

A Cross-Discipline Paper

The Influence of Tension on Intrinsic Tendon Fibroplasia

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For many years, the belief was held that flexor tendons are relatively avascular structures with sparsely sequestered cells having a structure similar to that of cartilage and therefore unable to initiate or partake in the healing process. It was believed that a new blood supply originating from adjacent tissue was essential for the reparative process and for new collagen to reach the damaged tendon ends by way of adhesions from the paratendinous tissues.

These misconceptions were based largely on some early studies made using the rabbit¹ and canine models.² It was argued that although healing could be demonstrated to take place in a tendon free of adhesion formation, these fibroblasts still arose from the sheath or from the vasculature. Recent evidence, however, suggests that tendons can heal without adhesion formation.³⁻⁷

Therefore, a model has been developed to

study the in vitro healing of flexor tendons and thus allow one to study the tendon isolated from the surrounding sheath and synovial influences. It has been demonstrated that tendons maintained in tissue culture medium are able to heal defects if they are provided with a plasma matrix into which fibroblasts can grow, proliferate, and produce collagen.^{6,7}

In this report, we present observations which show that fibroblasts respond to the effects of tension by dividing more rapidly and aligning themselves along the lines of tension.

Materials and Methods

Profundus tendons were removed from the legs of white leghorn chickens under sterile conditions. The tendons were rinsed in Dulbecco's Modified Eagle Medium (DMEM) and dissected cleanly to remove all mesotendon sheath and other synovial components. Two centimeter segments of tendons were then cut from each tendon and a 2 millimeter window was punched out of the center.

In one group of tendons, these segments were cultured in DMEM free of a plasma clot

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in the window (Fig 1). In a second group, a drop of freshly prepared chicken plasma was placed in the window and allowed to clot. In a third group of tendons, the segment with a clot in the center was placed on a traction apparatus having two rubber bands anchoring the tendon at each end (Fig 2). This apparatus applied approximately 3 g of force.

The specimens were all cultured in DMEM supplemented with 10% fetal calf serum. Under these conditions of cell culture, tendons remain viable for at least six weeks.⁶ Micrographs, electron microscopy, and timed lapse photography were used for recording observations and objective evaluations. Histologic sections were also taken at various timed intervals and stained with hematoxylin and eosin or trichrome reagents

for collagen. At two weeks, the contents of the windows were removed and analyzed for collagen synthesis by a method based upon the specific digestion of collagen using highly purified bacterial collagenase.⁸

Results

In a group incubated without a plasma clot in the window, tendon cells began actively dividing at the cut margins of the window after 4 to 6 days (Fig 3). The tendon fibroblasts became aligned in a palisade like fashion at the edge of the window and continued accumulating at the edge of the window, but they never completely filled the defect (Fig 4).

A totally different pattern was observed in the windows that were filled with the plasma clot. Tendon cells were observed migrating

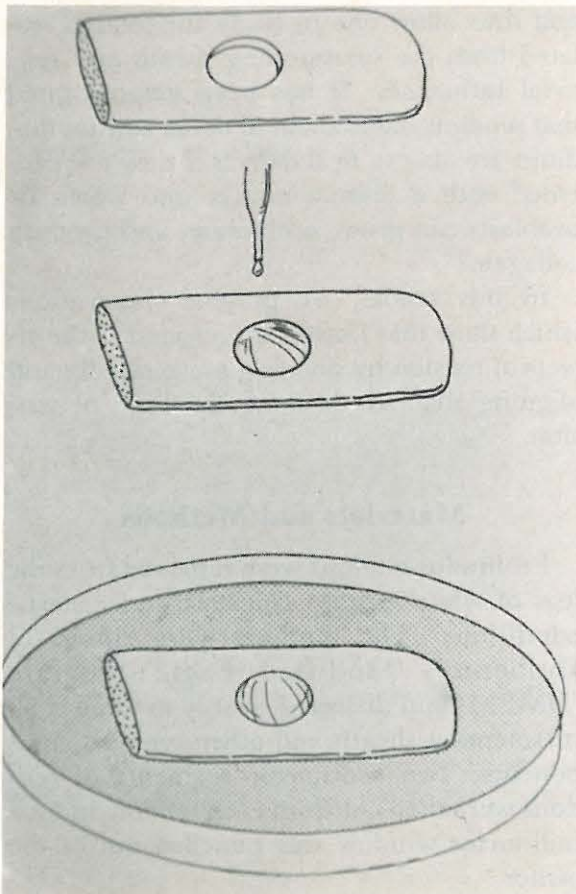


Fig 1. Preparation of the tendon specimen prior to placement in traction apparatus.

