Promotion of Acellular Dermal Matrix Resolution In Vitro by Matrix Metalloproteinase-2

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Objective: To determine whether acellular human dermis is degraded by matrix metalloproteinases (MMPs), a large class of matrix-degrading enzymes.

Methods: The degradation of acellular human dermis specimens was evaluated in vitro. Wild-type murine fibroblasts with a broad-spectrum MMP inhibitor, GM6001, and MMP-2–deficient fibroblasts were placed on the basement membrane and dermal surfaces of acellular human dermis. Matrix degradation and fibroblast infiltration into the matrix were assessed after a 20-day incubation period.

Results: The basement membrane thickness of the specimens cultured with wild-type fibroblasts was significantly less than that of specimens cultured with GM6001 (P<.001), and the infiltration of fibroblasts into the dermal surface was limited by the addition of GM6001 (P=.002). To determine whether MMP-2 was involved in this in vitro phenotype, MMP-2–deficient fibroblasts were assessed in comparison with wild-type fibroblasts. Wild-type fibroblasts degraded the basement membrane surface (P<.001) and infiltrated the dermal surface (P=.003) more efficiently than did MMP-2–deficient fibroblasts.

Conclusions: The results from our in vitro experiments suggest that MMPs and specifically MMP-2 may play an important role in the resorption of acellular human dermis. Addition of MMP inhibitors to implanted dermal matrices may slow fibroblast infiltration and improve their longevity in vivo.

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Implanted extracellular matrices (ECMs) used for reconstructive and cosmetic purposes are resorbed within weeks to months. Serial histological analyses of implanted acellular matrices have demonstrated resorption rates that exceed 50% within only 3 months (mean percentage persistence at 1 month, 83%; at 3 months, 48%). Jackson et al2 clinically noted a 40% to 80% resorption of an acellular human dermis (AHD) product (AlloDerm; LifeCell Corporation, Branchburg, NJ) 1 year after their patients underwent nasal dorsum augmentation. Improving the durability of implanted material requires an understanding of the mechanism by which implanted matrix resorption occurs. Collagen or dermal derivatives are some of the most commonly used implants, including hydrolyzed type I collagen and human or porcine dermis (Cymetra [LifeCell Corporation], Permacol [Tissue Science Laboratories plc, Covington, Ga], and AlloDerm). Collagen degradation of these implants over time is believed to occur as a result of fibroblast-derived proteases that are elaborated during the remodeling process.

Matrix metalloproteinases (MMPs) are a diverse group of zinc-dependent endopeptidases considered critical in the remodeling of ECM components. They are produced by a wide number of cell populations, including fibroblasts, tumor cells, mesenchymal cells, and glial cells. Matrix metalloproteinases play a role in wound healing, tumor proliferation, and tissue morphogenesis by degrading collagen and other components of the ECM. Recent observations also suggest that MMPs direct non-ECM molecules important in wound healing such as growth factors.3 To date, more than 25 mammalian MMPs have been identified. Of these, MMP-2 (gelatinase A, 72-kDa gelatinase) has been implicated in the normal turnover of ECM in wound healing and tumor cell invasion. Matrix metalloproteinase-2 (MMP-2) can degrade several matrix components (including collagen types I and IV and laminin) and has been implicated in tumor metastasis4 and wound healing.3

Human ADM (AlloDerm) is widely used in soft tissue augmentation as an allograft or an implantable material.5,6 Acellular dermis processed for this purpose

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contains an intact basement membrane and an overlying ECM (referred to as the dermal side). Descriptive studies1,11 have characterized the rate of resorption, histological characteristics, and tensile strength of ADM implanted in humans. The mechanism by which this occurs remains unknown. To this end, we assessed the role of MMPs in the resorption of ADM in vitro. Understanding the proteases involved in resorption may lead to improved durability of acellular dermis and other similar implantable biomatrices.

METHODS

CELL CULTURE

We used fibroblasts recovered from wild-type and gene-deleted murine (MMP-2) embryos. Fibroblast phenotype was confirmed by results of histological analysis. These cell lines were routinely kept in Dulbecco Modified Eagle Medium (In Vitrogen Corporation, Carlsbad, Calif) supplemented with penicillin (10,000 U/mL), streptomycin (10,000 μg/mL), and 20% fetal bovine serum (all from Mediatech Inc, Herndon, Va). All cultures were maintained at 37°C in a mixture of 5% carbon dioxide and 95% air.

