

Robust computational reconstitution – a new method for the comparative analysis of mRNA expression in tissues and isolated cell fractions

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1 Introduction.

The comparative analysis of gene expression in tissue samples and isolated cell fractions is pivotal for the understanding of healthy and diseased tissues because it allows to assess interactions between different cell types within the tissue. Isolation and amplification of mRNA from micro-dissected single tissue cells or cell-type specific sub-populations has recently been described in single publications [2, 5]. However, this method has still problems with exact dissection and representative mRNA extraction and amplification. Therefore, the present study compares gene expression profiles of whole tissue samples and cell fractions purified from the tissue. To this end it uses a computationally reconstituted tissue profile. Three main issues were addressed: 1) determination of the mRNA fractions that originate from different cell types, 2) identification of robustly expressed genes (having comparable expression in tissue and isolated cell fractions) and 3) biological interpretation of the results.

Methods for the reconstruction of cell-specific mRNA fractions have already been proposed by Venet et al. [6], Lu et al. [3], and Stuart et al. [4]. The methods of Venet et al. and Stuart et al. are exclusively based on tissue samples. In contrast, Lu et al. and the present study investigate tissue samples and their isolated cell-specific sub-populations at the same time. Whereas Lu et al. comparatively analyze synchronized and desynchronized yeast cells the current approach compares synovial tissues with the isolated cell fractions of macrophages, fibroblasts, and non-adherent cells obtained from patients with rheumatoid arthritis (RA) and osteoarthritis (OA), respectively (using Affymetrix HG-U95 GeneChips). The present study demonstrates for the first time that the determination of the mRNA fractions is only possible for a subset of robustly expressed genes. The accurate performance of the reconstitution algorithm was validated using two own mRNA mixing experiments and the mixing part of the GeneLogic dilution study [1]. The mRNA fractions obtained from robust computational reconstitution were compared to cell fractions assessed by immunohistochemistry as well as to mRNA fractions calculated from the expression of cell-specific marker genes. A probabilistic model completes the present approach.

2 Mathematical Model.

The present computational approach for determining the mRNA composition is based on a comparison between the measured expression profile S of the whole synovial tissue and the

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computationally reconstituted tissue profile

$$S^* = p_M M + p_F F + p_N N \quad (1)$$

which is additively composed from the measured expression profiles of the isolated cell fractions of macrophages M , fibroblasts F , and diverse non-adherent cells N , according to their respective computed fractions p_M, p_F and $p_N = 1 - p_M - p_F$ (Figure 1).

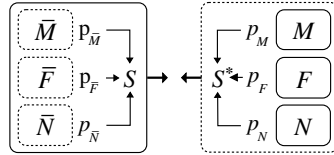


Figure 1: Schematic view of the gene expression in the synovial tissue (left) and the computationally reconstituted tissue profile (right). The expression of the synovial tissue is made up of contributions from macrophages \bar{M} , fibroblasts \bar{F} , and non-adherent cells \bar{N} . Only the overall tissue expression profile S has actually been measured. The reconstituted tissue profile is composed of the measured expression profiles of the isolated cell fractions, i.e. isolated macrophages M , fibroblasts F , and non-adherent cells N .

3 Conclusions.

Computational reconstitution can be used for the analysis of cell-cell interaction processes in tissues provided there is a sufficiently high number of stable genes. This number is estimated to be in the range of 1/4 to 1/2 of the genes represented on chip. A comparison with immunohistochemistry showed a noticeable correlation between the cell fractions and the computed mRNA fractions, especially for fibroblasts. Two-dimensional regression resulted in a higher and more significant correlation than in one dimension indicating that the total mRNA production rate may depend on the interaction with other cell types. Obviously, macrophages and non-adherent cells respond stronger to environmental changes than do fibroblasts. This is corroborated by the fact that a correspondence between the computed mRNA fractions and the mRNA fractions obtained from cell-specific marker genes was reasonable only for fibroblasts.

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