Dermatan Sulfate Proteoglycan and Glycosaminoglycan Synthesis Is Induced in Fibroblasts by Transfer to a Three-dimensional Extracellular Environment*

Composition and architecture of the extracellular matrix dictate cell behavior. Proteoglycans bind multiple components of the extracellular matrix by serving as important regulators of cell behavior. Given the influence of culture architecture on cell function, we investigated whether switching NIH3T3 fibroblasts from growth on type I collagen in monolayer to a collagen gel might influence dermatan sulfate expression. Immunofluorescent staining, immunoblot, and Western blot demonstrated an induction in decorin expression in cells switched to collagen gels. This induction was associated with a 40-fold increase in decorin transcript expression determined by quantitative real time PCR. Disaccharide analysis of extracted glycosaminoglycans from collagen gels showed an increase in total glycosaminoglycan and in the ratio of chondroitin sulfate to heparan sulfate compared with monolayer culture. The ratio of chondroitin sulfate to heparan sulfate likewise increased on syndecan-1 from gel culture. Digestion with chondroitinase B showed that this induced chondroitin sulfate was dermatan sulfate. Syndecan-1 extracted from wounded mouse skin also displayed an increase in dermatan sulfate synthesis compared with unwounded skin. Furthermore, glycosaminoglycans from collagen gel culture activated keratinocyte growth factor, whereas glycosaminoglycans from monolayer culture lacked this ability. These findings suggest that regulation of dermatan sulfate and dermatan sulfate proteoglycan is dependent on extracellular matrix architecture. The ability of collagen gel culture to mimic better the in vivo dermal environment may be due in part to this influence on dermatan sulfate and dermatan sulfate proteoglycan synthesis.

Cell behavior is dependent on its environment. Extracellular matrix (ECM) composition can dictate cell proliferation, movement, and morphology (1–3). Glycosaminoglycans (GAGs), such as heparan sulfate (HS) and chondroitin sulfate (CS), are important ECM components that can influence these cell behaviors. HS has been well described to be involved in a vast number of cell-cell and cell-matrix interactions ranging from processes such as development to microbial invasion (4–6). A growing body of evidence has identified another GAG, CS-B or dermatan sulfate (DS), as playing a role in a large number of similar cellular processes (7). The observation that DS is released at high concentrations during wound repair, and that it serves as a cofactor for several growth factors important to this process, has made it an attractive molecule for investigating cell-matrix interactions (8, 9).

DS is made up of repeating disaccharide units of iduronic acid and GalNAc and is the predominant GAG in the dermis (7, 10). The presence of GalNAc identifies DS as a chondroitin sulfate, whereas the presence of the iduronic acid sets DS apart from other chondroitin sulfates. DS and DS proteoglycans (DSPG) bind a long list of proteins influencing a large range of physiological processes. For example, binding of DS to heparin cofactor-II, thrombin, and activated protein C regulates the coagulation cascade (11–14). DS also interacts with basic fibroblast growth factor (FGF-2) and keratinocyte growth factor (FGF-7), promoting cell proliferation (8, 9). Furthermore, mice deficient in decorin, a DSPG that binds to collagen and fibronectin (15, 16), develop skin with increased fragility (17). These interactions demonstrate the importance of DS, particularly as a regulator of wound repair and skin strength.

Although much has been uncovered regarding the role of DS as a multifunctional co-factor, the regulation of DS synthesis is poorly understood. Changing the architecture of a culture environment from monolayer growth to three-dimensional (3D) growth has been shown to have profound effects on cell behavior. A switch to 3D culture architecture influences cell proliferation, matrix protein synthesis, cell-cell, and cell-matrix interactions (1, 2). Given that DS and DSPG are closely involved in these types of interactions, we hypothesized that the 3D culture environment might have an influence on DS and DSPG synthesis.

