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From Cell Biology to Tissue Engineering

The influence of Matrigel™ or growth factor reduced Matrigel™ on human intervertebral disc cell growth and proliferation

B.J. Desai, H.E. Gruber and E.N. Hanley Jr.
Department of Orthopaedic Surgery, Carolinas Medical Center, Charlotte, NC, USA

Summary. Matrigel™ (reconstituted basement membrane extract) is a potent inducer of cell growth and differentiation in vitro. This study examined phenotypic variation and proliferative responses of human annular intervertebral disc cells in vitro in Matrigel™ and Growth Factor Reduced Matrigel™ (GFR-Matrigel™). Cells from age- and gender-matched control subjects and patients with degenerative disc disease were grown either on the surface of, or suspended within, either matrices. Disc cells grew well on top of both matrices with cells spontaneously forming cell projections. Cells grown within either matrix migrated within the gel to form colonies. Increased colony formation within the matrices was seen with young control and patient cells ($p<0.05$). Old and young control and patient cells showed increased proliferation within GFR-Matrigel™ compared to Matrigel™. When grown on the matrix surface, young patient and control donor cells showed increased proliferation on GFR-Matrigel™ compared to Matrigel™. Cellular proliferation was significantly greater inside a 3-dimensional environment than a two-dimensional surface monolayer environment. Disc cells had increased proliferation when grown in or on GFR-Matrigel™ compared to Matrigel™. These studies serve as a baseline for subsequent investigations regarding effects of cytokines on disc cells and increase our knowledge of the influence of extracellular matrices on disc cell proliferation.

Key words: Matrigel, Disc, TGF-ß, EGF, IGF-1, PDGF, Cell proliferation

Introduction

The cell biology of the human intervertebral disc cell has been neglected compared to knowledge available on bone and chondrocyte cell populations. The etiology of degenerative disc disease remains unclear; however, disc cell research is of importance since low back pain and degenerative disc disease are the primary cause of disability in individuals under the age of 40 (Hanley, 1992). Studies indicate that since the adult disc is avascular (Crock et al., 1988), disc cells are kept viable by nutrients which move by diffusion from vasculature at the disc margin (Eyre et al., 1988). Estimation of diffusion gradients in explants of disc tissue has been carried out (Maroudas et al., 1975), but little is understood about individual disc cell nutrition in the healthy or diseased disc. We hypothesize that reduced availability of growth factors to disc cells (which follows as a consequence of the avascular state of the adult human disc) plays a major role in the etiology of degenerative disc disease and wish to investigate whether growth factor exposure improves cell proliferation in vitro.

Cell phenotypes are determined by internal genetic programs and also by important external signals which come to the cell from the organ and tissue micro-environment. Studies on cell interactions with the extracellular matrix show that the latter can be critical for differentiation of cultured cells (Hadley et al., 1990; Hohn et al., 1992). Matrigel™ matrices are commercially available as a soluble basement membrane extract of the Engelbreth-Holm-Swarm tumor; this product gels at room temperature to form a reconstituted basement membrane similar to the *in vivo* basement membrane. Matrigel™ is a potent in vitro inducer of cell growth and differentiation in a number of cell types (Hadley et al., 1985; Kubota et al., 1988). Matrigel™ contains multiple growth factors (0-0.1 pg/ml basic fibroblast growth factor; 0.5-1.3 ng/ml epidermal growth factor; 15.6 ng/ml insulin-like growth factor-I; 12 pg/ml platelet-derived growth factor; <0.2 ng/ml neuronal growth factor; and 2.3 ng/ml transforming growth factor-ß). Growth Factor Reduced Matrigel™ (GFR-Matrigel™) contains the same growth factors present in reduced concentrations (0-0.1 pg/ml basic fibroblast growth factor; <0.5 ng/ml epidermal growth factor; 5 ng/ml insulin-like growth factor-I; <5 pg/ml platelet-derived growth factor; <0.2 ng/ml neuronal growth factor; and

Offprint requests to: Helen E. Gruber, Ph.D., Orthopaedic Research Biology, Cannon 3rd Floor, Carolinas Medical Center, P.O. Box 32861, Charlotte, NC 28232, USA. Fax: (704) 355-2845. e-mail: hgruber@carolinas.org

1.7 ng/ml transforming growth factor-ß).

Evidence suggests that physical properties of the extracellular matrix are important, especially in three-dimensional flexible substrates which favor cell specific differentiation (Barcellos-Hoff and Bissell, 1989; Hohn et al., 1995). In order to determine the influence of the Matrigel™ set of growth factors on disc cells, we examined cell growth, proliferation and morphology in Matrigel™ or GFR-Matrigel™ with cells grown either on the surface of, or suspended within, each matrix.

Materials and methods

Clinical specimens

Cells were derived from intervertebral discs of age- and gender-matched control subjects and patients with degenerative disc disease. Studies were approved by the Institutional Review Board. Control specimens were obtained from the Cooperative Human Tissue Network. Young subjects studied in this presentation were a 42 year old male patient with a history of a herniated disc (L4-L5), age-matched with a 41 year old control male (cause of death adenocarcinoma of the colon). Older subjects were a 67 year old female patient with a lumbar interbody fusion (L3-L4, L4-L5), age-matched with a 75 year old control female (cause of death coronary artery disease).

