasonable.

Studies of the effect of pFN on healing of corneal lesions has been extensive, especially in Japan. Here again, it is important to consider the type of lesion when evaluating experimental results. Simple abrasions seem to heal well and the healing process is not enhanced by application of additional, exogenous pFN. Chronic ulcers, in contrast, seem to heal better if exogenous pFN is applied.

Watanabe, et al. (1988) reported that pFN stimulates corneal cells to attach and grow in culture. Soong, et al. (1989), however, reported that pFN does not cause corneal epithelial cells to migrate, as they do in covering a corneal defect; these authors concluded that their migration is controlled by epidermal growth factor. More recently, Nishida, et al. (1990) reported that pFN mediates corneal cell migration. Clearly, more work is necessary to resolve these issues.

Results from studies in rabbits have been conflicting. Spigelman, et al. (1985) reported that pFN aided the healing of alkali-induced corneal lesions, by improving the attachment of the new epithelium. Singh and Foster (1989) also found a beneficial effect, especially when pFN was combined with epidermal growth factor. Newton, et al. (1988), however, found no enhancement of healing by pFN in persistent corneal ulcers.

The results of treatment of corneal ulcers in humans have been more positive. Usually, pFN is prepared from the patients' own plasma, as described by Nishida, et al. (1982). These workers reported enhanced healing of herpetic keratitis using pFN prepared from patients' plasma (Nishida, et al., 1985). Several other groups have also found pFN from patients' plasma to be helpful in healing persistent corneal ulcers; the lesions were from a variety of causes, and the number of patients varied in each study (Harnisch and Sinha, 1985; McCluskey, et al., 1987; Phan, et al., 1987; and Spigelman, et al., 1987).

Ding and Burstein (1988) reviewed the use of pFN in treating corneal ulcers and pointed out the need for a commercial source of pFN for this use. It also seems necessary for a source of cFN for treating corneal ulcers, since cFN is involved in the longer-term reconstruction of the wound after the lesion has been reepithelialized.

Clark (1988) has called attention to the fact that many pFN preparations used by investigators treating wounds may contain growth factors that have not been purified out. Clearly, work on wound repair would benefit from having standardized, well purified, pharmaceutical grade pFN and cFN, with controlled amounts of collagen or procollagen, so that the materials could be applied to different types of wounds at different stages of repair.

Horowitz and Chang (1989) discussed the preparation of pFN for therapeutic use. For those uses where pFN is preferred, such as early treatment of burns, they estimated the need in the U.S. alone at about 100 kg/year. The need for cFN is surely close to that amount.

Summary

The fibronectin family of proteins consists of at least 20 varieties, and perhaps as many as 100, broadly divided into two major forms, pFN and cFN. These molecules have different physical and biological properties, derived directly from their different structure and composition. Their functions are different and it seems reasonable that there will be different therapeutic uses of each of the major forms. Wound healing requires both pFN and cFN to be applied at different times during the healing process. Treatment of malignancy requires cFN. There is a compelling need for commercial quantities of both pFN and cFN in pharmaceutical quality. The material is needed now by researchers investigating periodontal disease, the healing of wounds (corneal, surgical, chronic, and burns), and the malignant process and for the treatment of cancers.

In recent years, there has been a trend away from traditional pharmaceuticals, such as natural or synthetic chemical drugs, to biologically based drugs. These often are proteins or peptides occurring naturally in the body but that may be applied in higher concentration, controlled locations, or certain times, to achieve their therapeutic effect. Genetic engineering companies

are actively pursuing many such proteins. The FNs are too large and glycosylated for manufacture in bacteria, however.

In this era of organ transplant, wherein degenerated organs are replaced, it is inevitable that FN and its related structural molecules will be the next generation of molecular therapy. Chronic, degenerative diseases will be overcome by this innovative new therapy, which is, in a sense, the transplantation of new molecules at the needed site.

References

Akiyama SK, Yamada KM. (1987) Fibronectin. Adv. Enzymol. 59:1-57.

Asaga H, Kikuchi S, Yoshizato K. (1991) Collagen gel contraction by fibroblasts requires cellular fibronectin but not plasma fibronectin. Exp. Cell. Res. 193:167-174.

Balza E, Borsi L, Allemanni G, Zardi L. (1988) Transforming growth factor beta regulates the levels of different fibronectin isoforms in normal human cultured fibroblasts. FEES. Lett. 228:42-44.

