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Measuring Activity of Endothelial Nitric Oxide Synthase and Nanodisc Complex through Nitrate Production

College of Sciences and Health Professions

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<u>Abstract</u>

Nitric oxide is an important bioregulator generated in various regions throughout the body by a family of isozymes referred to as Nitric Oxide Synthases (NOS). Within vascular endothelial cells, nitric oxide is generated from oxygen and arginine (amino acid) by endothelial nitric oxide synthases (eNOS). Within this environment nitric oxide plays a critical paracrine role, mainly anithrombotic and anti-atherosclerotic. This is accomplished by vessel dilation and prevention of platelet and leukocyte aggregation and adherence to the vessel wall. The activity of the eNOS enzyme has been studied within solution and is well understood. However, the impact that the lipid bilayer of endothelial cells has on the activity is not known. To better understand this interaction, we have formed "nanodiscs" to bind to the eNOS. Nanodiscs have two components that combine and self-assemble when added to solution, POPC (a lipid) and MSP1E3D1 (Membrane Scaffold Protein). The nanodiscs help provide a better microenvironment to study the enzyme and its activity. Through reaction with an indicator dye in the Griess reagent system, activity levels, as calculated by nitrate production, reduced dramatically. Over a 50% reduction was seen when calculating specific activity of the eNOS enzyme when bound to nanodiscs. A possible indication that a lipid bilayer restricts activity of the eNOS enzyme.