Tissue culture

Primary cultures were grown as previously described (Gruber et al., 1997). Briefly, cells were grown from explants of minced portions of the outer annulus in sterile modified Minimal Essential Medium with Earle's salts (MEM, GIBCO BRL/Life Technologies, Gaithersburg, MD) containing 1% (v/v) L-glutamine, 1% (v/v) penicillin-streptomycin penicillin and 1% (v/v) non-essential amino acids (Irvine Scientific, Santa Ana, CA), in a humidified 37 °C atmosphere with 5% CO_2/95% air. For initial cell establishment, 20% fetal bovine serum (GIBCO BRL/Life Technologies, Gaithersburg, MD) was added. Primary cultures with confluent outgrowth of cells were trypsinised (1:250, trypsin (0.5 g/l), EDTA (0.2 g/l) (Irvine Scientific, Santa Ana, CA) and a split ratio of 1:4 used for subculturing. Cell viability was determined by trypan blue exclusion. Cells used in these experiments were passage four. Fifty thousand cells were seeded within or on the surface of Matrigel™ or Growth Factor Reduced Matrigel™ (GFR Matrigel™) (Collaborative Biomedical Products/Becton Dickinson Labware, Bedford, MA). Cultures were maintained for 4 or 8 days and fed every other day as described below.

Coating procedures using Matrigel™ or GFR Matrigel™

A thick gel coating method was used to coat 24 well plates or Costar Transwell Inserts (Costar, Cambridge, Mass.). The two matrices were allowed to thaw at 4 °C overnight. Matrices were mixed to homogeneity in cooled pipettes. <u>Cells layered on top of Matrigel™ or GFR Matrigel™</u>: Matrigel™ or GFR Matrigel™ matrix was diluted 1:1 (v/v) with cold MEM containing no serum (Serum Free Media; SFM) using cooled pipettes. 0.2 ml of either matrix were added per square centimeter. Plates were placed at 37 °C for 30 minutes to allow the matrices to gel. Trypsinised cell cultures were assayed for cell viability, the required volume of cell suspension centrifuged at 500 rpm for 5 minutes in an IEC MP4R centrifuge, and cells resuspended in MEM with 20% foetal bovine serum at a concentration of $1x10^6$ cells per ml. Cells were mixed by gentle thorough pipetting and 50 μl of the cell suspension placed on top of gelled Matrigel™ or GFR Matrigel™. Two ml of SFM were added to each well and cells left at 37 °C for 48 hours. Cells were assayed for subsequent studies as described below and fed with SFM every two days. <u>Cells suspended within Matrigel™ or GFR Matrigel™</u>: Matrigel™ or GFR Matrigel™ was diluted with SFM as described above. Trypsinised cell cultures were assayed for cell viability and the required cell suspension centrifuged and media aspirated off. An appropriate volume of the diluted Matrigel™ or GFR Matrigel™ solution was added to attain a concentration of $1x10^6$ cells/ml. Cells were mixed in either matrix with gentle thorough pipetting to ensure homogeneity. Costar Transwell Clear Inserts were placed in 24-well plates, and the desired amount of matrix/cell suspension was carefully added to the bottom of the membrane ensuring that no air bubbles were formed. Fifty μl of the matrix/cell suspension was added. Plates containing the inserts were placed at 37 °C for 30 minutes to allow the matrix/cell suspension to gel. Each insert was carefully lifted with sterile forceps and 2 ml SFM added to each well. Cells grew for 48 hours at 37 °C. Cells were fed with SFM every two days and assayed for subsequent studies as described below.

Recovery of cells from ungelled matrix/cell suspension

To obtain a cell suspension from the two matrices, wells were rinsed twice with Phosphate Buffered Saline (PBS), (1 min/rinse), and aspirate removed. For cells placed in the inserts, the rinse solution was not aspirated off. After the second rinse, liquid was wicked from the inserts using a sterile gauze. 0.2 ml per square centimeter of Dispase (Collaborative Biochemical Products/ Becton Dickinson, Bedford, MA) was added. Plates were incubated at 37 °C for 2 hours to ensure complete dissolution of either matrix, and the reaction stopped by addition of 300 μl (cells inside either matrices) or 600 μl (cells on top either matrices) 5 mM EDTA. Contents were transferred to a culture tube and assayed for DNA and cell proliferation as described below.

DNA assay

One ml of lysis buffer (1M Sodium Chloride/0.1%

Triton-X 100/0.01% Trypsin Inhibitor soybean Type II, Sigma Chemical Company, St. Louis, MO) was added to each sample and the tubes incubated at room temperature for 5 minutes. Samples were placed at -80 °C for 15 minutes, thawed, vortexed, and 200 μl Proteinase K solution (5 mg/ml)) Sigma Chemical Company) added. Tubes were incubated overnight at 60 °C in a shaking water bath and assayed for total DNA content using the Picogreen™ method (Molecular Probes Inc, Eugene, OR).