Barone MV, Henchcliffe C, Baralle FE, Paolella G. (1989) Cell type specific trans-acting factors are involved in alternative splicing of human fibronectin pre-mRNA. EMBO J. 8:1079-1085.

Bernard MP, Kolbe M, Weil D, Chu ML. (1985) Human cellular fibronectin: Comparison of the carboxyl-terminal portion with rat identifies primary structural domains separated by hypervariable regions. Biochemistry 24:2698-2704.

Blumenstock FA, Saba TM. (1982) Fibronectin: a mediator of phagocytosis and its potential in treating septic shock. Adv. Exp. Med. Biol. 155:613-621.

Burton-Wurster N, Lust G. (1989) Molecular and immunologic differences in canine fibronectins from articular cartilage and plasma. Arch. Biochem. Biophys. 269:32-45.

Burton-Wurster N, Leipold HR, Lust G. (1988) Dibutyryl cyclic AMP decreases expression of ED-A fibronectin by canine chondrocytes. Biochem. Biophys. Res. Comm. 154:1088-1093.

Caffessee RG, Smith BA, Nasjieti CE, Lopatin DE. (1987) Cell proliferation after flap surgery, root conditioning and fibronectin application. J. Periodontol. 58:661-666.

Cheng CY, Martin DE, Leggett CG, Reece MC, Reece AC. (1988) Fibronectin enhances healing of excised wounds in rats. Arch. Dermatol. 124:221-225.

Clark RA. (1988) Potential roles of fibronectin in cutaneous wound repair. Arch. Dermatol. 124:201-206.

Colvin RB. Fibronectin in wound healing. (1989) In: Mosher DF, editor. Fibronectin. New York: Academic Press, Inc; p. 213-254.

Ding M, Burstein NL. (1988) Fibronectin in corneal wound healing. J. Ocul. Pharmacol. 4:75-91.

Doillon CJ, Silver FH. (1986) Collagen-based wound dressing: effects of hyaluronic acid and fibronectin on wound healing. Biomaterials 7:3-8.

Doillon CJ, Silver FH, Olson RM, Kamath CY, Berg RA. (1988) Fibroblast and epidermal cell-type I collagen interactions: cell culture and human studies. Scanning Microsc. 2:985-992.

Doran JE, Lundsgaard-Hansen P, Rubli E. (1986) Plasma fibronectin: relevance for anesthesiology and intensive care. Intensive Care Med. 12:340-349.

Falcone PA, Bonaventura M, Turner DC, Fromm D. (1984) The effect of exogenous fibronectin on wound breaking strength. Plast. Reconstr. Surg. 74:809-812.

ffrench-Constant C, Van De Water L, Dvorak HF, Hynes RO. (1989) Reappearance of an embryonic pattern of fibronectin splicing during wound healing in the adult rat. J. Cell Biol. 109:903-914.

Grinnell F, Toda K, Takashima A. (1988) Role of fibronectin in epithelialization and wound healing. Prog. Clin. Biol. Res. 266:259-272.

Harnisch JP, Sinha PK. (1985) Fibronectins: a possible treatment of therapy-resistant corneal ulcers. Klin. Monatsbl. Augenheilkd. 187:53-56.

Holman JM Jr, Saba TM, Lewis E. (1988) Effect of fibronectin-rich human cryoprecipitate on fluid volume requirements in sheep during postoperative sepsis J. Trauma 28:571-581.

Horowitz B, Chang MY. (1989) Preparation of fibronectin for therapeutic administration. In: Mosher DF, editor. Fibronectin. New York: Academic Press Inc p. 441-455.

Hynes, R. Molecular biology of fibronectin. (1985)

Ann. Rev. Cell Biol. 1:67-90.

Kornblihtt AR, Vibe-Pedersen K, Baralle FE. (1984) Human fibronectin: molecular cloning evidence for two mRNA species differing by an internal segment coding for a structural domain. EMBO J. 3:221-226.

Kubo M, Norris DA, Howell SE, Ryan SR, dark RA. (1984) Human keratinocytes synthesize, secrete, and deposit fibronectin in the pericellular matrix. J. Invest Dermatol. 82:580-586.

