Profiling Nitric Oxide metabolites in endothelial dysfunction using a 3D blood vessel model

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Endothelial dysfunction is the prime cause of many pathological conditions such as atherosclerosis, thrombosis, platelet aggregation, inflammation, and defective oxygen mechanism due to hypoxic conditions. The natural mechanism in maintaining vascular integrity involves various signaling compounds and mechanistic factors such as fluid flow and shear stress. Nitric Oxide (NO) is considered an essential signaling compound in maintaining vascular homeostasis. Hence, analysis of the NO level is necessary for understanding pathological conditions. Evaluating the level of NO to predict the risk of endothelial rupture is crucial for patient management, yet current two-dimensional endothelial cell culture models and methods suffer from several limitations due to NO's short half-life and lack of fluid flow in the models. This results in less NO expression and displays a non-physiologic phenotype.

This study's focus is to compare two-dimensional (2D) and three-dimensional (3D) cell culture platforms in terms of their NO-specific metabolite levels. To measure NO metabolites using UPLC-MS/MS, we developed a tracer-based metabolomics strategy in a three-dimensional micro vessels-on-a-chip model with a microfluidic pump that maintains a unidirectional fluid flow. We investigated how specific isotopically labelled marker metabolites involved in NO production can be used to determine the eNOS (endothelial nitric oxide synthase) activity by tracking the conversion of the NO substrate L-Arginine to L-citrulline and L-ornithine. We detected significant changes in the downstream metabolite levels during stimulation of NO. Compared to the 2D culture, the augmented effects of the NO specific metabolite L-citrulline in 3D blood vessels was due to the presence of hemodynamic shear stress. We also studied the impact of oxygen in endothelial dysfunction and NO metabolism with an in-line oxygen measurement system. Our 3D model with a unidirectional fluid flow produces a more physiologically relevant environment and clearly demonstrates the importance of using 3D culture models.