Tritiated thymidine incorporation assay

2 μCi/ml [^{3}H]-thymidine were added 24 hours prior to sampling. Cells were solubilized in 200 mM sodium hydroxide (Sigma) at 58 °C overnight before the assay was performed. Solubilized cell layers were placed at 4 °C and precipitated by the addition of an equal volume of ice-cold 10% trichloroacetic acid (Sigma). Precipitates were collected on glass filters, rinsed twice with ice-cold 5% trichloroacetic acid and [^{3}H]-thymidine incorporation determined by liquid scintillation spectrometry. Quantitative proliferation results were expressed as counts per minute (cpm) [^{3}H]-thymidine incorporation per μg DNA.

% Colony Forming Unit Assay (%CFU)

Fifty thousand cells in or on the surface of Matrigel™ or GFR Matrigel™ were utilized and cultured for 8 days as described above. Phase contrast photomicrographs were taken to record the same sites at daily intervals. Photomicrographs (x335 magnification) were scored and the number of single cells or colonies (two or more cells) recorded. %CFU was determined by the following equation;

$$\%CFU = \frac{\text{Number of multi-celled colonies}}{\text{Total number of cells or colonies}} \times 100$$

Morphological studies

Transmission electron microscopy studies utilized preparations fixed in one tenth strength Karnovsky's fixative, post-fixed in osmium tetroxide supplemented with 0.1% (w/v) ruthenium red, embedded in Spurr resin, thin sectioned with an LKB ultramicrotome, grid stained, and viewed in a Phillips CM10 electron microscope. For cells grown on top of the two matrices preparations were fixed in one tenth strength Karnovsky's fixative, post-fixed in osmium tetroxide supplemented with 0.1% (w/v) ruthenium red, pelleted at 8,000 rpm in a microcentrifuge for 2 minutes, embedded in Spurr resin, thin sectioned with an LKB ultra-microtome, grid stained, and viewed in a Phillips CM10 electron microscope.

Statistical analysis

Data are presented as mean±SEM (n) derived from 3-5 replicate samples/treatment. Statistical analyses utilized Student's t-test for proliferation data and ANOVA for CFU data. If a p value of 0.05 or below was found, cells were further analyzed by Student-Newman-Keuls test for pairwise differences. $p<0.05$ was considered significant. SAS, version 6.11, was the statistical computing package employed.

Results

Differences in disc cell growth with Matrigel™ or GFR-Matrigel™ were detected when cells were grown either on the surface or suspended within each of the matrices.

Cells on top of Matrigel™ or GFR-Matrigel™

Both patient and control human disc cells grew well on top of Matrigel™ (Fig. 1a), and GFR-Matrigel™

Fig. 1. Photomicrographs of cells from the young control donor growing on top of Matrigel™ **(a)** or GFR-Matrigel™ **(b)**, 7 days in culture. Note the presence of long thin cytoplasmic projections which extend to neighboring cells. x 335

(Fig. 1b). Within 48 h after plating, control and patient disc cells on Matrigel™ or GFR-Matrigel™ spontaneously formed colonies and individual cells had thin cytoplasmic projections. This feature was present through 7 days of culture; by day 8 cells had migrated within the gel surface and appeared more as monolayer cultures. Electron micrographs of cells grown on top of either matrix possessed thin cytoplasmic projections which appeared less extensive than the in vitro patterns because of pelleting of these cultures during cell processing (Fig. 2).

Quantitative analysis of %CFU did not demonstrate any significant differences over 7 days for proliferation either on Matrigel™ or GFR-Matrigel™ (Fig. 3A,B). Both patient and control cells formed CFU on both matrices. By day 3, cells from the young control donor showed an increase in%CFU when grown on Matrigel™ or GFR-Matrigel™ (40±4.6%, and 50±7% respectively; mean±SEM). This increase continued through day 5 and 6, but by day 7 decreased to 41±6% (Matrigel™) and 43±4% (GFR-Matrigel™). Similarly, cells from the young patient showed an increase in %CFU at day 3 on