The findings in this report indicate that the transfer of fibroblasts from monolayer to 3D culture induces the synthesis of the DSPG decorin. This change in culture architecture also stimulates an increase in the DS GAG chain synthesis on the fibroblast growth factor; FGF-7, keratinocyte growth factor; 3D, three-dimensional; TFGR3, transforming growth factor-β; FGFR2 IIIb, fibroblast growth factor-2 IIIb; PCS, fetal calf serum; MWCO, molecular weight cut-off; PBS, phosphate-buffered saline; BSA, bovine serum albumin; RT, reverse transcription; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-3, interleukin-3.
cell surface PG syndecan-1. The observation that a similar change is found in skin after wounding demonstrates the biological relevance of this finding. Finally, the influence of 3D culture on fibroblast behavior appears to be functionally important, as GAG from fibroblasts in 3D culture acquired the ability to promote growth factor activity.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**

NIH3T3 Fibroblasts in Monolayer Culture—Cells were cultured in “NIH3T3 media” composed of Dulbecco’s minimal Eagle’s medium with 10% FCS, 2.92 mg/ml L-glutamine, 100 units/ml penicillin, 50 µg/ml streptomycin, and 1 mM sodium pyruvate on plastic coated with 490 µg/ml type I rat tail collagen (Sigma). All cell cultures were incubated at 37 °C, 5% CO₂. For treatment of monolayer culture with TGFβ, fibroblasts were cultured with 1–10 ng/ml TGFβ for 15 min at 4 °C, and the supernatant was also transferred by this dot assay as described below.

For immunoblot and Western blot analysis, 2 µg of cellular extracts were transferred to an Immobilon-N membrane (Millipore Corp., Bedford, MA). GAG in 500 µl of water was applied to a filter and centrifuged at 14,000 rpm (Eppendorf 5415c) at 4 °C until the complete volume had passed through retaining only the GAGs. Samples were washed twice more with 500 µl of RPMI with L-glutamine, penicillin, and streptomycin and then were resuspended in media for proliferation assay as described below.

For immunoblot, 20 µg of cellular extracts were transferred to an Immobilon-N membrane (Millipore, Bedford, MA) using a MiniTuff I dot blot system (Schleicher & Schuell). 1 ml of culture medium from fibroblasts grown on collagen for 48 h (cells were 90% confluent, total media volume was 10 ml) was centrifuged at 14,000 rpm (Eppendorf 5415c) for 15 min at 4 °C, and the supernatant was also transferred by this dot blot system to an Immobilon-N membrane. Both dot blot and Western blots were blocked with 3% nonfat dry milk, 0.5% BSA, 0.15 mg/ml NaCl, 0.03% Tween 20 in Tris-buffered saline at room temperature for 2 h, incubated with primary antibody (mouse monoclonal anti-human DSPG antibody at 1:1000 or rat anti-mouse syndecan-1 antibody at 1:2000) overnight at 4 °C, washed with 0.3% Tween 20, Tris-buffered saline, and then incubated with goat anti-mouse antibody-horseradish peroxidase at 1:2500 or goat anti-rabbit antibody-horseradish peroxidase at 1:2000 (Dako, Carpinteria, CA) for 40 min at 4 °C. Western Lightning chemiluminescence reagent (PerkinElmer Life Sciences) was used to detect electrochemiluminescence.

**Chondroitinase Digestion and Nitrous Acid Treatment**

Prior to PAGE, some extract samples were digested with a chondroitinase and/or treated with nitrous acid. For chondroitinase ABC digestion, extract samples were lyophilized, resuspended in chondroitinase A buffer (50 mM Tris base, 60 mM sodium acetate, pH 5.0) plus 0.625 milliuunits/µl (final concentration) of chondroitinase ABC (Sigma), and incubated overnight at 37 °C. For chondroitinase B digestion, extract samples were lyophilized and resuspended in chondroitinase B buffer (chondroitinase ABC buffer plus 0.05% BSA) plus 40 milliuunits/µl (final concentration) of chondroitinase B (Sigma). For nitrous acid digestion, equal parts of 1 M H₂O₂, 0.5 M barium nitrite hydrate at room temperature, and the precipitate was separated by centrifugation at 10,000 rpm (Eppendorf 5415c) for 5 min at room temperature. Lyophilized extract samples were resuspended in the supernatant (nitrous acid, pH 1.5), incubated for 30 min at room temperature, and then neutralized with a volume of 1 M NaHCO₃.

