Fibroblast Gel Culture: A Model for Biochemical Investigations of Dupuytren's Contracture

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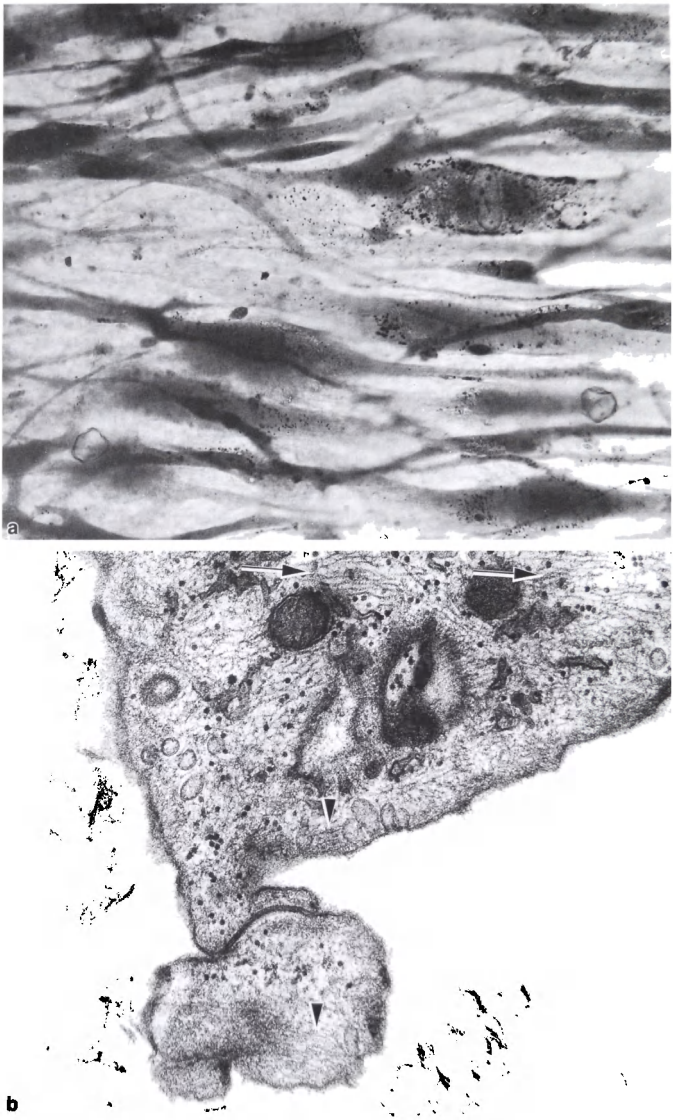
Introduction

In Dupuytren's contracture a more or less heterogeneous cell population proliferates and infiltrates the palmar fascia. To investigate the biochemistry and metabolism of a distinct cell population involved in the pathogenesis of Dupuytren's contracture, cell culture systems offer the opportunity to study metabolism independent of local or exogenous factors interfering with the regulation of cell functions. Monolayer cell cultures are widely used to study reaction mechanisms on the cellular level, in particular in fibroblasts. However, the morphologic phenotype of fibroblasts, their arrangement within the extracellular matrix, and their metabolic characteristics differ in monolayer cultures compared to in connective tissues in situ. In monolayer cultures, fibroblasts are confluent with extensive side to side contacts without the appropriate extracellular matrix between the cells. By contrast, in situ, fibroblasts are embedded in and surrounded by a specific matrix and the cells have cell to cell contact via thin processes which extend into the matrix, touching the neighboring cells with their tips. Therefore, a culture system which offers all the requirements mentioned above would be useful. A fibrin gel culture system proved to be the most suitable system for growing fibroblasts in an in vivo like fashion.

Methods

Isolation of Cells from Human Connective Tissue. The cells were incubated with collagenase for varying times corresponding to the age of the donor (3 × 30–40 h, 37°C), filtered through glass fibers, centrifuged and washed with minimum essential medium (MEM) containing 10% fetal calf serum (FCS). They were then resuspended in MEM/FCS and counted in a cell counter.

