Measuring Black Carbon Particles in the Human Placenta

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INTRODUCTION

The relationship between exposures to ambient particulate matters (PM) and pregnancy outcomes has been well established with reports of pre-term births and low birth weights (Hu et al., 2014). A major source of the concerning air pollution is Traffic Related Air Pollution (TRAP), conventionally measured and indicated by the concentration of black carbon (BC) particles. Although it is known that the placenta can serve as a sieve, in which it prevents translocation of certain materials from the maternal to the fetal circulation, recent literature has demonstrated transplacental movement of BC particles (Bove et al., 2019). From our NIH ECHO (Environmental Influences on Child Health Outcomes) UPSIDE Study in Rochester, New York, we have replicated these findings, detecting BC particles in the three areas of placentae: maternal blood, fetal blood, and fetal tissue. Here, we present our imaging technique and analysis protocol established for BC identification and quantification in human placental tissue.

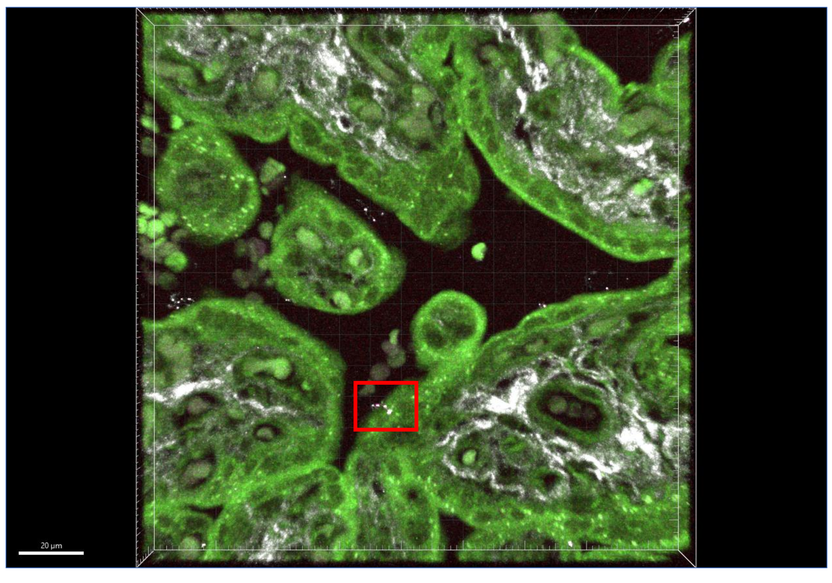

Figure 1: Image obtained from two-photon laser microscopy of human placental tissue. Areas in green are cross-sections of chorionic villi, which include fetal capillaries and stromal tissue. Black spaces represent the maternal blood space. RBCs can be observed in both the maternal blood space and within fetal capillaries. The red box represents the area shown in Figure 2 where this group of particles has been highlighted.

METHODS DEVELOPMENT

Tissue sections were imaged using a two-photon microscope (Olympus FVMPE-RS) with an 810 nm laser excitation and four bandpass emission channels (370-410, 425-465, 575-630, and 645-685 nm). This pilot study examined slides prepared from 5 placentae, with 60 fields of view (170 x 170 µm) collected per slide. Imaris software was then used to identify candidate BC particles based on the following criteria: (i) present in all four emission channels, and (ii) to be of ≤ 0.3 µm in diameter (Figure 1). A 3-dimensional surface model was generated to visualize the spatial location of the BC particles with respect to placental tissue (Figure 2).

In this pilot study, we investigated the following questions: 1. how many views are needed to provide a quantitative estimate of the particle number density in each of the tissue types, and 2. how many tissue samples need to be evaluated to estimate the total deposition in a given placenta.

Prior to these questions, we had to assess other related issues. Tissue samples fixed in formalin have been known to form black precipitates, a potential confounder. However, we found that the emissions from formalin precipitates do not produce the same spectral emission response as do true BC. Additionally, we have found that tissue samples stained with hematoxylin and eosin, a stain used traditionally for histological analyses, led to false positives. Given these findings, we have examined unstained, formalin-fixed tissue samples.

