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Enhancing transdermal drug transport with low-frequency ultrasound

The recent completion of the human genome project and advances in molecular biology techniques have enabled the discovery and characterization of many peptides, proteins or polynucleotides as potentially novel drugs. Because these molecules are metabolically labile and undergo extensive enzymatic degradation, they cannot be administered to patients by the traditional oral route. Hence, pharmaceutical scientists have explored alternative modes of administration, such as the transdermal route, for the effective systemic delivery of these compounds.

Transdermal drug delivery offers several advantages over traditional drug delivery systems such as oral delivery and injections: the attractive attributes of transdermal drug delivery include avoidance of first-pass metabolism, elimination of pain that is associated with injection and the opportunity for the sustained release of drugs. However, the efficacy of the transdermal transport of molecules is low because the stratum corneum of the human skin is an effective and selective barrier to chemical permeation [1]. Indeed, the low permeability of the stratum corneum is the key reason that only a small number of low molecular weight drugs are currently administered using this route [2]. In a recent issue of *Drug Discovery Today*, Lavon and Kost [3] provide an excellent and comprehensive overview on the use and mechanism of low-frequency ultrasound to promote the transdermal transport of drugs, which could have applications in drug delivery as well as transdermal monitoring.

The biophysical modes of ultrasonic action on a biological system can be classified into two categories – thermal mechanisms and non-thermal mechanisms [4,5]. The thermal effects of ultrasound, which is directly related to the intensity of the ultrasound, results from the transfer of energy from the vibrating pressure waves to the objects as the waves propagate through the medium. In transdermal applications, this energy is absorbed by the skin, which results in a rise in skin temperature. Although literature supports the observation that increasing temperature leads to enhanced skin permeability [6], recent studies indicate that thermal effects play an insignificant role in promoting transdermal drug transport that is effected using low-frequency ultrasound [7,8]. For example, low-frequency ultrasound (20 KHz at 15 W cm^{-2} for 2 h) caused a 20°C rise in temperature that resulted in 35-fold increase in the level of mannitol delivered across porcine skin *in vitro*. By contrast, when the skin was heated (in the absence of ultrasound) to produce a thermal profile that is comparable to the thermal profile generated by ultrasound, the permeability of mannitol increased by only 25% [8]. These data indicate that the key mechanism responsible for the observed skin permeability is related to the non-thermal effect of ultrasound. Although the mechanism for improved transdermal transport by ultrasound is not well understood and has yet to be characterized fully, a consensus has been reached that acoustic cavitation is responsible for low-frequency sonophoresis.

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Gene expression analysis enriched

The use of DNA microarrays to identify genes that are upregulated or downregulated is a crucial component in

the early phase of drug target development, optimization and clinical validation. It is well recognized that a significant change that is revealed by statistical methods, even if fitted to the experimental objectives, does not always unlock all the biologically significant changes that are related to disease or treatment. Novel approaches to enrich expression analysis by including information on pathways, biomedical literature or phenotype have therefore increased interest in this approach.

The analysis of expression levels of thousands of genes remains a daunting task, not only because of the increasing number of transcript probes on microarrays that have now reached genome-wide coverage, as well as variability between individuals and limited sample sizes, but also as a result of the limitations of well-established statistical methods, such as differential expression and clustering. Typically, the course of an experiment includes pooling of specimens, and optimal pooling schemes are achieved by balancing statistical control with minimal total costs. Subsequent fold-analysis facilitates the identification of 'top-ranking' genes, whereas cluster analysis exposes patterns that indicate functional relationships. However, pooling schemes and 'gene-centric' statistics can obscure relevant information, particularly when the variability between individuals is high and the changes are modest.

Mootha *et al.* [1] have recently published an alternative strategy for gene-expression analysis that uses *a priori* sets of genes as part of the data analysis process; this method was referred to as gene set enrichment analysis (GSEA) [1]. The goal in this study was to determine whether *a priori* defined sets of genes are enriched at the top of the list of genes that are listed in order based on the expression difference between classes, such as normal and disease. Functionally related genes, for example, genes that belong to pathways, constitute such predefined sets. The application of this methodology to a large collection of individuals in a diabetes study enabled Mootha and co-workers [1] to detect modest, but coordinate, changes in the expression of oxidative phosphorylation-coregulated genes, the expression of which is mediated by a peroxisome proliferator-activated receptor-γ coactivator-α, a finding that would not have been revealed using traditional fold-analysis. The GSEA approach appears to be particularly applicable when it is considered that microarrays are progressing into areas such as aging and metabolic disorders.

Limitations of current statistical approaches also include cluster analysis. Quality cluster results are obtained when the optimum cluster algorithm is chosen, the design of which biases and the assumptions of which meet the underlying distribution of the data set; the results will be poor if the assumptions are violated in the data set. However, the underlying distribution of the gene expression data sets is typically unknown, and a variety of clustering algorithms is available. Combining multiple clustering algorithms – cluster ensembles – improve the results. Interpretations can be enhanced through the identification of relationships between genes or proteins that form a cluster, a process that is time-consuming and notoriously limited by incomplete functional annotations. A new generation of automated text-mining algorithms has been developed that automatically queries and analyzes relationships between genes or proteins based on published scientific literature [2]. Automated text-mining holds the promise to consolidate the large amounts of biological knowledge that have been published in an estimated 12 million abstracts and articles to make them an integral part of analytical procedures.

The inclusion of phenotypical data as covariants in transcriptomics opens another approach for enriching microarray analysis. Phenotypical data sets include metabolomics or structural high-content image data based on emerging high-throughput technologies, for example, multiparameter flow cytometry (Cytomics; http://www.cytomics.info) and automated tissue-histomorphometry (Tissomics), all of which will make phenotypical data more readily available. In particular, microscopic tissue-screening combined with image informatics supplements a visually performed histopathological diagnosis and is equipped to work in regulated environments (e.g. Title 21 Code of Federal Regulations Part 11).

When the goal of the research is to correlate gene expression with variables that are measured at the subject level or to identify gene profiles that help to classify individual subjects and predict their membership in groups, pooling of mRNA would not be appropriate. Adequate statistical methods consider these covariants by using rank correlations rather than fold-analysis of pooled samples [3]. As a result, genes that would otherwise be missed can now be unlocked. Statistical considerations that preserve individual responses will probably be more relevant for all phases of drug development and for the creation of a personalized medicine.

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