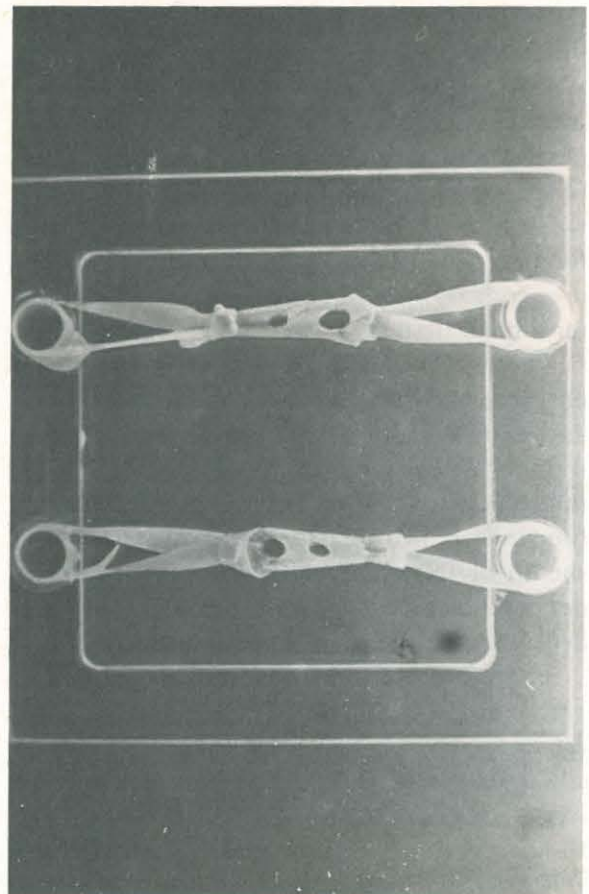


Fig 2. Traction apparatus used to apply tension to tendon healing model in tissue culture.

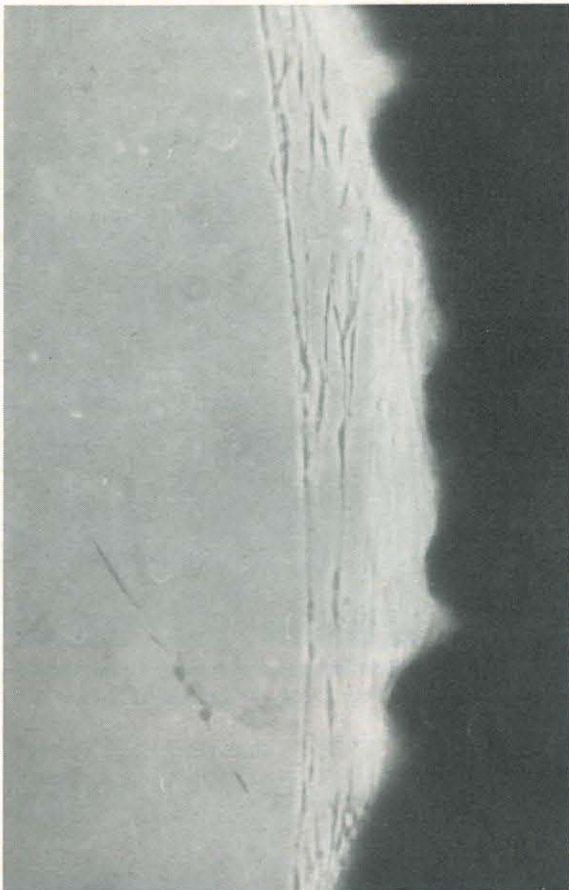


Fig 3. Tendon window incubated without plasma clot. At six days, the fibroblasts are proliferating at the edge of the window.

into the plasma clot in the window by 48 hours (Fig 5). By three weeks, the windows were confluent with tendon fibroblasts (Fig 6), and at six weeks, they were opaque. Electromicrographs of the plasma demonstrated that the cells migrating from the tendon were fibroblasts, easily identified by the characteristic spindle shape morphology, well developed rough endoplasmic reticulum, prominent nucleus containing dark staining nucleoli and the presence of adjacent extracellular collagen (Fig 7).