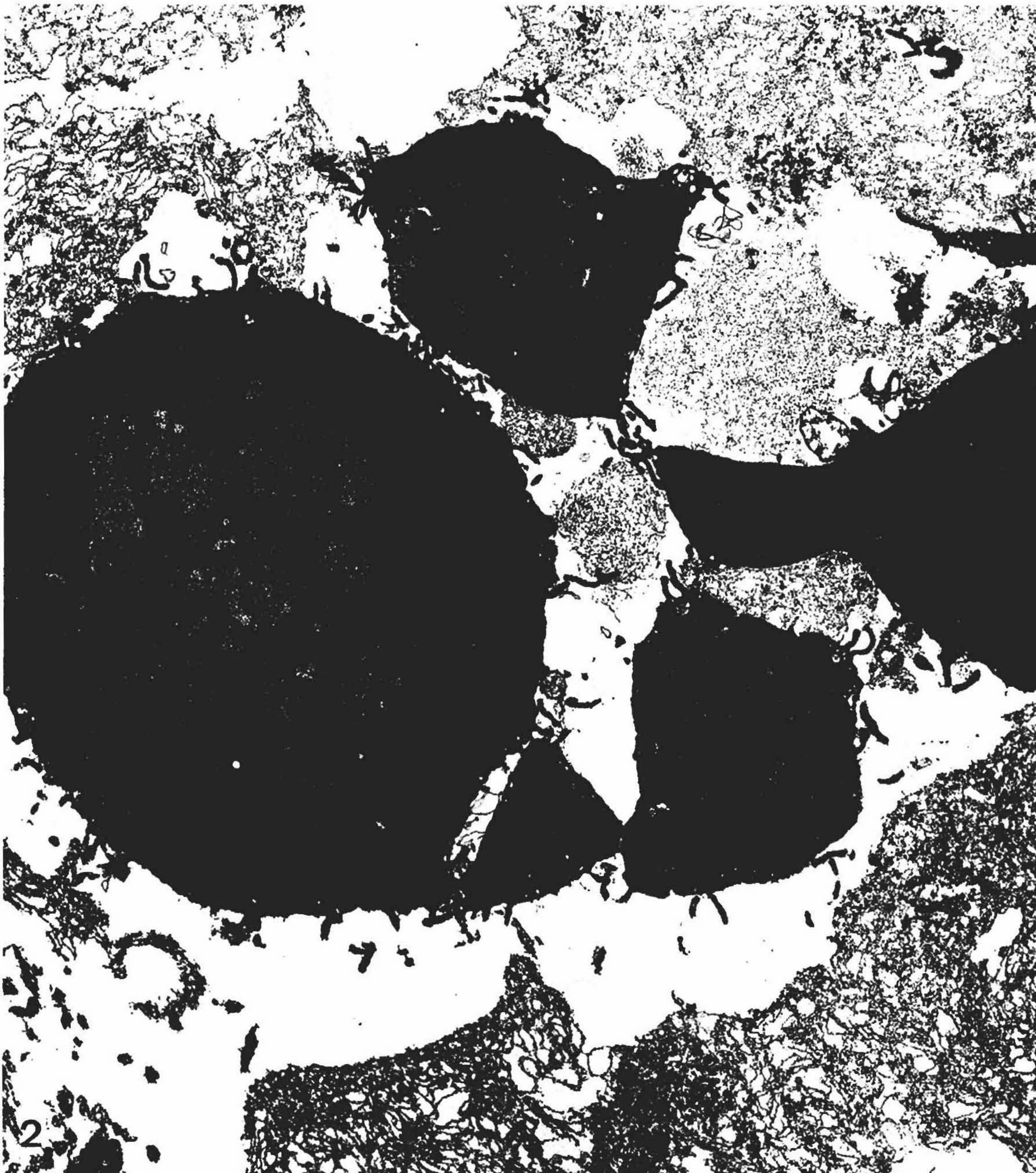

Fig. 2. Electron micrograph of disc cells from the young control donor growing on top of Matrigel™, 8 days in culture. Cells show cytoplasmic projections. x 10,725

Matrigel™ (44±13%) and GFR-Matrigel™ (36±5%); however, no significant changes were present, and %CFU values at day 7 reached 49±8% (Matrigel™) and 56±25% (GFR-Matrigel™) (Fig. 3A). Cells from the old control donor showed no significant differences over the growth period studied and reached CFU values of 62±16% (Matrigel™) and 54±10% (GFR-Matrigel™) respectively at day 7. Disc cells from the older patient showed CFU ability which by day 7 reached 37±5% (Matrigel™), whilst on GFR-Matrigel™ values reached 54±15% (Fig. 3B).

When the proliferative responses of both old and young patient and control donor cells were assessed, significant changes were observed for young control and patient cells on GFR-Matrigel™ after 4 days in culture. Cells from the young patient showed significantly increased proliferation on GFR-Matrigel™ compared to Matrigel™, ($p<0.001$, Table 1). Cells from the young control donor also showed significantly increased proliferation on GFR-Matrigel™ compared with the response on Matrigel™; $p=0.006$ (Table 1). The older patient and control donor cells showed some proliferative response on Matrigel™ at day 4 (6007±2005 and 1814±488, respectively), which dropped to 2839±303 (patient cells) and 800±185 (control cells) on GFR-Matrigel™, but no significant differences from control was seen in growth of these cells on the two matrices. By day 8 no significant differences in tritiated thymidine incorporation/μg DNA were seen for the young patient or young control donor cells between the two matrices (Table 1). Only the older patient cells showed a significantly greater proliferative response at day 8 on Matrigel™ compared to GFR-Matrigel™ ($p=0.005$). When comparing the growth of these cells on

Table 1. Proliferative response of cells Grown on the surface of Matrigel™ or GFR-Matrigel™.