Litvinov RI, Izmailov SG, Zinkevich OD, Kochev OS, Ermolin GA. (1987) Effect of exogenous fibronectin on the healing of skin wounds as evidenced by tensiometric data. Biull. Eksp. Biol Med. (USSR) 104:727-730.

Lukinmaa PL, Mackie EJ, Thesleff I. (1991) Immunohistochemical localization of the matrix glycoproteins - tenascin and the ED-sequence-containing form of cellular fibronectin - in human permanent teeth and periodontal ligament. J. Dent. Res. 70:19-26.

Lydon MJ, Foulger CA. (1988) Cell-substratum interactions: serum spreading factor. Biomaterials 9:525-527.

Martin DE, Reece MC, Maher JE, Reese AC. (1988) Tissue debris at the injury site is coated by plasma fibronectin and subsequently removed by tissue macrophages. Arch. Dermatol, 124:226-229.

McCluskey P, Wakefield D, York L. (1987) Topical fibronectin therapy in persistent corneal ulceration. Aust. NZ J. Ophthalmol. 15:257-262.

Nagelschmidt M, Becker D, Bonninghoff N, Engelhardt GH. (1987a) The effect of fibronectin therapy and fibronectin deficiency on healing of rat burns and excision wounds. Res. Exp. Med. 187:217-223,

Nagelschmidt M, Becker D, Bonninghoff N, Engelhardt GH. (1987b) Effect of fibronectin therapy and fibronectin deficiency on wound healing: a study in rats. J Trauma 27:1267-1271.

Newton C, Hatchell DL, Klintworth GK, Brown CF, (1988) Topical fibronectin and corneal epithelial wound healing in the rabbit. Arch. Ophthalmol. 106:1277-1279.

Nishida T, Nakagawa S, Awata T, Nishibayashi C, Manabe R. (1982) Rapid preparation of purified autologous fibronectin eyedrops from patient's plasma. J. J. Ophthalmol. 26:416-424.

Nishida T, Nakagawa S, Manabe R. (1985) Clinical evaluation of fibronectin eyedrops on epithelial disorders after herpetic keratitis. Ophthalmology 92:213-216.

Nishida T, Nakamura M, Mishima H, Otori T. (1990) Differential modes of action of fibronectin and epidermal growth factor on rabbit corneal epithelium. J. Cell Physiol 145:549-554.

Oyama F, Murata Y, Suganuma N, Kimura T, Titani K, Sekiguchi K. (1989) Patterns of alternative splicing of fibronectin pre-mRNA in human adult and fetal tissues. Biochemistry 28:1428-1434.

Pande H, Calaycay J, Lee TD, Legesse K, Shively JE, Siri A. (1987) Demonstration of structural differences between the two subunits of human-plasma fibronectin in the carboxyterminal heparin-binding domain. Eur. J Biochem. 162:403-411.

Paul JI, Schwarzbauer JE, Tamkun JW, Hynes RO. (1986) Cell-type-specific fibronectin subunits generated by alternative splicing. J. Biol. Chem. 261:12258-12265.

Phan TM, Foster CS, Boruchoff SA, Zagachin LM, Colvin RB. (1987) Topical fibronectin in the treatment of persistent corneal epithelial defects and trophic ulcers. Am. J. Ophthalmol. 104:494-501.

Reilly JT, Nash JR. (1988) Vitronectin (serum spreading factor): its localisation in normal and fibrotic tissue J Clin Pathol. 41:1269-1272.

Rutka JT, Giblin JR, Apodaca G, DeArmond SJ, Stern R, Rosenblum ML. (1987) Inhibition of growth and induction of differentiation in a malignant human glioma cell line by normal leptomeningeal extracellular matrix proteins. Cancer Res. 47:3515-3522.

Ryan PC, Waring C, Seymour GJ. (1987) Periodontal healing with citric acid and fibronectin treatment in cats. Aust. Dent. J. 32:99-103.

Schwarzbauer JE, Paul Jl, Hynes RO. (1985) On the origin of species of fibronectin. Proc. Natl. Acad. Sci. 82:1424-1428.

Sekiguchi K, Siri A, Zardi L, Hakomori S. (1985) Differences in domain structure between human fibronectins isolated from plasma and from culture supernatants of normal and transformed fibroblasts. Studies with domain-specific

antibodies. J. Biol. Chem. 260:5105-5114.