In addition, specific biochemical analysis confirmed that the tendon fibroblasts observed in the window were synthesizing collagen at the rate characteristic of fibroblasts;

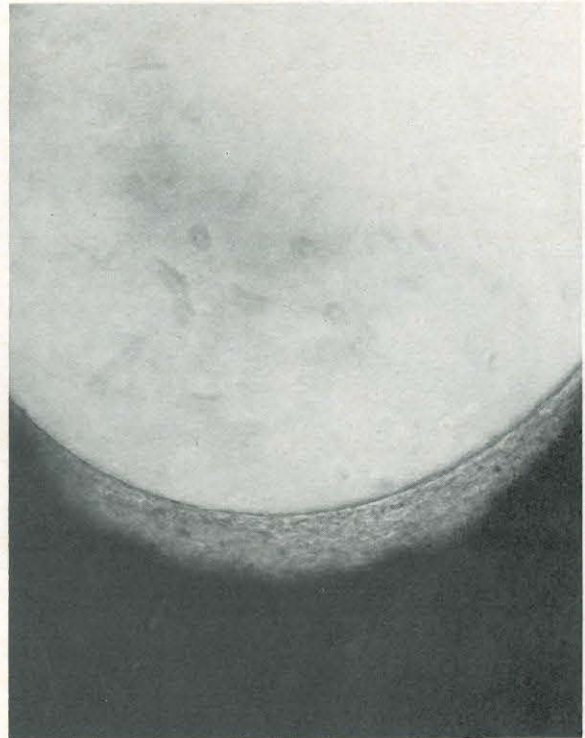


Fig 4. Tendon window incubated without plasma clot. At three weeks, a rim of fibroblasts has formed at the edge of the tendon window.

6% to 10% of the total protein synthesized was represented by collagen.

When the tendon window model was placed under approximately 3 g of tension, it was immediately apparent that the round window was transformed into an oval configuration and fibrin strands became aligned longitudinally along the line of tension (Fig 8a). Fibroblasts were noted to appear at the edge of the window by 48 hours, and the process of proliferation and migration into the clot appeared to be enhanced approximately two-fold in those specimens which were placed under tension (Fig 8b).

Hematoxin and eosin stains of the tendon window sections under tension showed increased cellularity compared to control window sections (Fig 8c).

In addition, these thin sections also clearly demonstrate that fibroblasts are stretched out

