Supplemental Data

Methods

Microarray experiments and analysis

Triplicate samples of hSMM cells were cultured on each of three coatings: collagen, gamma KOS, or Matrigel™, or on uncoated tissue-culture plates (control). Gamma KOS was chosen as the keratin treatment based on the results of the adhesion assays (Figure 3), which showed that this fraction of keratin led to the only significant change in adhesion compared to collagen. After 10 days, media was removed from the wells, rinsed once with PBS, and frozen at -80 for subsequent RNA isolation. Total RNA isolation, RNA QC using the Agilent 2100 Bioanalyzer and gene expression assays were performed on Illumina HT-12 BeadChip microarrays, one array per sample, at Expression Analysis (Durham, NC). In order to determine the overall similarity between samples, raw data were subjected to Principal Components Analysis (PCA; Qlucore Omics Explorer software). No filtering was applied to the profile level data prior to PCA. Raw gene expression data were uploaded into the GeneSifter® Analysis Edition (Geospiza, Inc, Seattle, WA) software suite for a pair-wise analysis to determine differentially-expressed transcripts. For all pair-wise analyses (3 total: cells grown on each of three surfaces versus cells grown on uncoated plates) the data sets were compared using the Student's t-test, with Benjamini-Hochberg FDR (False Discovery Rate) correction, to generate lists of differentially-expressed transcripts (DETs). The fold-change threshold was set at ≥ 2.0 and the data were considered significant if the comparison had an associated log ratio adjusted p-value ≤ 0.05. The overlap of differentially-expressed transcripts from the three pair-wise comparisons was visualized by using a Venn diagram and some key transcripts that exhibit differential expression on each coating are discussed.

Results

Based on the observation that gamma KOS led to significant increases in adhesion, microarray analysis was used to assess gene expression profiles of skeletal muscle progenitor cells following interaction with keratin, Matrigel™ and collagen. Triplicate samples of hSMM cells were cultured on each of three coatings: collagen, gamma KOS, or Matrigel, or on uncoated tissue-culture plates (control). After 10 days, RNA was isolated from the cells and gene expression assays were performed. In the unsupervised data reduction analysis (principal components analysis; PCA) displayed in Figure S1A, the entire gene expression profile for each individual sample is represented by a colored sphere. The cells grown on uncoated plates showed the lowest between-sample variability in gene expression. Cells grown on Matrigel also demonstrated relatively low between-sample variability, however their gene expression profiles were quite different from the other three groups. In cells grown on each of the other two coatings (collagen and gamma KOS), one individual replicate was highly different from the other two. Overall, cells grown on gamma KOS and collagen had more similar gene expression profiles to each other and to cells grown on uncoated plates than to cells grown on Matrigel™.

Figure S1B shows the results of pair-wise statistical comparisons between gene expression in cells grown on each coating versus cells grown on uncoated plates returned modest numbers of differentially-expressed transcripts (DETS). Gamma KOS versus uncoated had 30 DETS; collagen versus uncoated had 20 DETS whereas Matrigel versus uncoated had 90 DETS and there was almost no overlap between the three lists (Figure S1B).

The single transcript change that overlapped between cells grown on Matrigel and gamma KOS (Figure S1B) is ILMN_1791634. This corresponds to a DNA microarray element

(XM_941911) Homo sapiens gene similar to Plectin 1 (PLTN or PCN). (Hemidesmosomal protein 1 is the designation used by Illumina).

The top genes changed in keratin relative to the uncoated control included LIF (a gene involved in differentiation, down 8.02-fold) and FOXC2, SP2, and ZNF200, which are transcription factors. The top genes affected by collagen also included transcription factors (SREBF2, ZFY, and HIC1), the translation factor eIF5, and genes involved in lipid metabolism (SREBF2 and BMOAT2). The top DETs in the Matrigel™ to uncoated comparison had greater fold-changes than in the other comparisons, suggesting that the differences observed in Matrigel™ are more likely to be real. The top genes (e.g., FNDC1 and SPINT2) changed in the Matrigel™ group were associated with functions of the extracellular matrix, cell-cell interactions, and cell growth. It is interesting to note that one of the down-regulated genes in the keratin group was LILRA5 (down 5.23-fold, p value <0.0001). This gene is increased in expression in rheumatoid tissue, and may be involved in inflammation (Mitchell et al, LILRA5 is expressed by synovial tissue macrophages in rheumatoid arthritis, selectively induces pro-inflammatory cytokines and IL-10 and is regulated by TNF-alpha, IL-10 and IFN-gamma, European journal of immunology, 38(12): 3459-3473, 2008), so its down-regulation may be an advantageous cellular response. While this result is intriguing, down-regulation of a single gene does not imply a mechanism of action and could simply be a structural phenomenon of the biomaterial. Taken as a whole, however, these results seem to indicate that gamma keratose acts in a manner similar to collagen. Matrigel has an undefined nature, indicating that its multiple components could be responsible for the changes in gene expression.

Supplemental Figure 1. Gene array results for hSMM on keratin coatings.

Figure S1A. Principal components analysis; PCA representing the entire gene expression profile. Each individual sample is represented by a colored sphere: collagen (green), gamma KOS (red), Matrigel (white), and Uncoated control (blue).
Figure S1B. Venn Diagram analysis. Pair-wise analyses were performed between gene expression data from cells grown on each of three coatings versus cells grown on uncoated tissue-culture plates. The degree of overlap between the lists of DETs (@ fold change ≥ 2; adjusted p ≤ 0.05) is displayed.
Figure S1C. Molecular functions analysis. Pairwise analysis (Student's t-test) was used to compare gene expression in cells grown on keratin vs. uncoated control tissue culture plastic. Differential expression was measured at a fold change of ≥ 1.7 and a p value ≤ 0.05. In the comparison between cells plated on gamma KOS there were 776 DETs (differentially expressed transcripts), representing a number of molecular functions.

Supplemental Figure 1