

**Title:** Monitoring Inflammation Using THP-1 Monocytes and Magnetic Resonance Imaging

**Trainee Name:** Praveen Dassanayake

**Supervisor(s):** Dr. Donna Goldhawk

**Structured Abstract:**

**Introduction:** After acute myocardial infarction (AMI), inflammatory response(s) stabilize the region of infarction by strengthening the heart muscle. Nevertheless, an unrestricted inflammatory response leads to excessive left ventricular remodeling and, eventually, heart failure. Differentiating between pro- and anti-inflammatory responses may help discern when interventions should be introduced to curb unwanted tissue remodeling [1]. Monocytes are the precursors of pro- (M1) and anti- (M2) inflammatory macrophages. In each cell type, the iron handling activity is distinct, with M1 macrophages largely displaying an iron storage phenotype while M2 macrophages mainly exhibit an iron recycling phenotype. Hepcidin is a hormone expressed post AMI (unpublished results); is induced by pro-inflammatory signaling; and down regulates ferroportin (FPN), an iron export protein found in monocytes and macrophages [2][3]. We are investigating the correlation between cellular iron content and transverse relaxation rates.

**Hypothesis:** Changes in monocyte iron regulation during an inflammatory response are mediated by hepcidin and influence both cellular iron content and MR relaxation rates.

**Methods:** Human THP-1 monocytes were cultured for one week in the absence (-Fe) and presence (+Fe) of iron-supplemented medium, containing 25uM ferric nitrate. Upon withdrawal of iron supplement, cells were cultured a further 1(Fe-1h) and 2(Fe-2h) hours. At harvest, cells were lysed in RIPA/protease inhibitors (Roche) and sonicated. Expression of iron export protein was assessed by Western blot using rabbit  $\alpha$ -FPN (Invitrogen) as the primary antibody [1]. Transverse relaxation rates ( $R2^*$ ,  $R2$ ) were measured at 3T in MR phantoms [4].

**Results:**To examine the influence and regulation of iron export activity in monocytes, THP-1 cells were cultured with or without iron supplementation and assessed either immediately or 1 to 2 hours after removal of iron supplement. Both  $R2^*$  and  $R2$  decreased in the presence of iron supplementation, returning to baseline non-supplemented values within 1 hour of withdrawal of extracellular iron. The level of FPN expression was similar +/- Fe.

**Discussion:** THP-1 monocytes are an iron exporting cell type and, as such, may respond to pro-inflammatory signaling mediated by hepcidin. Consistent with this, we show that, in response to an increase in extracellular iron, THP-1 monocytes predominantly decrease the  $R2$  component of transverse relaxation rate. A return to baseline values within 1 hour raises the possibility that THP-1 cells autoregulate iron export post-translationally through paracrine or autocrine secretion of hepcidin[5].

**References:** [1] Fang, Lu et al., 2015, Journal of Geriatric Cardiology 12; [2] Goldhawk et al., 2015, MR Insights 8(S1): 9; [3] Alizadeh (2017) MSc thesis, Western University; [4] Sengupta et al., 2014, Front Microbiol 5: 29; [5] Theurl, I et al. 2008. Blood 111(4).