

# Methods for the Quantitative Analysis of Diabetic Fibroblast Migration

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**Abstract:** Normal wound healing is a complex process involving the coordinated growth and migration of many different cell types. Fibroblasts play an important role by migrating to the injury site and secreting necessary materials to promote cell growth. Individuals suffering from diabetes are known to have compromised wound healing capabilities. This study focuses on developing methods to compare the migration of diabetic and non-diabetic fibroblasts. A Matlab® program was created to calculate two mathematical parameters of migration, cell speed and directional persistence. These parameters were calculated by fitting experimental data to a persistent random walk model through non-linear regression. Preliminary results imply that diabetic fibroblasts migrate with greater speed and increased persistence compared to those isolated from non-diabetic individuals.

## 1. INTRODUCTION

In 2000 there were 171 million reported cases of diabetes worldwide, and it is expected that this figure will double by the year 2030 [5]. According to the World Health Organization, in 2000 alone, 3.2 million individuals died from diabetes related complications [6]. Type II diabetes, also known as adult onset diabetes, is caused by a resistance of tissues within the body to insulin, and accounts for approximately 90% of diabetes cases worldwide. Diabetics have increased risk for cardiovascular disease, blindness [3], kidney failure, and amputation [4].[6]

An important complication of diabetes mellitus is a reduced ability to recover from injuries and infections due to cellular abnormalities, impaired release of growth factors, and compromised operations of healing-related cells [4]. There are multiple players involved in normal wound healing, including fibroblasts, inflammatory cells, endothelial cells, growth factors, and enzymes [4]. Within this cellular response, fibroblasts play an essential role by migrating to the wound site where they secrete extracellular matrix and growth factors necessary to stimulate further cellular growth [1]. Diabetics can also suffer from hyperglycemia which, when uncontrolled, can have devastating effects on the body possibly by altering cell function. Previous research has shown that glucose concentration in culture media influences cell migration [3].

Cell migration can be characterized through the usage of two major parameters: the cell speed,  $S$  ( $\mu\text{m}/\text{min}$ ), and the persistence time of the cells,  $P$  (min). Persistence can be qualitatively described as a characteristic time over which cell movement is not random. These parameters can be calculated by fitting experimental cell tracking data to a persistent random walk model:

$$\langle \underline{d} \cdot \underline{d} \rangle = nS^2 P(t - P(1 - e^{-t/P})) \quad (1)$$

where  $\langle \underline{d} \cdot \underline{d} \rangle$  is mean squared displacement (i.e., the square of distance),  $t$  is the time increment of cell tracking, and  $n$  is the number of dimensions. The mean squared displacement can be determined from experimental data for a time interval  $t_d = n_d \Delta t$  (where  $n_d$  is the number of steps at  $t_d$ ) tracked for a total of  $N \Delta t$  min and it is calculated by:

$$\langle d \cdot d(t_d) \rangle = \frac{1}{(N - n + 1)} \sum_{i=0}^{N-n} [(\bar{x}_{i\Delta t} - \bar{x}_{(n+i)\Delta t}) \cdot (\bar{x}_{i\Delta t} - \bar{x}_{(n+i)\Delta t})] \quad (2)$$

where  $\bar{x}_i$  is the position vector at time  $i$ . These values are calculated for all intervals and fit to Equation 1 to determine  $S$  and  $P$  for an individual cell.

The primary objectives of this research were to 1) write Matlab® code to compute the speed and persistence of migration from cell tracking

data in 2D culture and to 2) apply this code to compare migration characteristics of fibroblasts isolated from diabetic and non-diabetic patients. This would aid in determining if there are migrational differences between fibroblasts isolated from the limbs of diabetic patients compared to non-diabetic fibroblasts. As such, this research should provide some insight into explaining the differences between the compromised wound healing in diabetic patients in contrast to normal wound healing. This may also help lead to improvements in wound healing therapies.

## 2. MATERIALS AND METHODS

### 2.1 Cell Culture and Migration Assay Setup

Standard cell culture techniques were used to grow human dermal fibroblasts isolated from both diabetic and non-diabetic patients. All protocols received approval from the Hines V.A. Hospital IRB. Cells were grown in Dulbecco's modified eagle medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS), 50 µg/mL gentamicin, and 2 mM L-glutamine. Cells were seeded (500 cells/well) into standard 24 well plates.

