The Effect of Collagen Glycation on Fibroblast Migration
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Introduction
People with diabetes make up a significant portion of the patient population in need of regenerative medicine therapies. While cell dysfunction in people with diabetes has received significant attention, alterations in the extracellular matrix (ECM) have not. Chronic exposure to reducing sugars due to diabetes can permanently modify ECM proteins. This non-enzymatic glycosylation, or glycation, can lead to the formation of advanced glycation end products (AGE) and crosslinking of the ECM. This study investigates the effects of collagen glycation on fibroblast migration.

Materials and Methods

Cell Isolation
All procedures received approval from the Institutional Review Board. Fibroblasts were isolated from skin of people with type II diabetes and non-diabetic, age-matched controls.

Collagen Glycation and Characterization
Collagen gels were prepared from rat tail collagen type I and crosslinked with glucose-6-phosphate (G6P). Following gelation, collagen gels were incubated in 100 or 375mM G6P. Gels were incubated for various times for glycation consistent with people with diabetes. Gels were characterized using confocal reflectance microscopy (fiber density), AGE ELISA, amine content (glycation), collagenase degradation, and rheometry (storage modulus).

Cell Migration
Cells were seeded on the gels and monitored using time-lapse microscopy. Cell centroids were tracked and fit to a persistent-random walk model of cell migration to determine migration speed and persistence time:

\[ \langle d \cdot d(t_d) \rangle = nS^2P[t - P(1 - e^{-t/P})] \]

Statistical Analyses
Statistical comparisons were analyzed using students t-test. A p value < 0.05 was considered statistically significant.

Results

Properties of Glycated Collagen
Collagen gels incubated in G6P exhibited glycation, AGE formation, resistance to degradation, and increased stiffness consistent with levels in people with diabetes (Table 1) without altering fiber density.

<table>
<thead>
<tr>
<th>G6P (mM)</th>
<th>0</th>
<th>100</th>
<th>375</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber Density (mm/mm²)</td>
<td>1.8 ± 0.2</td>
<td>1.4 ± 0.3</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>AGE (pmol/mg)</td>
<td>48 ± 73</td>
<td>498 ± 737</td>
<td>666 ± 481</td>
</tr>
<tr>
<td>Glycation (%)</td>
<td>0</td>
<td>13 ± 2</td>
<td>26 ± 8*</td>
</tr>
<tr>
<td>Degradation time (s)</td>
<td>2731 ±182</td>
<td>2810 ± 164</td>
<td>3783 ± 357*</td>
</tr>
<tr>
<td>Storage modulus at 1.6 Hz (Pa)</td>
<td>46 ± 2.0</td>
<td>55 ± 4.4</td>
<td>111 ± 9.4*</td>
</tr>
</tbody>
</table>

Table 1: Influence of G6P Concentration on Collagen Gels. * indicates significance from 0 mM.

Cell Migration
There were no differences in migration parameters between cells from people with and without diabetes. However, migration speed for both cell types decreased with increasing collagen glycation under both high and low soluble glucose levels (Figure 1).

Discussion and Conclusions
These results show that collagen glycation alters fibroblast migration from people with and without diabetes. There were no differences between the cells from the two different patient populations. These results suggest that alterations in the ECM may play an important role in altered cell behavior and wound healing.

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Disclosures
Authors have no conflicts of interest to disclose.