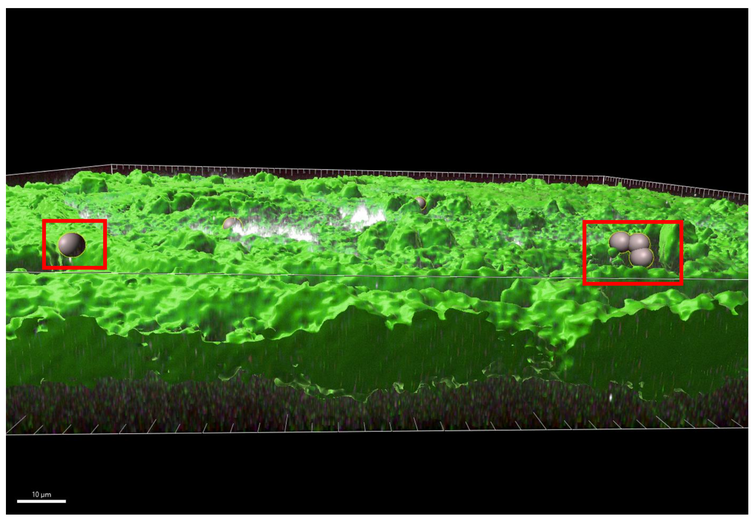

Figure 2: 3D model of the tissue grcaptured in Figure 1. The grey, oversized spheres represent BC as detected by Imaris software. The box on the right shows the oup of particles shown in Figure 1.

RESULTS

Of the examined 5 randomly selected placentae, typical numbers per view were 4.52 ± 3.66, 2.83 ± 2.48, and 2.60 ± 2.30, in the material blood, fetal tissue, and fetal blood, respectively. Figure 3 shows the distributions of particles observed per view in one tissue sample. Converting these numbers to particle density (particles per unit volume), we obtained 11,450 ± 9,230, 7,220 ± 6,650, and 6,670 ± 5,920 particles per cubic millimeter for one specific placenta. Further analyses of these results should answer the question of how many images are needed to characterize the BC tissue dose in each placenta.

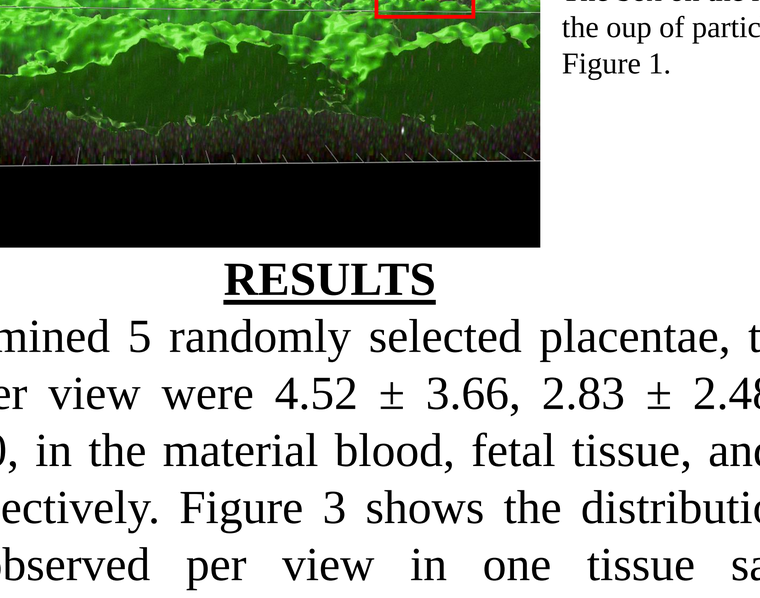

Figure 3: Histograms of the distributions of particles per view in the 3 tissue types in one specific tissue sample.

FUNDING AND REFERENCES
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