	DAY 4			DAY 8		
	Matrigel™	GFR-Matrigel™	p value	Matrigel™	GFR-Matrigel™	p value
Young Patient	1274±180	7762±487	< 0.001*	7035±1145	8710±1241	NS
Old Patient	6007±2005	2839±303	NS	18747±2721.2	4352±747	0.005*
Young Control	896±9264	6945±1188	0.006*	9263.6±1053.4	12492±1019	0.059
Old Control	1814±488	800±185	NS	7956±1657	5911±1894	NS

Data are mean±SEM of 3-5 replicate wells. *: denotes significantly different proliferation of each subject's cells when grown on the surface of Matrigel™ versus GFR-Matrigel™. NS: not significant

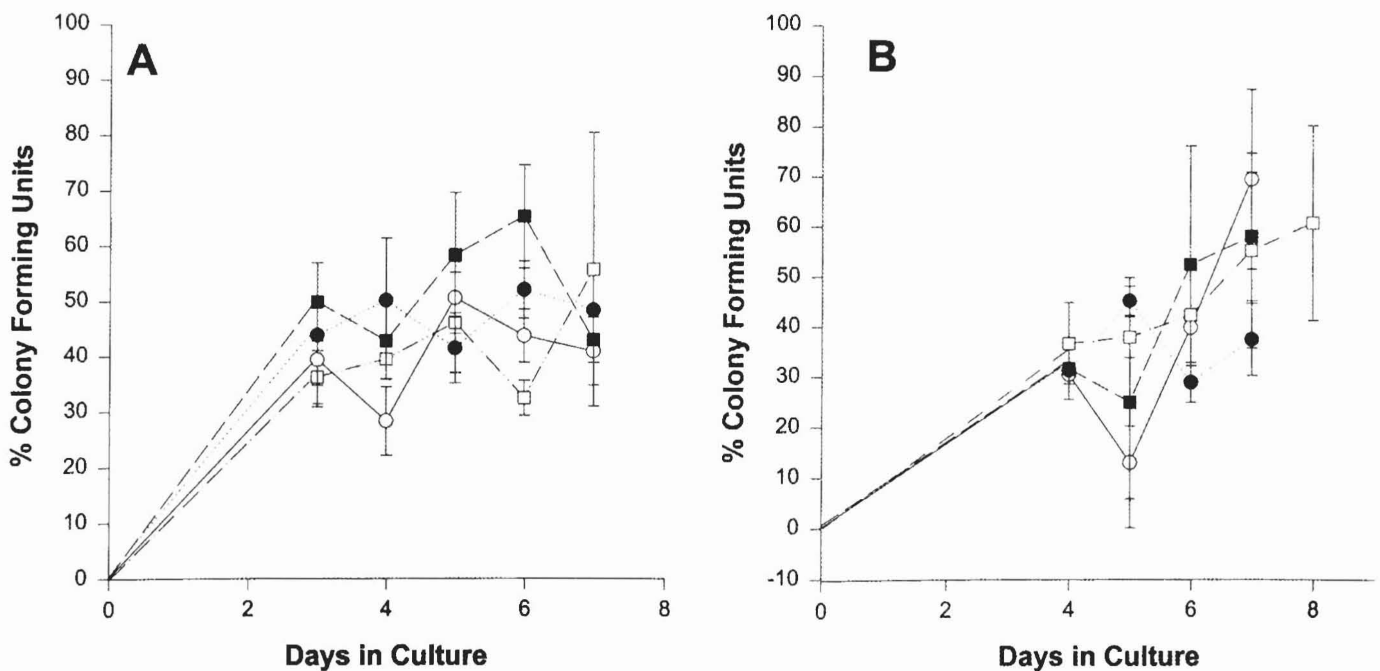

Fig. 3. %CFU for cells from the young patient and young control donor **(A)**, and the old patient and old control donor **(B)** layered on top of Matrigel™ or GFR-Matrigel™. Young and old patient cells on Matrigel™ (solid circle), young and old control donor cells on Matrigel™ (open circle), young and old patient cells on GFR-Matrigel™ (open square), and young and old control donor cells on GFR-Matrigel™ (solid square). Values are mean±SEM of 3-5 replicate wells.

the two matrices, cells from older subjects responded better on the Matrigel™ than GFR-Matrigel™, and the patient cells showed a significant proliferative response ($p=0.005$).

Cells inside Matrigel™ or GFR-Matrigel™

Morphologic observation showed that both patient and control human disc cells grew well within Matrigel™ (Fig. 4a) and GFR-Matrigel™ (Fig. 4b). After 48 h of culture, patient and control donor cells within the two matrices were present as single cells or as colonies which remained distinct over the 8 day growth period. Cells within Matrigel™ (Fig. 4a) formed small colonies and cells possessed short cytoplasmic surface projections. Cells within GFR-Matrigel™ were present as single cells with thin projections extending into the gel. Network-like colonies persisted through 8 days in culture and cells appeared to migrate within either type of matrix gel to form spreading colonies of cells (Fig. 4B). Electron micrographs of cells grown in Matrigel™ or GFR-Matrigel™ demonstrate short cytoplasmic projections from cells within the matrices (Fig. 5).

