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A novel approach for noninvasive drug delivery and sensing through the amniotic sac

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ABSTRACT

Current invasive prenatal tests (amniocentesis and chorionic villus sampling) are known for their risk to the fetus. In the last decade, the use and awareness of these prenatal tests have increased, resulting in growing demand for a safe, non-invasive, and accurate prenatal test. Chemical penetration enhancers (CPEs) have long been used to increase transport phenomena across skin and other membranes (e.g., tympanic membrane). The amniotic sac membrane is called the chorioamnion (CA) membrane and serves as the physical barrier between the fetus and the mother. In this research, the effect of CPEs on human CA mass transport was evaluated both *in vitro* and *ex vivo*. The results show that the tested CPEs exhibit an enhancing effect on CA mass transport. Based on the permeability results, two mechanisms of action were suggested: "extractors" and "fluidizers". Fourier transform infrared (FTIR) and rapid colorimetric screening measurements supported the mechanisms, based on which, more potent compounds were designed and tested for their enhancing effect. The enhancing mass transport effect of CPEs on CA membrane may be used both for sampling of cell-free DNA and for noninvasively administering drugs and other biological agents to the amniotic sac.

1. Introduction

In the past two decades, prenatal tests have become more and more frequent and available to pregnant women. These tests can be divided into two main groups: invasive and noninvasive. The noninvasive tests, mainly maternal blood screening and ultrasound imaging, present almost no risk to the fetus or the mother, but are not very accurate [1]. Second trimester maternal serum screening (MSS) for Down syndrome (DS) and open neural tube defects (ONTDs) has become routine obstetric practice in many countries. The commonly used triple screen that uses maternal serum alpha-fetoprotein (AFP, 69 kDa), unconjugated estriol (uE3, 288 Da), and human chorionic gonadotrophin (total hCG, 25.7 kDa) in combination with maternal age can detect 60% to 65% of fetuses with DS and 80% of fetuses with ONTD [2]. On the other hand, the invasive tests, mainly amniocentesis (AMN) and chorionic villus sampling (CVS), are very accurate (98–99% for both CVS and AMN) [3] but do present a potential risk for the fetus and the mother (approximately 1% increase in miscarriages following AMN and higher for CVS [4]). Recently, a new prenatal test was suggested in which fetal cell free DNA (cfDNA) fragments are detected in the maternal blood for the detection of Trisomy 13, 16, 18, and 21 [5,6]. The authors emphasize the need for construction of a library to account for all fetal genetic material. Also, there is still a debate between researchers regarding the origin of the cfDNA; while some suggest that cfDNA exists in maternal blood even decades after giving birth, others refute this finding [7,8]. A recent study reviewed the clinical validation of studies of maternal blood cfDNA analysis in screening for aneuploidies [9]. The study gives three main limitations for implementing this method: the 1–2 weeks needed for the results to be returned (due to the small sample size); the 1–5% rate of failure to provide results, again due to the small sample size; some fetal diseases cannot be detected or the accuracy for the detection of these diseases is reduced also due to the small sample size. Thus acquiring higher concentrations and variety of cfDNA may improve the accuracy and applicability of this method. These data only emphasize the growing need for reliable, safe, noninvasive, and accurate prenatal detection.

Researchers have long been seeking and developing new noninvasive methods in every field of medicine where there was a need for it. These methods include, but are not limited to, low and high intensity focused ultrasound (LIFU and HIFU), sonophoresis, electrophoresis, iontophoresis, chemical penetration enhancers (CPEs), and micro-needles.

One of the most commonly used methods for mass transport increase is CPE application. Williams and Barry, in their excellent

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review on CPEs, categorized 12 different groups of chemical enhancers that are known to increase mass transport across skin [10]. Among these groups of CPEs one can find pyrrolidones, surfactants, alcohols and glycols, fatty acids, and metabolic intervention agents. The main challenge for using CPEs is the fact that it is almost impossible to predict if and which CPEs will have an effect on mass transport, since their effect varies for different membranes and permeates and is also concentration dependent. Different modes of action have been identified for CPEs on skin: the intercellular lipid matrix in which the CPEs may disrupt their organized packing; the intracellular keratin domains, through increasing drug partitioning into the tissue by acting as a solvent for the permeate; and "pooling" effect. "Pooling" effect occurs when CPEs heterogeneously concentrate within domains of the bilayer lipids. Such a "pooling" phenomenon has been shown for Oleic acid and Azone, and is likely to occur (in skin) for most CPEs, considering the range of packing and different molecular domains in the stratum corneum (SC) lipids [10]. Until today, the effects of the permeability enhancers described above were described mainly for skin and, to a lesser extent, other "peripheral" membranes such as the tympanic membrane, buccal mucosa, nasal membrane, and even the blood retinal barrier [11–15].

The chorioamnion (CA) membrane constitutes the sac enveloping the fetus. It is essentially composed of two membranes attached together solely by the amniotic fluid pressure exerted on it. The membrane facing the maternal side is called the chorion membrane. The other membrane, which faces the fetal side, is called the amnion. The amnion is mainly composed of a collagenic network that gives the entire CA membrane its mechanical strength. In general, the sac surrounding the fetus does not develop intact all at once but rather in stages and layers over weeks, somewhat similar to two balloons gradually being inflated inside of a rigid sphere. The final arrangement of membranes is present for at least the last half of the pregnancy. While the chorion is a moderately organized tissue without blood vessels (less organized than skin), the amnion is composed mainly of fibrillar collagen [16]. The chorion thickness is 431 ± 113 μm, amnion 111 ± 78 μm (skin is 1000–1500 μm) [17,18]. The higher organization and thickness of skin may explain why the skin possesses a greater barrier for mass transport. The fact that the chorion is also composed of lipid membranes may indicate a similar effect of CPEs. A histological section of the chorioamnion is presented in (Fig. 1).