**RT-PCR and Quantitative Real Time PCR**

Total RNAs were extracted from the monolayer and 3D cultured fibroblasts at different time points in the culture by using TRIzol Reagent (Invitrogen) per the manufacturer’s instructions. After DNase digestion, RNA samples were passed through RNasey Mini Spin columns for DNA/RNA extraction (Qiagen, Valencia, CA) per the manufacturer’s instructions. RT-PCR was performed on 2 µg of RNA using Retroscript kit (Ambion, Austin, TX). Briefly, 4 µl of 50 µM Random Decamers were combined with RNA, brought up to 24 µl with water, and incubated at 80 °C for 3 min. The reaction was iced, and 4 µl of 10× RT buffer, 8 µl of dNTP mix, 2 µl of RNase inhibitor, and 2 µl of Moloney murine leukemia virus-reverse transcriptase were added. Reactions were incubated at 42 °C for 1 h and 92 °C for 10 min. Real time PCR was performed on an ABI Prism 7700 sequence detector (Applied Biosystems) using fluorescently labeled probes (PioGen, Valencia, CA) for 40 cycles of 95 °C for 15 s and 60 °C for 1 min.
performed using a Prism 7000 Sequence Detection System from Applied Biosciences (Foster City, CA). GAPDH-F (5′-TTACAGCCUCCTGGCC-AAG-3′) and GAPDH-R (5′-TGTGCTAGATCTTCCAGCAG-3′) were used to amplify GAPDH. DECORIN-RT-F1 (5′-ACGTACCTGGCCACGGAT-3′) and DECORIN-RT-R1 (5′-AGGAACATTGCGGACAGCT-GC-3′) were used to amplify decorin. mTGFβ-RealT-F (5′-TGGAGCTGGTAAAGGGAG-3′) and mTGFβ-RealT-R (5′-ACAGGATCTGGCCACGGAT-3′) were used to amplify mTGFβ. 1 μl of reverse transcriptase reaction was added to 24 μl of SYBR Green PCR Master Mix (Applied Biosystems) and 0.25 μl of each 20 μm primer. The thermal profile was as follows: 50 °C for 2 min, 95 °C for 10 min, and 40× (95 °C for 15 s, 60 °C for 1 min). The melting temperature profiles of amplicons were determined to show the specificity of amplification. Results were analyzed using the comparative Ct method, where ΔCt = Ct (mCRAMP) − Ct (GAPDH) and ΔΔCt = ΔCt (K14-Cath-mCRAMP mice) − ΔCt (wild-type mice). Relative expression was calculated as 2−ΔΔCt. All real time PCRs were performed in triplicate.

**Wounded Mouse Skin Experiment**

After back hair was removed by shaving and chemical depilation from Balb/c mice, multiple full-thickness 1-cm incisions were made in their back skin. After 48 h, back skin was excised from mice with wounded skin and from control mice with unwounded skin. No evidence of infection was seen at the incision sites. Skin tissue samples were processed for PG/GAG isolation as described above. Animal procedures were approved by the Veterans Affairs San Diego Healthcare System subcommittee on animal studies (protocol 02-037).