Fibrin Gel Preparation. A suspension containing 1.5 mg/ml human fibrinogen, 6.0 µg/ml human fibronectin, 1 U/ml factor XIII, 2 mmol/l calcium, MEM/FCS 10%, and 150 000 cells/ml was dispensed into culture dishes coated with a thin film of high temperature agarose. Coagulation was started by adding 0.1 U/ml of thrombin.


a
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Incubation. The cell/fibrin gel system was incubated at 37°C under 5% CO_2/95% air, with a medium change every third day.

Results

Using collagenase to free the cells gives a high yield of viable cells. Suspending the cells in a fibrinogen-MEM solution is necessary to get an equal three-dimensional distribution of cells in the gel. Gel formation is initiated by the addition of thrombin, immediately after dispensing the fibrinogen/cell suspension, and completed by factor XIII, present in the suspension. The gel is stable and easy to handle for medium changes, etc. The protein gel matrix facilitates reisolation of the fibroblasts or separation of glycosaminoglycans for further analysis.

Figure 1a shows a fibroblast gel culture taken at the 110th day of culture, Fig. 1b an electron micrograph of the cells in culture. The figure clearly demonstrates the in vivo like three-dimensional arrangement of the Dupuytren's contracture cells embedded within the extracellular matrix. The cells exhibit all the morphological characteristics of viable fibroblasts including gap junctions between the tips of the cell extensions and contractile microfilaments. The fibroblasts have been continuously cultured without subculturing for more than 130 days. Reisolation of the cells after 80 days and reestablishing the gel culture allowed continuation of the cell culture with the still viable cells.

Employing labeled precursors it could be shown that the cultured fibroblasts synthesize glycosaminoglycans and collagen at a considerable rate (Fig. 2). Incorporation of label into DNA reflects the growth activity of the cells. After a growth period of about 50 days, [^{3}H]thymidine incorporation reaches a steady state, indicating that cell replacement rather than proliferation is occurring. Proteoglycan and collagen synthesis remain unchanged, as demonstrated by the incorporation rates of the respective precursors [^{35}S]sulfate and [^{3}H]-hydroxyproline. Therefore, we conclude that, as in connective tissues in vivo, fibroblasts in gel culture reach a steady state of cell growth and death but continue to synthesize extracellular matrix. Thus, the fibrin gel culture system affords a preincubation time of at least 50 days before the cells reach steady state and experiments can be started. Due to the limited number of experiments carried out so far, a result of the lengthy culture times and labor intensive handling involved, it is not possible to interpret the data as to possible metabolic differences between Dupuytren's contracture cells and

Fig. 1a,b. Culture of cells from Dupuytren's contracture in a fibrin gel (110th day of culture). The cells were fixed with glutaraldehyde in sodium cacodylate buffer and then with osmium tetroxide in the same buffer. **a** The myofibroblasts form a three-dimensional network. **b** Gap junctions connect the cells which contain, beside organelles, abundant thin (*arrowheads*) and intermediate (*arrows*) filaments. **a** ×310; **b** ×40000

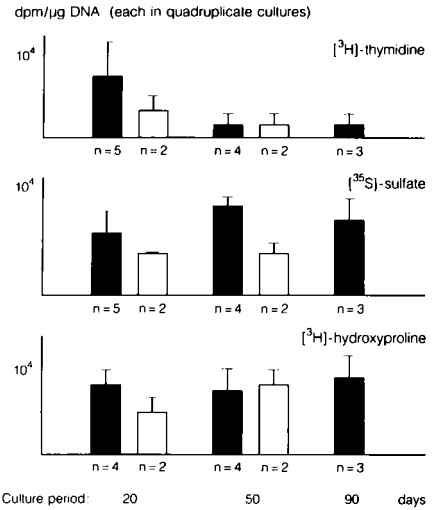

Fig. 2. Labeled precursor incorporation into extracellular components and DNA in fibrin gel cell culture of cells (90 days continuous culture) from Dupuytren's contracture. *Filled columns*, Dupuytren's contracture cells; *open columns*, palmar fascia cells

controls. It may be that Dupuytren's contracture cells show a slightly higher [^{35}S]sulfate incorporation than palmar fascia cells.