### 2.2 Cell Tracking

Wells were imaged every 20 min over a 24 h time period using a Zeiss Axiovert 200M microscope and Zeiss AxioCam Mrm camera (Zeiss). Either one or two positions were chosen for observation within each well. Criteria for position selection included cell position, proximity to other cells, and the total amount of cells in the field of interest (FOI). Positions were observed using a 10x objective where 1 pixel = 0.624963 µm. Each movie was viewed and individual cells were tracked using Axiovision software. Tracking was done by marking cell centroids for each 20 min time interval. The collected X and Y centroid coordinates were saved as a data file and input into the Matlab® program to plot mean squared displacement and calculate speed and persistence time by fitting the data to the persistent random walk model.

### 2.3 Nonlinear regression

Matlab® 6.1 code was written that allows the user to load pixel coordinates collected from tracked cells. The program then calculates the speed and persistence time by providing a fit for the mean squared displacement through a nonlinear regression. The Levenberg-Marquardt algorithm was used to perform the nonlinear regression.

As long as the time interval is much less than the persistence time, the cell speed is calculated explicitly from the first time point. The persistence time is then determined using the non-linear regression. The initial estimate of the persistence time is 100 min based on previously published results for human fibroblasts. The actual persistence time is then determined from the nonlinear regression. The goal of a regression is to minimize the sum of the squares:

$$S = \sum_{i=1}^N \left( \langle d \bullet d(t_d) \rangle_{ex} - \langle d \bullet d(t_d) \rangle_p \right)^2 \quad (3)$$

where the subscripts ex and p indicate from experimental data (Equation 2) and predicted from the persistent random walk model (Equation 1), respectively. Based on this sum, a new guess for the persistence time is determined by solving the equation:

$$\left( J^T J + \lambda I \right) q = -J^T f \quad (4)$$

where J is the Jacobian, I is the identity matrix, λ is a damping factor, and q is the iterative change in persistence time. Initially, λ=1 and Equations 3 and 4 solved iteratively to minimize S. The next step found S using λ/v, where v=2. If S did not decrease λ was replaced with λv<sup>k</sup>, for k=1, 2, ...,10 until a minimum was found. The persistence time is the value at the minimum of S. The code was written in a flexible fashion allowing users to input the unit conversions for the data, the time increment, and initial guess for p, allowing the code to be applied to many different experimental conditions.

### 3. RESULTS

The Matlab® program was utilized to analyze migration of fibroblasts isolated from both diabetic and non-diabetic patients. Cells were grown on polystyrene tissue culture plates under standard glucose (5 mM) concentrations and imaged every 20 min for 24h. Example fibroblast migration paths of the analyzed data are presented in Figure 1. The mean square displacement was automatically calculated for each cell (Figure 2) and fit to equation one (Figure 3). The parameters of cell speed and persistence were then determined for each cell based on the regression. Multiple cells were analyzed for each patient to give mean speed and persistence times. Results for three patients are summarized in Table 1.