The %CFU values for cells grown within Matrigel™ or GFR-Matrigel™ are shown in Fig. 6. By day 4, cells from the young control donor showed an increase in %CFU within Matrigel™ and GFR-Matrigel™ (49±6% and 40±5% respectively; $p<0.05$ vs day 3 values; Fig. 6A). This increase persisted through day 8 and then decreased to 24±5% (Matrigel™) and 25±3% (GFR-Matrigel™). This change in % CFU for young control cells within Matrigel™ was significant at days 4, 5, 6, and 7 when compared to day 3 values ($p<0.05$). Similarly, young control donor cells grown within GFR-Matrigel™ showed a significant increase in% CFU at days 4, 5, 6, and 7 when compared with day 3 values. Cells from the young patient also showed an increase in % CFU at day 4 within Matrigel™ (51±8%) and GFR-Matrigel™ (51±6%), which fell to 15±5%, $p<0.05$ (Matrigel™) and 32±6% (GFR-Matrigel™) by day 8 (Fig. 6A). Only the older patient cells showed a significant CFU response when grown within Matrigel™ at day 7, which reached 50±6%, $p<0.05$ vs day 3 (14±4%) (Fig. 6B). No significant changes in CFU were observed in the old control donor cells grown within either of the matrices.

When the proliferative responses of both old and young patient and control donor cells were evaluated, significant changes were observed for cells in GFR-Matrigel™ after 4 and 8 days in culture (Table 2). Cells from the young patient expressed a significant increase

Table 2. Proliferation response of cells grown within Matrigel™ or GFR-Matrigel™.

	DAY 4			DAY 8		
	Matrigel™	GFR-Matrigel™	p value	Matrigel™	GFR-Matrigel™	p value
Young Patient	1001±154	4510±464	< 0.001*	2562±243	4962±345	< 0.001*
Old Patient	3142±259	16326 ±1367	0.002*	5641±677	35676±19157	NS
Young Control	1382±678	3505±587	0.04*	1920±258	4595±529	0.003*
Old Control	212±11	11656 ±2988	0.01*	6714 ±2239	16480±2364	0.02*

Data are mean±SEM of 3-5 replicate wells. *: denotes significantly different proliferation of each subject's cells when grown within Matrigel™ versus GFR-Matrigel™. NS: not significant

Fig. 4. Photomicrographs of the patterns of cell division within Matrigel™ **(A)** and GFR-Matrigel™ **(B)** after 7 days in culture. **A.** Cells from the young patient formed small colonies with slight cytoplasmic projections. **B.** Cells from the young patient showed distinct, long cytoplasmic projections. x 335

in proliferation in GFR-Matrigel™ at day 4 when compared with growth in Matrigel™ (4510±464, GFR-Matrigel™; 1001±154, Matrigel™; p<0.001). Similarly, cells from the control expressed a significant proliferative response of 3505±587 at day 4 in GFR-Matrigel™ compared with the response in Matrigel™ (1382±678; p<0.05). The older patient and control donor cells showed significant proliferation at day 4 when grown inside of GFR-Matrigel™ when compared with the growth observed in Matrigel™ (16326±1367 vs 3142±259; p=0.002 (patient cells); 11656±2988 vs 212±11; p=0.0186 (control cells)). By day 8 both the young control and patient disc cells as well as the old control disc cells, continued to show a significant proliferative response in GFR-Matrigel™ when compared with growth in Matrigel™, (4962±345 vs 2562±243; p<0.001 (young patient), 4595±529 vs 1920±258; p=0.004 (young control), and 16480±2364 vs 6713±2239; p=0.02 (older control); Table 2).