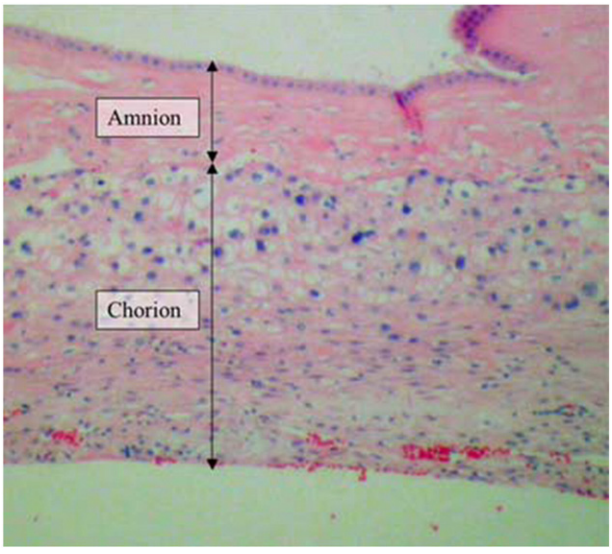

Fig. 1. Hematoxylin and eosin (H&E)-stained histological section of the CA membrane at 39-weeks gestation [16].

Only a handful of articles were published on the permeability properties of the CA membrane. For example, Guiet-Bara et al. [19] evaluated the passive permeability of the CA membrane (or just amnion or chorion) toward ions, particularly monovalent cations. Menjoge et al. recently tested the passive transport of poly-amidoamine (PAMAM) dendrimers through the CA membrane [19,20]. The chorion is less permeable than the amnion as was shown by Guiet-Bara [19] and Battaglia [21]. Moreover, in 2001 it was also discovered through FTIR measurements by Nishsida et al. [22] that there is a great variety in CA membrane properties, not only for different donors but also for different sites of the same donor. The thickness of the CA membrane varies from mother to mother and also depends on its location (distance from placenta).

Most drugs with a molecular weight of <500 Da readily cross the placenta and enter the fetal circulation. Substances with a high molecular weight (e.g., protein-bound drugs) usually do not cross the placenta [20,23]. Today two strategies exist for the treatment of fetal tachycardia/bradycardia: administering drugs to the mother or direct fetal therapy (by either intra-umbilical or intramuscular injection). Maternal drug treatment does not work well when the fetus has fetal hydrops (a serious fetal condition defined as abnormal accumulation of fluid in 2 or more fetal compartments, including ascites, pleural effusion, pericardial effusion, and skin edema, [24]) and only allows control in 50–70% of fetuses without hydrops [25]. As for direct fetal treatment, it may cause many complications such as bleeding, preterm labor, chorioamniotic membrane separation, and premature rupture of these membranes [26]. The main problem with these fetal diseases is the fact that the mother is healthy while the fetus is not. For example, fetal tachycardia requires drugs that decrease heart rate, which is not desirable for the healthy mother. As of today, there are no approved drugs for application onto the CA membrane.

Thus, the goal of this work was to evaluate the effect of CPEs on mass transport across CA membranes in order to draw (through the cervix) amniotic fluid's cfDNA and enable sampling in a non-invasive manner for abnormality detection. We also set out to understand the mechanism by which the CPEs act upon the CA membrane.

2. Materials and methods

PBS tablets, d-limonene, polyoxyethylene octyl phenyl ether (TX-100), 1-dodecyl-2-pyrrolidinone (1D2P), ethylene glycol (EG), bupivacaine, lidocaine, iso-stearic acid (ISA), decanoic acid, oleic acid, cetyltrimethylammonium bromide (CTAB), n-methyl-2-pyrrolidone (NMP), sodium lauryl sulfate (SLS), and fluoro isothiocyanate (FITC)-Dextran (77 kDa) were all purchased from Sigma–Aldrich, Israel. Chloroform and EtOH (AR grade) were purchased from Bio-Lab Ltd., Jerusalem, Israel. 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), was purchased from Avanti Lipids (Alabaster, AL, USA). The diacetylenic monomer 10,12-tricosadiynoic acid was purchased from Alfa Aesar (Karlsruhe, Germany).

The diaphragm vacuum pump used in CA membrane FTIR measurements was made by Vacubrand GMBH CO + KG model ME 2C, the volumetric flow rate was 1.7 $m^3\ h^{-1}$. All solutions were prepared in PBS (0.01 M, using double distilled water). All CPE solution concentrations were below their known skin irritation level. All concentrations in this paper are given as percentage volume for liquid CPEs and as w/v for powder/solid CPEs.

2.1. Diffusion experiments

CA membranes were kindly provided by "Hillel Yaffe" Medical Center (and authorized for experiments by the "Hillel Yaffe" Helsinki Committee). In order to preserve the CA membranes, they were cut into 5 cm × 5 cm squares and then kept in −20°C until