ACELLULAR DERMAL INVASION ASSAY

In separate experiments, we assessed the ability of the fibroblasts to invade the dermal and basement membrane surfaces of the ADM. There were a total of 3 experimental groups: wild-type fibroblasts (control), wild-type fibroblasts with GM6001, and MMP-2–deficient fibroblasts. Each experiment was performed in triplicate. Acellular dermis (AlloDerm) was washed in phosphate-buffered saline solution for 15 minutes and then, under sterile conditions, cut into squares (5.0 × 5.0 mm). The squares were then placed with the dermal or basement membrane surface facing outward on freshly prepared rat-tail tendon type I collagen plugs within the upper chamber of transwell dishes (Corning Inc, Corning, NY). Fibroblasts from each cell line (2.5 × 10⁶ cells/well) were then added to the surfaces of the ADM specimens and incubated at 37°C for 2 hours to allow complete gelling. A total of 3 mL of 20% fetal bovine serum and Dulbecco Modified Eagle Medium was then added to each transwell dish. We subsequently added GM6001 (Galardin; Chemicon International, Inc, Temecula, Calif) at a 5µM concentration to the appropriate dishes. Because of the minimal degradation seen with MMP-2–deficient fibroblasts, GM6001 was not added to these cultures. Media were changed every 2 to 3 days, and 5µM GM6001 was again added to the 2 respective chambers at this time. The cells were incubated for a total of 20 days. Specimens were then removed and processed for histological evaluation and stained with hematoxylin and eosin (Figure 1). We used the Axioplan 2 imaging microscope and accompanying software program, Axiowiew, version 3.1 (Carl Zeiss Microimaging, Thornwood, NY)

For the sections in which the basement membrane was up, the slides were viewed at 400X power, and the depth of the basement membrane was measured 20 times per high-power field (HPF). Ten such HPFs per sample were measured, yielding a collection of 200 depths per sample. These values were averaged to obtain a mean. For comparison, the basement membrane depth of ADM without fibroblasts was similarly measured. For the specimens with the dermal surface up, the slides were viewed at 200X power. The number of invading cells was counted on each HPF. Ten such fields were viewed and the values were averaged to obtain a mean.

STATISTICAL ANALYSIS

Means and standard deviations were calculated for the control and experimental groups. We used unpaired t tests to detect differences between the control (wild-type) and experimental (MMP-2 deficient and GM6001) groups for the basement membrane measurements. The Wilcoxon signed rank test was used to detect differences for fibroblast invasion into the dermal surface.

RESULTS

BROAD-SPECTRUM MMP INHIBITOR

Wild-type fibroblasts derived from late-gestation mouse embryos were cultured on the basement membrane surface or the dermal surface of the acellular dermis for 20 days (Figure 1). To determine whether fibroblast-mediated invasion was MMP dependent, the invasion assay was conducted in the presence of GM6001, a broad-spectrum MMP inhibitor (Figure 2). Although cultured fibroblasts did not invade the basement membrane surface of the dermal matrix, they eroded the matrix, which could be measured. Fibroblast-mediated erosion of the basement membrane was inhibited by GM6001. The basement membrane thickness of the specimen cultured with wild-type fibroblasts (mean, 14.14 μm; range, 6.19-27.36 μm; SD, 3.62 μm) measured significantly less than specimens cultured with GM6001 (mean, 22.4 μm; range, 10.37-42.78 μm; SD, 6.29 μm [P<.001]) (Figure 2A).
Because fibroblast invasion is considered to depend on matrix degradation, we measured fibroblast invasion into the dermal aspect of the ADM as a surrogate of matrix degradation. Fibroblast invasion into the matrix was 6 cells/HPF in the presence of MMP inhibition compared with 11 cells/HPF on the dermal surface ($P = .002$) (Figure 2B and C). To determine whether GM6001 is toxic to the cells or whether MMP-2–deficient fibroblasts have a decreased growth rate, we assessed the number of fibroblasts on the matrix surface in the histological sections obtained from the in vitro experiments. No significant differences in cell number were identified.