Sekiguchi K, Titani K. (1989) Probing molecular polymorphism of fibronectins with antibodies directed to the alternatively spliced peptide segments. Biochemistry 28:3293-3298.

Singh G, Foster CS. (1989) Gross factors in treatment of nonhealing corneal ulcers and recurrent erosions. Cornea 8:45-53.

Skorstengaard K, Jensen MS, Sahl P, Petersen TE, Magnusson S. (1986) Complete primary structure of bovine plasma fibronectin. Eur. J. Biochem. 161:441-453.

Smith BA, Smith JS, Caffesse RG, Nasjieti CE, Lopatin DE, Kowalski CJ. (1987a) Effect of citric acid and various concentrations of fibronectin on healing following periodontal flap surgery in dogs. J. Periodontol. 58:667-673.

Smith B, Caffesse R, Nasjieti C, Kon S, Castelli W. (1987b) Effects of citric acid and fibronectin and laminin application in treating periodontitis. J. Clin. Periodontol. 14:396-402.

Somerman MJ, Sauk JJ, Foster RA, Norris K, Dickerson K, Argraves WK. (1991) Cell attachment activity of cementum: bone sialoprotein II identified in cementum. J. Periodont. Res. 26:10-16.

Soong HK, Hassan T, Varani J, Huang SC, Brennan M. (1989) Fibronectin does not enhance epidermal growth factor-mediated acceleration of corneal epithelial wound closure. Arch. Ophthalmol. 107:1052-1054.

Spigelman AV, Deutsch TA, Sugar J. (1987) Application of homologous fibronectin to persistent human corneal epithelial defects. Cornea 6:128-130.

Spigelman AV, Vernot JA, Deutsch TA, Peyman GA, Molnar J. (1985) Fibronectin in alkali burns of the rabbit cornea. Cornea 4:169-172.

Tamkun JW, Hynes RO. (1983) Plasma fibronectin is synthesized and secreted by hepatocytes. J. Biol. Chem. 258:4641-4647.

Thompson EW, Seymour GJ, Whyte GJ. (1987) The preparation of autologous fibronectin for use in periodontal surgery, Aust. Dent. J. 32:34-38.

Tressel T, Shively JE, Pande H. (1988) Hurhan placental fibronectin: Demonstration of structural differences between the A and B chains in the extra domain-A region. Arch. Bioch. Biophys. 266:639-643.

Uehara A, Isaka Y, Hashikawa K, Kimura K, Kozuka T, Kamada T. (1988) Iodine-131-labeled fibronectin: potential agent for imaging atherosclerotic lesion and thrombus. J. Nucl. Med. 29:1264-1267.

Vaheri A, Keski-Oja J, Vartio T. Fibronectin and malignant transformation. (1989) In: Mosher DF, editor. Fibronectin. New York: Academic Press, Inc.; p. 255-271.

Varani J, Schuger L, Fligiel SE, Inman DR, Chakrabarty. S. (1991) Production of fibronectin by human tumor cells and interaction with exogenous fibronectin: comparison of cell lines obtained from colon adenocarcinomas and squamous carcinomas of the upper aerodigestive tract. Int. J. Cancer 47:421-425.

Vasil'ev SA, Dzhumabaeva BT, Kolesnikova AS, Ermolin GA, Efremov EE. (1987) Plasma heparin precipitate as a source of fibronectin in the treatment of patients with trophic skin lesions. Ter. Arkh. 59:127-130.

Watanabe K, Nakagawa S, Nishida T. (1988) Chemotactic and haptotactic activities of fibronectin for cultured rabbit epithelial cells. Invest. Ophthalmol. Vis. Sci. 29:572-577.

Wikesjo UM, Claffey N, Christersson LA, Franzetti LC, Genco RJ, Terranova VP. (1988) Repair of periodontal furcation defects in beagle dogs following reconstructive surgery including root surface demineralization with tetracycline hydrochloride and topical fibronectin application. J. Clin. Periodontol. 15:73-80.

Wysocki AB, Grinnell F. (1990) Fibronectin profiles in normal and chronic wound fluid. Lab. Invest. 63:825-831.

Yamada KM, Kennedy DW. (1979) Fibroblast cellular and plasma fibronectins are similar but not identical. J. Cell Biol. 80:492-498.