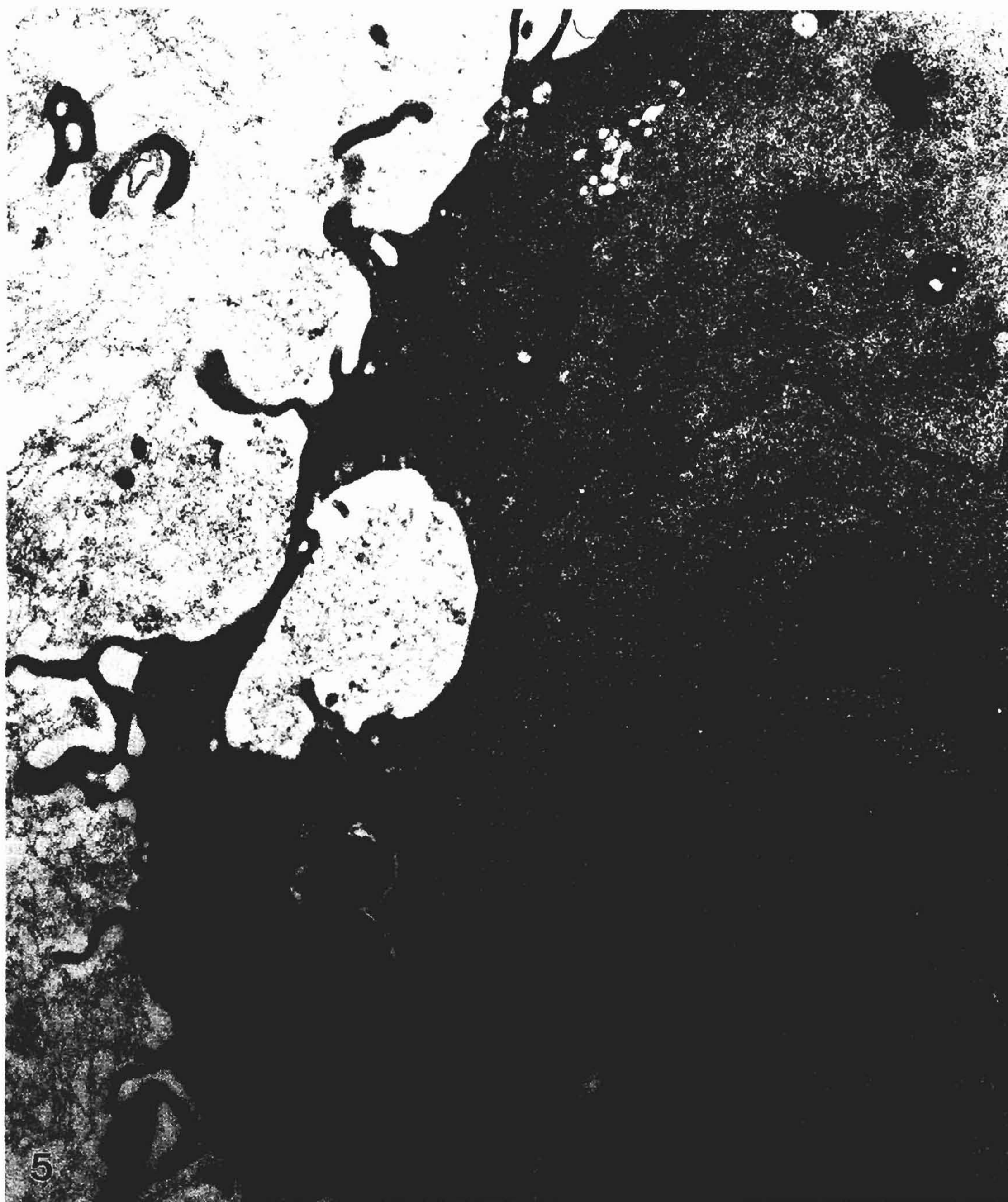

Fig. 5. Electron micrograph of the young patient cells grown within Matrigel™. Disc cells show a rounded shape with slight cytoplasmic projections. x 18,178

Discussion

The importance of the cellular interactions with basement membrane in the maintenance of the differentiated phenotype has been documented (Kleinman et al., 1987). To determine the phenotypic variation of human intervertebral disc cells in vitro we evaluated cell proliferation, a major criteria of cell function, in Matrigel™ or GFR-Matrigel™. Control donor and patient disc cells grown on Matrigel™ and GFR-Matrigel™ showed formation of interconnecting cytoplasmic cell projections with no apparent difference between the two matrices. Similar cell process formation on Matrigel™ in vitro has been previously documented in rat primary calvarial osteoblasts, in mouse osteoblast-like cell line MC3T3-E1 (Vukicevic et al., 1990, 1992), and fetal lung cells (Liu et al., 1995).

In contrast, disc cells grown within Matrigel™ or GFR-Matrigel™ (in a three-dimensional micro-environment) formed single cells or colonies which slowly migrated to form clusters of colonies. Such growth patterns within basement membranes have been shown with rat adipocytes (Brown et al., 1997) and fetal lung cells (Liu et al., 1995). These differences in growth pattern when grown on top versus within the two matrices are important since such differences may determine the type of cellular response observed. Cell shape, determined by the cytoskeletal structure of the cell, is an important physiological growth-control element (Folkman and Moscona, 1978) and cells are believed to be able to change their shape in order to minimize stress. Thus, disc cells grown on top of, or within, both matrices may transmit physical force through different intracellular networks, which initiate different signal transduction pathways leading to distinct cellular responses to internal or external stimuli.

Distinct cellular responses were observed when human intervertebral disc cells were grown on top of or within each matrix. %CFU data showed no significant changes in young or older patient and control donor cells when grown on top of Matrigel™ or GFR-Matrigel™. In contrast, young patient and control disc cells showed significant %CFU over an 8 day growth period when cultured within the two matrices. Cells from older patients or donors did not respond significantly within these matrices.

Similarly, marked differences were observed in cell proliferation rates when cells were grown on top of or within each matrix. Young patient and control donor cells showed a significant proliferative response on top of GFR-Matrigel™ when compared with Matrigel™ after 4 days in culture. This response was no longer significant after 8 days in culture. In contrast, all cultures tested appeared to show increased proliferation within GFR-Matrigel™ compared with Matrigel™ over the 8 days growth period.