**MMP-2–DEFICIENT FIBROBLASTS**

Because a broad-spectrum MMP inhibitor reduced fibroblast-mediated invasion and basement membrane degradation, we considered the possibility that this process was mediated by MMP-2, which is capable of type I collagen degradation (dermal surface) and type IV collagen degradation (basement membrane). Using fibroblasts derived from MMP-2 knockout mice, we assessed the potential of MMP-2 to degrade the ADM. The basement membrane thickness of the specimen cultured with wild-type fibroblasts (mean, 14.14 µm; range, 6.19-27.36 µm; SD, 3.62 µm) measured significantly less than specimens cultured with MMP-2–deficient fibroblasts (mean, 19.16 µm; range, 8.73-37.54 µm; SD, 5.06 µm [$P < .001$]) (Figure 3A). The dermal surface was more readily infiltrated by wild-type fibroblasts (11.4 cells/HPF; range, 8.15 cells/HPF) than MMP-2–deficient fibroblasts (6.4 cells/HPF; range, 4.10 cells/HPF [$P = .003$]) (Figure 3B and C), suggesting the requirement for MMP-2 in this process.

**COMMENT**

The term resorption is derived from the Latin *resorber*e, which means “to swallow again.” The objective of this study was to investigate a role for MMPs in implantable ECM resorption. To this end, we used an in vitro model to assess fibroblast penetration into human ADM. Treat-
ment of ADM with an MMP inhibitor (GM6001) impeded in vitro fibroblast penetration into the dermal surface and reduced thinning of the ADM basement membrane. We found similar but less pronounced results when using fibroblasts deficient in MMP-2.

The ECM is a complex structural entity surrounding and supporting cells that are found within mammalian tissues. The ECM is often referred to as connective tissue. The ECM is composed of 3 major classes of biomolecules: (1) structural proteins including collagen and elastin, (2) specialized proteins (eg, fibrillin, fibronectin, and laminin), and (3) proteoglycans, which are composed of a protein core to which is attached long chains of repeating disaccharide units termed glycosaminoglycans. Although the total thickness of the ADM specimen may vary, we measured the dermal basement membrane thickness, which is unlikely to vary significantly,13 especially within a particular sheet of AlloDerm. We measured matrix degradation on both sides of the ADM to determine whether the same process occurred on the basement membrane surface as on the dermal aspect.

As a model of in vivo degradation, we assessed fibroblast-mediated degradation and cellular penetration of a commercially available ADM. Cellular invasion through complex matrices has been shown to be an MMP-dependent process.14-16 In fact, fibroblast invasion has been shown to require proteolytic degradation.17 Matrix metalloproteinase–dependent cellular penetration precedes remodeling in physiological conditions such as angiogenesis and wound healing.18,19 We chose MMP-2 from among the available MMPs because it degrades the type IV collagen found in the basement membrane and the type I collagen present in the dermal surface of the dermal matrix. In our study, wild-type fibroblasts degraded the basement membrane significantly more than did the MMP-2–deficient fibroblasts or those cultured with GM6001. Similarly, MMP-2–deficient fibroblasts and those treated with the broad-spectrum MMP inhibitor showed a reduced ability to invade the dermal surface when compared with wild-type fibroblasts.

It is possible that MMP-2–deleted fibroblasts do not migrate as efficiently as wild-type fibroblasts; however,
this would not affect the degradation rate. In addition, multiple MMPs acting in concert could be responsible such that deletions of other MMPs may limit matrix degradation or cellular penetration. Other possible mediators of ADM degradation include serine proteases and, in animal models, the host's immune response to the injury incurred from implanting the material. Future experiments that include in vivo models would further enhance our understanding of the role of MMPs in implantable matrix resorption.

In conclusion, although it is unlikely that a single MMP is responsible for the resorption of bioimplantable matrices, these results suggest that ADM is resorbed in part by an MMP–2–dependent process. Matrix metalloproteinase inhibitors may be important in future attempts to improve the longevity of implantable matrices.

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Additional Information: Wild-type fibroblasts were provided by Henning Birkedal-Hansen, PhD, National Institute of Dental and Craniofacial Research, Bethesda, Md; and MMP-2–deficient fibroblasts, by Lynn Matrisian, PhD, Vanderbilt University, Nashville, Tenn. The AlloDerm was a gift of LifeCell Corporation, Branchburg, NJ.

REFERENCES