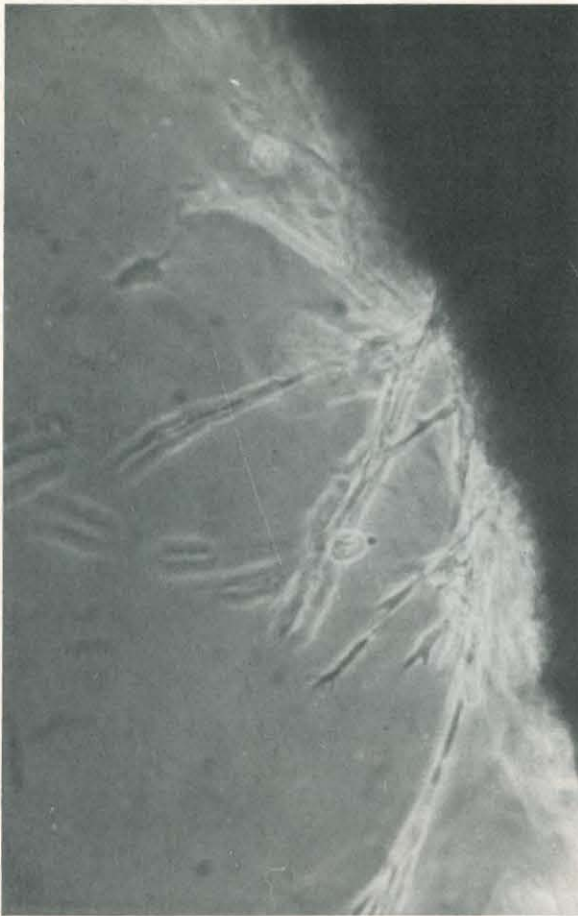


Fig 5. Tendon window filled with plasma clot. At 48 hours the fibroblasts arise from the tendon edge and proliferate and grow into the clot.

parallel to the direction of tension (Fig 9a) compared to the random orientation of the cells and control tendons without tension (Fig 9b).

Transmission electromicrographs revealed highly active fibroblasts in the windows of the tendons under tension which were oriented parallel to the lines of stress and surrounded by numerous collagen fibers, also aligned in the direction of the cell membranes (Fig 10).

Discussion

There has been considerable controversy concerning the capacity of tendons to heal in-

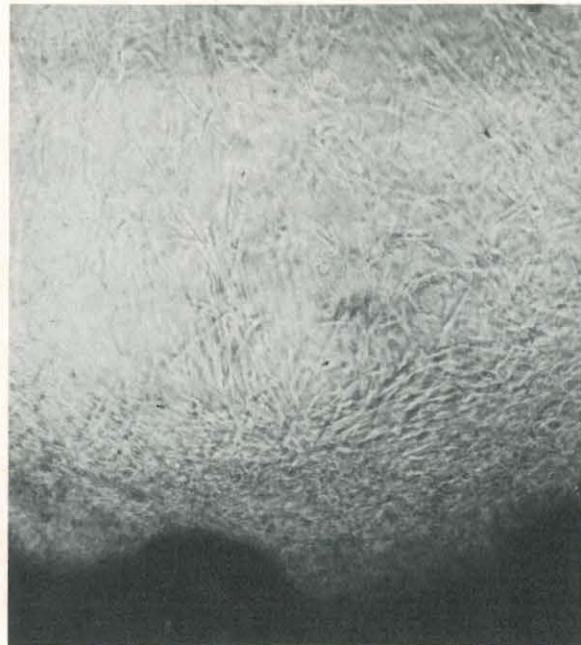


Fig 6. Tendon window incubated with plasma clot. At three weeks, the window is confluent with tendon fibroblasts and collagen.



Fig 7. Electron micrograph shows the fibroblasts. No tension has been applied.