As shown by Flint et al. [1] and our laboratory [2], the glycosaminoglycan pattern in palmar fascia undergoes characteristic changes during development of the contracture. In comparing the glycosaminoglycans in biopsies from Dupuytren's contracture, from healthy palmar fascia (Fig. 3), and in the respective cell culture matrices prepared according to Gurr et al. [3], the glycosaminoglycan distribution in the gel culture matrix differs from that found in the tissues of both Dupuytren's contracture and palmar fascia. Thus, cells in fibrin gel culture express a unique phenotype, determined solely by the experimentally provided extracellular matrix. Cells from healthy palmar fascia and Dupuytren's contracture exhibit almost the same pattern of glycosaminaglycan expression (Fig. 4).

The pattern of glycosaminoglycan expression in cells in culture resembles that occurring in fetal connective tissues and in the early stages of wound healing. The remarkably small proportion of dermatan sulfate more or less indicates the absence of small proteoglycans in the extracellular matrix of the respective cell cultures. This may be due to the lack of an appreciable amount of collagen fibers synthesized at this stage of culture, as shown in the electron microscopic examinations. However, preliminary data from our attempts to isolate proteo-

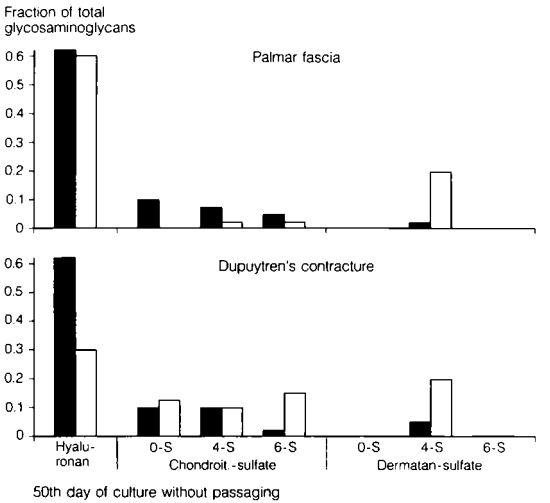

Fig. 3. Glycosaminoglycan patterns in fibroblast gel culture (*filled columns*) and tissue biopsy (*open columns*)

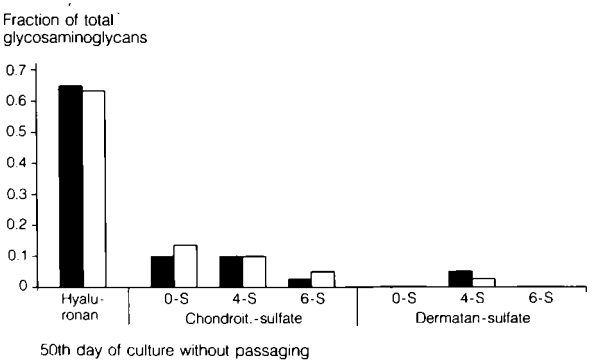

Fig. 4. Glycosaminoglycan patterns in fibroblast gel cultures and biopsies of healthy palmar fascia and Dupuytren's contracture. *Open columns*, Dupuytren cells; *filled columns*, palmarfascia cells

glycans from the gel culture matrices suggest the presence of both large and small proteoglycans.

Discussion

In the experimental extracellular matrix, the cells are surrounded by and under the influence of a large amount of fibrin, some fibronectin and factor XIII, beside the fetal calf serum. The former three components are known to stimulate proliferation of fibroblasts, thus providing an environment for the cells which is very close to the one found at the early stage of wound healing. In addition to the proliferation stimulating activity of fibrin, fibronectin and factor XIII, other, so far unknown modulating activities, may contribute to the expressed phenotype. Factors present in fetal calf serum act on the cells in a similar way. The result of the multifactorially determined interaction between extracellular matrix and the cells under investigation in this system is, among others, aberration of glycosaminoglycan synthesis, as demonstrated in the expression pattern observed in these experiments. If this is indeed true, then cells isolated from Dupuytren's contracture biopsies have not undergone permanent alterations of their metabolic and morphological characteristics in vivo. Instead, they remain able to express a different phenotype depending on the environment they are allowed to grow in.

Further investigations should answer the numerous questions emerging from these findings and further characterize this model for studies on the cellular level in connective tissue research. A variation in the composition of the experimental gel matrix should provide an improved in vivo like model for growing cells from Dupuytren's contracture and, at the same time, yield more information about mediators which actually determine the phenotype of these cells in situ and in vitro.

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