Abstract: **P271**

**Hanging drops: a novel in vitro method to analyze human monocytes of relevance for atherosclerosis**

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**Topic(s):**
Basic Science - Cardiac Biology and Physiology: Leukocytes, Inflammation, Immunity

**Citation:**
Cardiovascular Research (2018) 114 (Supplement 1), S69

**Background/ Purpose:** Monocytes play a key role in the development of atherosclerosis due to their accumulation within the vessel wall and their differentiation into macrophages or dendritic cells. Furthermore, recent studies demonstrated that monocytes can act pro- and anti-inflammatory, depending on their differentiation either into M1 or M2 macrophage-phenotype. The main bottleneck to proper analyze the exact behavior of monocytes in vitro is their cultivation. Upon contact with a plastic surface during cultivation, they immediately change their phenotype as well as their expression pattern of cytokines. Aim of our present study was therefore to evaluate a novel method to cultivate human primary monocytes in hanging drops.

**Methods:** Three different methods were compared: cell adhesion, negative selection by magnetic beads and hanging drops. Human peripheral monocytes were isolated from healthy individuals, proceeded in accordance with the individual methods, and the expression of various cytokines was analyzed by quantitative RT-PCR at different time points (0h, 2h, 6h, 12h, 24h): CD14, CD16, CD68, IL-1beta, -6, -8, -10, -12, TNF-alpha, TGF-beta, IFN-gamma, MMP12, CD206, YKL-40, PTX3.

**Results:** Expression of CD14, which represents the undifferentiated state of monocytes, was highest when using hanging drops compared to adhesion or magnetic beads method. No significant difference was observed for the expression of CD68. In contrast, IL8 expression was significantly down-regulated using the hanging drop approach compared to adhesion or magnetic beads. IL-12 expression was found to be the lowest when using hanging drops.

**Conclusion:** Our results indicate that in absence of cell-plastic contact (hanging drops method) monocytes do not significantly switch phenotype and maintain their undifferentiated state at least for 24 hours. Thus, hanging drops may be a helpful tool to analyze differentiation of monocytes into specific cell types.