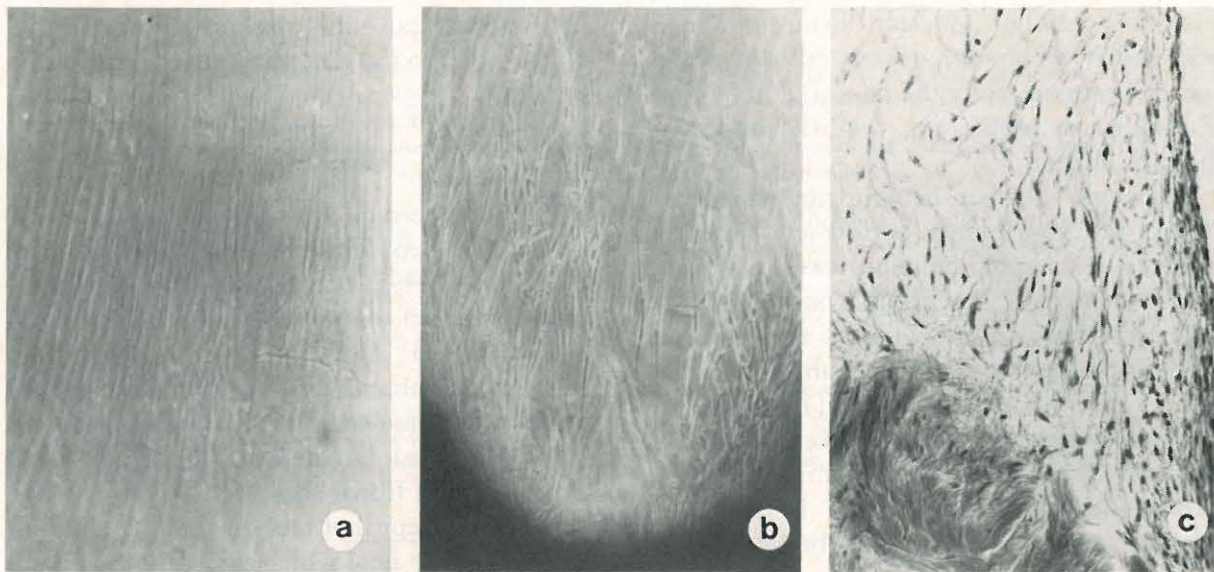


Fig 8. Fibrin strand (a) becomes aligned longitudinally when tension is applied to the clot. When placed under tension, the circular window (b) assumes an oval shape and fibroblasts align themselves in a parallel fashion in the direction of tension. (c) Tension has been applied. Note fibroblasts are more numerous, are aligned in a parallel fashion and the collagen deposition is in progress.

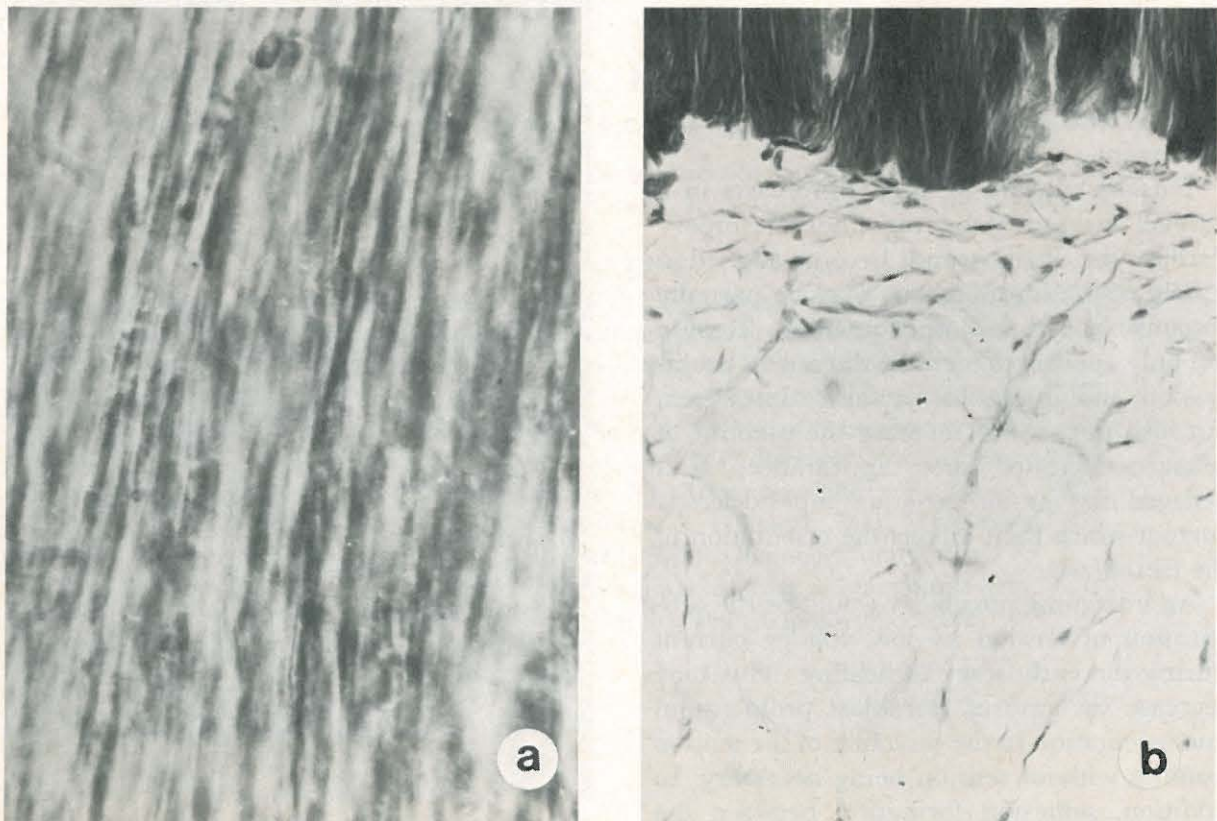


Fig 9. (a) Higher power magnification of the window at three weeks. Tension has been applied. Note fibroblasts are more numerous. They are aligned in a parallel fashion and the collagen deposition is in progress. (b) No tension has been applied here.