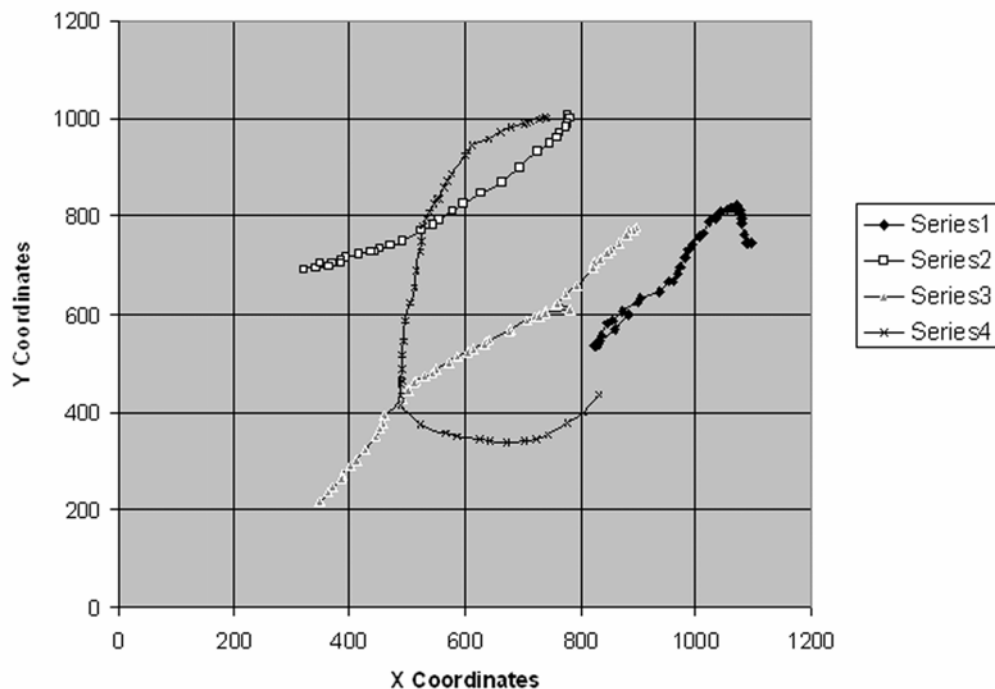
### 4. DISCUSSION

The Matlab® code was successfully developed and applied to the analysis of 2D cell migrations comparing fibroblasts isolated from diabetics and non-diabetics. Initial results suggest that fibroblasts isolated from diabetic patients migrate at a higher speed and with an increased

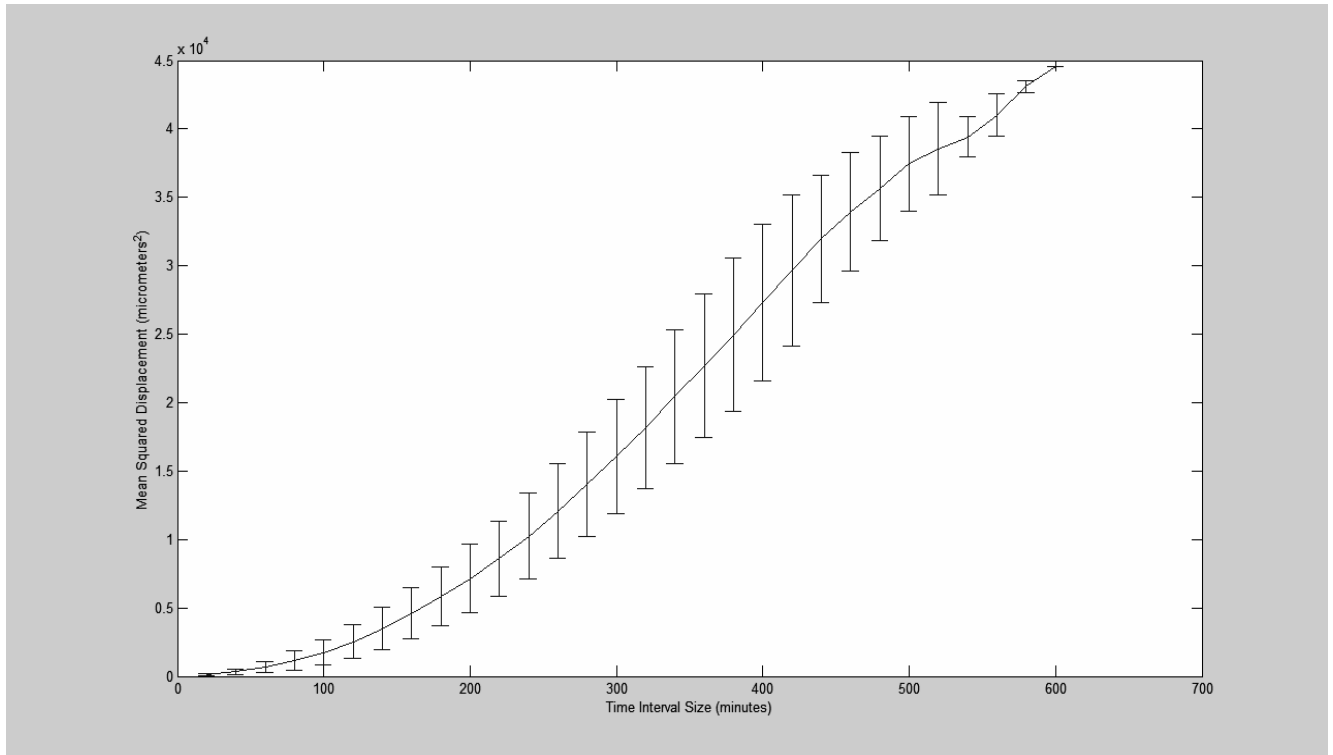
persistence when compared to those isolated from a non-diabetic individual. Current work focuses on applying this approach to compare baseline migration characteristics of cells from diabetics and non-diabetics. Future work will be to apply this model to the development of analysis of new wound healing therapies in regenerative medicine.