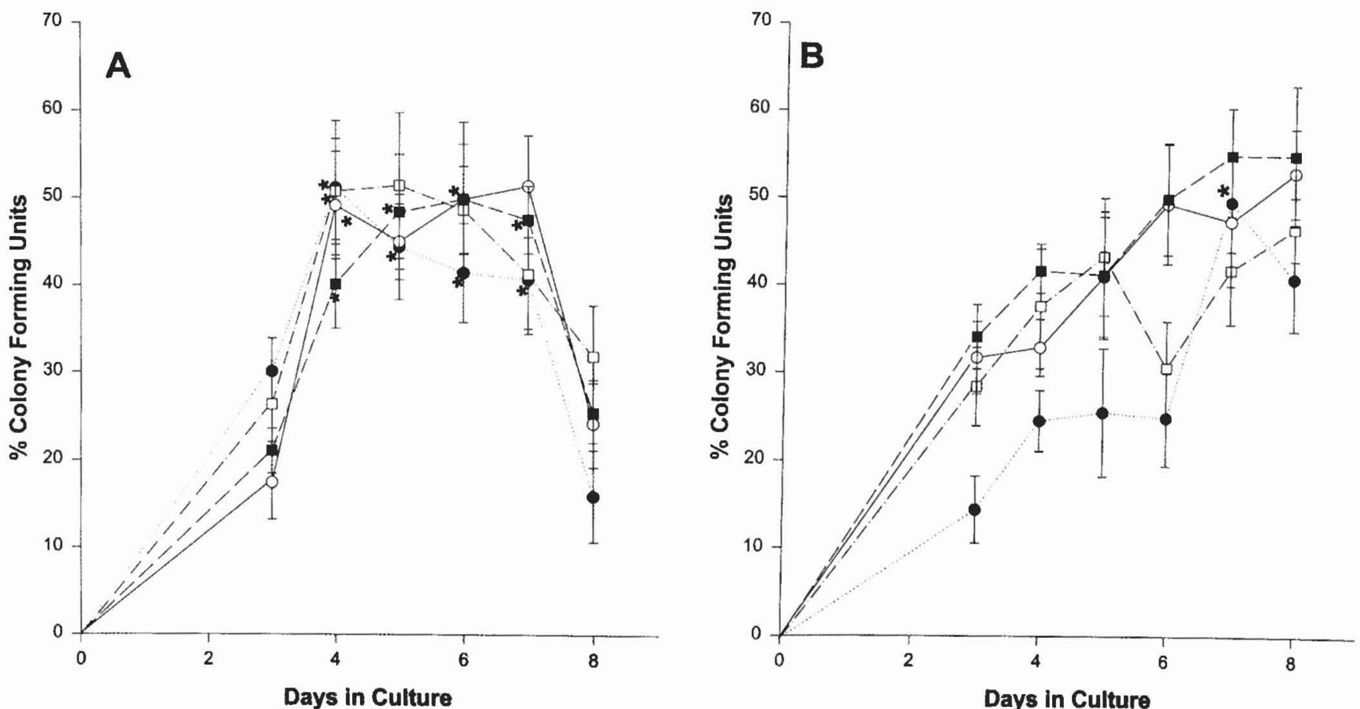

Fig. 6. %CFU for cells from the young patient and young control donor **(A)** and the old patient and old control donor **(B)** within Matrigel™ or GFR-Matrigel™. Young and old patient cells on Matrigel™ (solid circle), young and old control donor cells on Matrigel™ (open circle), young and old patient cells on GFR-Matrigel™ (open square), and young and old control donor cells on GFR-Matrigel™ (solid square). Values are mean±SEM of 3-5 replicate wells. *: indicates significant difference ($p<0.05$) when compared with day 3 values.

These observations are interesting from several perspectives. First, cellular responses were significantly increased when disc cells were grown within a specific matrix and second, proliferation rates were consistently higher when cells were exposed to GFR-Matrigel™ than with Matrigel™. Many studies have demonstrated such differences between two-dimensional and three-dimensional culture systems (Lui et al.,1995; Brown et al., 1997). Usually, in two-dimensional culture systems cells do not retain their in vivo relationships. Under three-dimensional culture conditions, however, there is a matrix against which cells can re-aggregate to form structures similar to the in vivo environment (Simpson et al., 1985). A two-dimensional culture environment may not be optimal for studying cell division since cell division is inhibited by cell-cell contact (Lui et al., 1995). Our studies here demonstrate the preference of disc cells for a three-dimensional culture environment, similar to the preferred culture method for chondrocytes, a cell type very similar to the disc cell and known to dedifferentiate in two-dimensional culture. This dedifferentiation results in production of low levels of type II collagen, a phenotypic marker for these cells, but cells can re-express the characteristic type II collagen extracellular matrix when placed in an agarose three-dimensional culture system (Benya and Shaffer, 1982; Bonaventure et al., 1994; Kolettas et al., 1995).

Our results show that cellular proliferation of human disc cells significantly increased with GFR-Matrigel™ but not with Matrigel™. Possible explanations may be too high a growth factor content of Matrigel™, or the effect of many growth factors in combination. As yet poorly defined antagonistic or synergistic effects of growth factors may influence disc cell proliferation. Growth factors influence a variety of cell responses including differentiation, metabolism and growth. The effect of growth factors at the cellular level and the relation of this to disc health is an important new area of research. Several studies have shown growth factor effects on disc cells. Gruber et al. (1997) showed modulation of proteoglycan expression by TGF-ß1 in cells from the annulus. TGF-ß1 has also been shown to result in mitogenic stimulation of cells in the nucleus and the transition zone of explant slices of canine disc (Thompson et al., 1991). Disc cells are now known to produce IL6, phospholipase A_2 and fibroblast growth factor (Weinstein et al., 1996).

The model and results presented here serve as a baseline for subsequent investigations on the effects of cytokines/growth factors on disc cell growth. Present data expand our knowledge of the relationship between disc cells and their interaction with and cellular response to their surrounding matrix.

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