**DNA Assay for Determination of Cell Number**

Cell count of fibroblasts in monolayer culture or 3D collagen gel culture was determined by release of cells from collagen by trypsinization and collagenase treatment, respectively, followed by determination of DNA content using a Hoechst dye (33258, Sigma) DNA assay. Hoechst dye (or bisbenzimide) has been shown to be useful for determining the quantity of DNA in cellular extracts (20). The quantity of DNA per fibroblast was determined by collecting −5 × 106 cells from the monolayer culture on collagen and then re suspending and sonicating cells in 4 ml of DNA assay buffer (50 mM NaH2PO4, 2 M NaCl, 2 mM EDTA). The sample was then diluted 1:40 in DNA assay buffer to a total volume of 1 ml and combined with 50 μl of Hoechst dye. 100 μl of sample was transferred to a 96-well plate, and fluorescence was quantified on a fluorescent spectrophotometer (excitation wavelength, 460 nm; emission wavelength, 480 nm). Values were compared with the standard curve of known amounts of DNA. For release of fibroblasts from collagen gel culture, gels were transferred from media to 500 μl of 0.05% collagenase B (Roche Applied Science) in PBS. After incubation at 37 °C for 1 h with occasional mixing by inversion, cells were pelleted by centrifugation. The supernatant was removed, and the cells were re suspended in 1 ml of DNA assay buffer, and Hoechst dye DNA assay was carried out as described above.

**Disaccharide Analysis**

The Glycotechnology Core at the University of California, San Diego performed all steps of the disaccharide analysis. After complete digestion of GAG samples with either chondroitinase ABC or the combination of heparinase I, heparinase II, and heparitinase, the resulting disaccharides were purified by 10,000 MWCO Microcon centrifugal filters (Millipore Corporation, Bedford, MA). Analysis and separation of disaccharides were performed by using reverse phase ion pair chromatography (C18 Tosohaas ODS-120T column, 4-μm particle size) with post-column derivatization based on a method described previously (21).

**Cell Proliferation Assay**

BaF3/KGFR cells were washed in PBS three times prior to use to remove traces of IL-3. Cells were then transferred to 384-well plates at 5 × 103 per well in a final media volume of 25 μl with varying concentrations of GAG and/or human recombinant FGF-7 (R & D Systems, Minneapolis, MN) in RPMI containing 10% FCS, 1-glutamine, penicillin, and streptomycin. Purified dermatan sulfate (chondroitin sulfate B, Seikagaku America, Falmouth, MA), molecular mass range of 11 to 25 kDa, at 3 μg/ml was used as a positive control. Cell proliferation was determined using the Cell Titer nonradioactive cell proliferation kit (Promega, Madison, WI) after incubation for 72 h at 37 °C, 5% CO2.

**RESULTS**

**Switch to 3D Culture Environment Induces Decorin Expression by Fibroblasts**—DS is an abundant product of fibroblasts in the dermis, representing 0.3% of the dry weight of the skin (7). To investigate the mechanisms that regulate DS and DSPG synthesis by fibroblasts, decorin expression by NIH3T3 cells, either cultured as a monolayer on type I collagen or in a 3D type I collagen gel, was evaluated. After 7 days of culture, fibroblast monolayers or sections of 3D collagen gels were analyzed by immunofluorescent staining with an antibody against decorin. Sections from 3D collagen gels showed increased staining for decorin compared with monolayer culture (Fig. 1A). Fluorescent staining of decorin in 3D culture formed a reticular pattern exemplifying the structure of the ECM in the collagen gel.
FIG. 2. Kinetics of decorin expression in monolayer cultured fibroblasts transferred to 3D culture environment. RT-PCR was performed on RNA samples extracted at multiple time points from fibroblasts cultured in 3D collagen gels. Quantitative real time PCR was performed with primers specific for decorin. Data were analyzed by using the comparative C\textsubscript{T} method as described under “Experimental Procedures.” Values are means \pm S.D. of three separate experiments.