### ACKNOWLEDGEMENTS

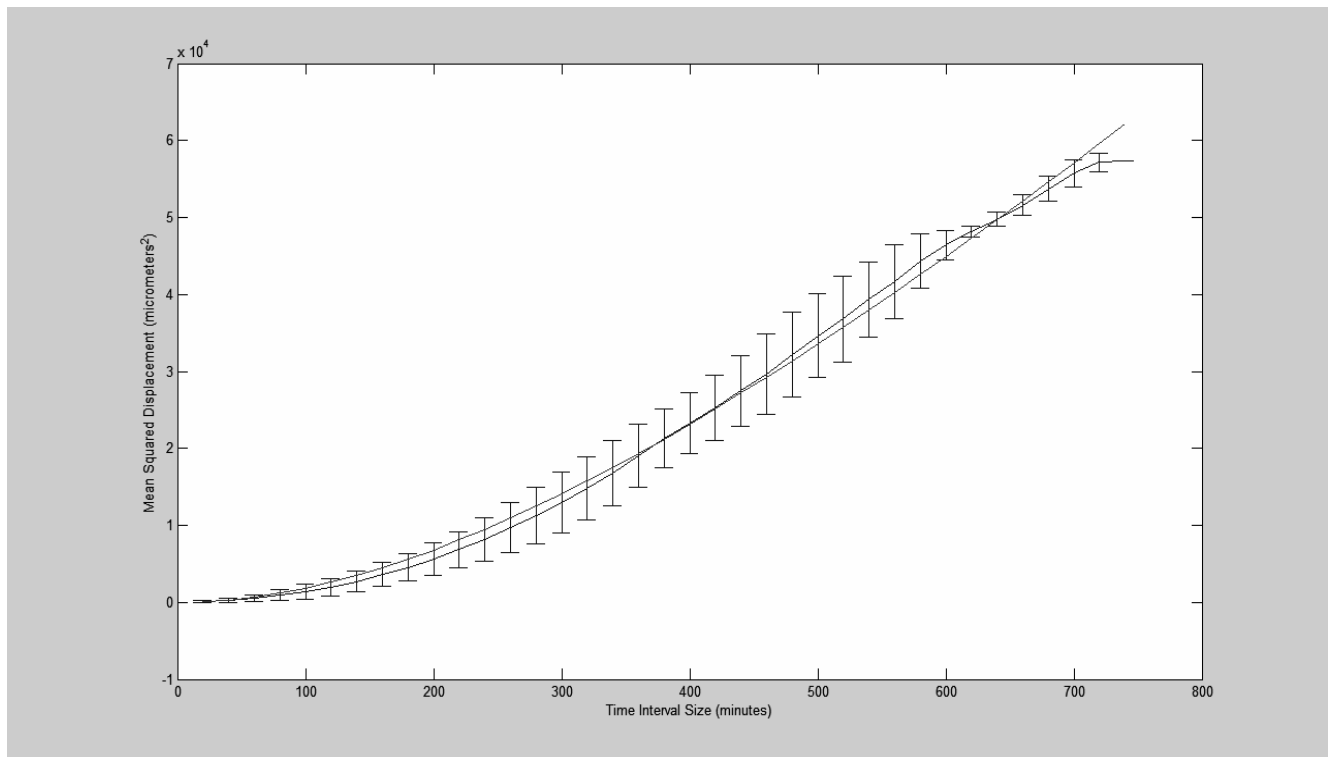
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**Figure 1** Example diabetic fibroblast migration paths.



**Figure 2** Mean square displacement for a tracked diabetic fibroblast.



**Figure 3** Regression showing fit of the persistent random walk model to experimental displacement data.

Type	N	average s ( $\mu\text{m}/\text{min}$ )	average p (min)
Diabetic	49	0.5122	220.05
Diabetic	45	0.577	171.81
Non-diabetic	37	0.3804	95.437

Key	
N	# of cells tracked
s	Speed
p	persistence

**Table 1** Summarized speed and persistence values for two diabetic patients and one non-diabetic patient.

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