trinsically without adhesion formation.¹⁻⁷ To help clarify this issue, a tissue culture model was designed to study the potential of tendon fibroblasts to proliferate, migrate, and synthesize collagen.^{6,7}

Although intrinsic healing may have occurred in experiments performed by early workers,^{1,2} this phenomenon was completely overshadowed by the prolific paratendinous tissue reaction. Recent evidence suggests that tendons possess an intrinsic healing potential which can only be demonstrated under exacting conditions. Tendons have also been shown to heal when placed in an isolated environment (prepatellar bursa).⁵ In addition, intrinsic tendon healing has been demonstrated to occur in repaired tendons with early active motion applied.⁹

The present study further demonstrates that tendons contain active fibroblasts which are able to proliferate, migrate from the cut edge of the tendon, and then produce collagen. This process of fibroplasia is enhanced by the presence of a plasma clot which provides a fibrin matrix for cell attachment and movement.

Tension appears to align fibroblasts in a parallel fashion along the lines of force.¹⁰ Perhaps the fibrin strands become aligned in a field of tension and thus may be partially responsible for this phenomenon. Tension not only appears to increase the rate of proliferation and migration of these fibroblasts, but also appears to increase the amount of collagen deposited (gross appearance). It is believed that tension generates a piezoelectric current which then directs the orientation of the fibroblasts.

An intriguing possibility would be the substitution of tension by low voltage current during the early stage of healing. This may increase the rate of fibroblast proliferation and orientation in the direction of the tendon bundles without tension being necessary. In addition, adhesion formation between the tendon and surrounding sheath would be dis-

couraged because this alignment would be at right angles to the direction of the force of the current.

Conclusions

Tendons contain fibroblasts that have the potential to proliferate, migrate, and produce collagen. These processes of fibroplasia occur in the absence of an intact circulation and synovial fluid. Also, the tendon fibroblastic response is enhanced by the presence of a fibrin rich plasma clot. It has also become evident that tension enhances fibroplasia and orientation of fibroblasts. These studies support the concept that tendons have an intrinsic capacity to heal which may be modulated by tension (piezoelectric) or electric forces.



Fig 10. *Highly active fibroblasts under tension parallel to lines of stress. Collagen fibrils are also in a parallel fashion.*

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Emmett M. Lunceford, Jr., Heads Eastern Orthopaedic, Annual Meeting and Hands-on-Course October in Mexico

Emmett M. Lunceford, Jr., MD is the new president of the Eastern Orthopaedic Association. He replaces William T. Green, Jr., MD who is with the Children's Hospital in Pittsburgh, Pennsylvania.

Dr. Lunceford who is with the Moore Clinic in Columbia, South Carolina, will serve until the 15th Annual Meeting of the EOA which will be held October 10-14 in Acapulco, Mexico. The annual meeting will be preceded by a hands-on-course: "Intramedullary Fixation of Fractures," that will be held October 8-10.

Other newly elected officers are: First Vice President, John F. Mosher, MD, Upstate Medical Center, Syracuse, New York, who will become president next year. Second Vice President, B. David Grant, MD, Broomall, Pa. Secretary, Murray R. Glickman, MD, Philadelphia, Pa. Treasurer, Henry R. Cowell, MD, Wilmington, Delaware. Historian, Andrew G. Hudacek, MD, Morristown, N.J. Managing Director, Robert N. Richards, MD, Chambersburg, Pa.

For additional information about the annual meeting and the course write: Eastern Orthopaedic Association, 301 S. 8th St., Philadelphia, Pa. 19106.