To further evaluate and quantify the induction of decorin, NIH3T3 fibroblasts, after 7 days of either monolayer culture or 3D collagen gel culture, were extracted and partially purified by anion exchange chromatography to enrich for GAGs and PGs. These extracts were first analyzed by decorin immunoblot showing a dramatic increase in decorin expression from fibroblasts in the 3D culture environment compared with monolayer culture, where decorin was minimally detectable (Fig. 1B). In contrast, the relative expression of the cell surface proteoglycan syndecan-1 was largely unaffected by culture architecture. Decorin was not detected in culture media from fibroblasts in monolayer culture (data not shown). Next, these extracts were analyzed by Western blot to evaluate the nature of GAGs on these PGs. Consistent with the immunofluorescence staining and dot blot analysis, no decorin was detected in extracts from monolayer culture, whereas decorin expression was abundant in extracts from 3D culture, appearing at a mass consistent with the decorin core protein at \ \~48\ kDa and a proteoglycan at \ \~200\ kDa (Fig. 1C).

The smear seen for decorin represented heterogeneity in the length of its one dermal sulfate GAG chains as digestion with chondroitinase ABC, which cleaves all chondroitin sulfate GAG chains to disaccharides, left only the core protein. Equivalent digestion with chondroitinase ABC (Fig. 1, C and D) or chondroitinase B (Fig. 1D) confirmed that this CS GAG was DS.

To determine whether the induction in decorin PG was accompanied by a change in transcription of the decorin core protein, fibroblasts cultured in 3D collagen gels were evaluated by quantitative real time PCR. Decorin gene transcription increased 40-fold over the first 48 h of 3D collagen gel culture and then plateaued (Fig. 2).

Analysis of GAGs from Fibroblasts Switched to 3D Culture Environment—The amount, relative CS composition, and sulfation pattern of CS influences cellular behaviors in response to GAGs produced during development, coagulation, and wound repair (7, 22–24). Therefore, we chose to characterize CS induced in fibroblasts switched from monolayer to 3D culture. The total quantity of sulfated GAG synthesized by fibroblasts after 7 days of culture was measured and normalized to cell number. Total sulfated GAG per cell increased more than 2.5-fold in fibroblasts from 3D compared with monolayer culture (Table I). Analysis of disaccharide composition revealed that extracted sulfated GAGs had 7 or 21.5% CS in total GAG when coming from fibroblasts in monolayer or 3D culture, respectively. Sulfated GAG from fibroblasts also displayed a 4-fold increase in CS to HS ratio compared with total GAG from monolayer culture. Moreover, the switch to a 3D culture environment also influenced CS sulfation patterns. Percent 4-O-sulfated disaccharide in CS was decreased in 3D collagen gel culture associated with a large increase in unsulfated CS.

TABLE I

<table>
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<tr>
<th>Monolayer</th>
<th>3D</th>
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<td>CS/HS ratio</td>
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*Extracts were collected after 7 days of culture. Total sulfated GAG was determined by Blyscan assay, and the disaccharide composition was determined by following the enzyme digestion and high pressure liquid chromatography separation as described under “Experimental Procedures.” The CS/HS ratio was based on the determination of %CS and %HS in the total GAG sample. Monolayer indicates the extract from fibroblasts in monolayer culture; 3D indicates the extract from fibroblasts of collagen gel cultures; NDA indicates no detectable amount.*
collagen gels also shifted the migration of syndecan-1 (Fig. 3C). Digestion with chondroitinase B or a combination of chondroitinase B and nitrous acid showed that the increase in CS on syndecan-1 is largely DS GAG chains. Therefore, switching fibroblasts from a monolayer to 3D culture environment stimulated the DS GAG chains on a predominantly heparan sulfate PG. This stimulation was accompanied by a transient but significant change in the amount of fibroblast TGFβ transcript in 3D versus monolayer culture (Fig. 4). However, treatment with TGFβ neutralizing antibody did not inhibit the induction of DSPG nor did treatment of monolayer culture with TGFβ induce DSPG synthesis.

Wound Repair Environment Stimulates DS GAG Chain Synthesis on Syndecan-1—To see whether these changes in the glycosylation of syndecan-1 might be relevant in vivo, we evaluated syndecan-1 from both normal skin and skin undergoing wound repair. PG extracts from unwounded mouse skin and mouse skin 48 h after aseptic wounding were analyzed by Western blot. Syndecan-1 from unwounded skin ran as a smear suggesting a considerable degree of heterogeneity in GAG chain composition (Fig. 5A). However, this heterogeneity appeared to represent varying lengths of exclusively HS GAG chains as it was reduced to a protein core by nitrous acid treatment. In contrast, syndecan-1 from wounded skin ran larger in size, a difference attributable to an increase in non-HS GAG chains given that a residual band at 220 kDa was seen after nitrous acid treatment. Further analysis of syndecan-1 from wounded skin showed that these non-HS GAG chains represent DS GAG chains, as the combination of chondroitinase B digestion and nitrous acid treatment reduced the majority of this syndecan-1 to its protein core (Fig. 5B). Decorin from normal skin is exclusively a DSPG with no change seen following stimulation by injury (27, 28).

GAGs Isolated from Fibroblasts in 3D Culture Environment Potentiate FGF-7-dependent Cellular Proliferation—DS is the principal cofactor for FGF-7 activity (9), a growth factor important to the wound repair process. Given that DS synthesis was stimulated by switching fibroblasts to 3D culture, we hypothesized that GAGs from 3D culture would be superior in promoting FGF-7 activity compared with GAGs from monolayer culture, and thus better able to approximate the GAGs induced in vivo. To test this, we used the lymphocyte cell line BaF3, lacking cell surface GAG expression and engineered to stably express the FGFR2 IIIb receptor (referred to as BaF/KGFR cells). The FGFR2 IIIb receptor binds FGF-7, an interaction promoted by the presence of DS (18, 29). BaF/KGFR cells were incubated with increasing concentrations of GAGs from 3D or monolayer culture with and without 20 ng/ml FGF-7, and cell proliferation was assessed after 72 h. In the presence of 50 μg/ml of GAGs from 3D culture, FGF-7 stimulated cell proliferation, an effect comparable with that seen after incubation of FGF-7 with 3 μg/ml purified DS (Fig. 6). In contrast, neither GAG from monolayer culture with FGF-7, GAG alone, or FGF-7 alone stimulated proliferation above background levels. The unsulfated GAG hyaluronic acid (HA) is a likely component of the GAG preparations from these cells but was not measured in the current study.

Switching fibroblasts from monolayer to 3D collagen culture allows them to take on functional properties that more closely mirror fibroblasts in vivo (2, 30). The present study sheds light on why this transition in cellular morphology and function might occur. The switch to 3D culture, despite initially culturing in type I collagen, induces a dramatic increase in the expression of the ECM DSPG, decorin, while simultaneously inducing the expression of DS on the cell surface PG syndecan-1. When compared with in vivo PG profiles, this shift appears to resemble skin in the state of wound repair. Consistent with this, GAG extracts from 3D culture have the ability to potentiate growth factor activity important in wound repair, an activity that is not seen from GAG extracts from fibroblasts in monolayer culture.

A large increase in expression of decorin from fibroblasts following the switch to 3D culture was shown through multiple modalities, and at both the protein and transcript level. Immunofluorescent staining for decorin not only displayed greater staining for decorin in 3D fibroblast culture but also provided a view of the ECM network of collagen gel. Given the intricacy of this network, it is not surprising that cell-cell and cell-matrix interactions would differ in 3D culture compared with traditional monolayer culture. Through more quantitative analysis by immunoblot, this increase in decorin was a selec-

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**Figure 4.** mTGFβ expression in fibroblasts switched to 3D culture environment. RT-PCR was performed on RNA samples extracted at multiple time points from fibroblasts grown in monolayer or 3D collagen gel culture. Quantitative real time PCR was performed with primers specific for mTGFβ. Data were analyzed by using the comparative Ct method as described under “Experimental Procedures.” Values are means ± S.D. of three separate experiments for 3D culture.

**Figure 5.** Expression of dermatan sulfate GAGs on syndecan-1 is induced during in vivo wound repair. A, Western blot of extracts from unwounded mouse skin or mouse skin 48 h after aseptic wounding. Cell extracts underwent no treatment (--), nitrous acid treatment (NA), or after digestion with chondroitinase B (b), or after the combination of chondroitinase B digestion and nitrous acid treatment (b/NA). Blots were probed with antibody to syndecan-1.
The finding that the expression of an ECM PG was regulated by environmental cues such as TGFβ, FGF-2, and insulin levels (26, 34, 35). The influence of 3D culture on syndecan-1 led to an increase in the ratio of CS to HS GAG side chains. Further analysis of the nature of the induction in CS GAG chains revealed that these syndecan-1 chains were more specifically DS GAG chains. To our knowledge this is the first report of the expression of DS GAG chains on syndecan-1. The mechanism behind this switch remains unknown. A transient increase in TGFβ transcript was observed, but this factor alone was not capable of mimicking the effects of 3D collagen gel culture. The influence of 3D culture also led to a decrease in the size of syndecan-1, similar to previous studies (36, 37) showing that syndecan-1 size can be altered because of changes in the culture milieu. This change in size may be partially due to shorter HS chains or a smaller number of HS chains. This observation warrants further examination.

Multiple studies have demonstrated the importance of syndecan-1 and its GAG chains in wound healing. Syndecan-1 is induced in the skin within 12 h of injury, and levels remain elevated until re-epithelialization has occurred (38, 39). Syndecan-1 knockout mice show delays in epithelial cell proliferation and decreased expression of the α6 integrin during wound repair (40). Furthermore, syndecan-1 GAG chains are shed into dermal wound fluids where they regulate protease inhibitor activity (41). The ratio of CS/HS GAG chains on cell surface PGs, including syndecan-1, has been shown to increase by treatment of fibroblasts with growth factors important in wound repair (26, 34). Analysis of syndecan-1 GAG chains in the murine skin wound model used in our study showed a dramatic shift from almost exclusively HS side chains to a mixture of CS and HS. As in the case of the collagen gel culture, the increased CS expression was selectively an increase in DS expression, suggesting that DS may serve an important role in the wound repair process.

DS binds FGF-7 and is the principal cofactor for its activity (9). FGF-7 is highly expressed after skin injury and stimulates keratinocyte proliferation (42–44). Therefore, we tested whether GAGs extracted from fibroblasts induced by culture in the 3D environment might be superior to GAGs from monolayer culture in supporting FGF-7-dependent cell proliferation. GAGs from 3D culture potentiated FGF-7-induced proliferation in a dose-dependent fashion, while GAGs from monolayer culture lacked this ability. Thus, switching fibroblasts from monolayer to 3D culture environments promotes a GAG expression profile better equipped to activate FGF-7, an important growth factor in the process of wound healing. We have shown previously that GAGs extracted from the wound environment possess the capacity to potentiate FGF-7 (9), an activity consistent with our finding that wound GAGs, like 3D fibroblasts GAG, shift to DSPGs.

3D collagen gels continue to be used as models for both the dermal ECM environment in general and for wound repair. Our findings provide some insight as to why 3D culture might be a superior model system compared with traditional monolayer culture. The GAG and PG profiles in 3D culture, resembling in vivo profiles, are better suited for biologically relevant growth factor function and cell-cell and cell-matrix interactions.

These findings underscore the importance of the extracellular environment in influencing cell behavior. Traditionally, with regard to cell culture, much emphasis has been placed on media composition, ambient gas mixture, and incubator tem-
perature to better mimic in vivo conditions, but less consideration has been given to the potential influence of culture architecture on cell behavior. ECM architecture has been shown to affect a large range of cellular activity including but not limited to the morphology, proliferation, and collagen metabolism of stellate cells (1), the fibroblast expression of matrix metalloproteinases (45), and the chondrocyte response to growth factors (46). In the current study, the dramatic influence of 3D culture demonstrates how ECM architecture controls GAG synthesis, a central regulator of multiple cell behaviors.

REFERENCES

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