Introduction
Atherosclerosis is associated with initial endothelial dysfunction followed by accumulation of smooth muscle cells in the sub-intimal layer of the arterial wall. Nitric oxide produced by nitric oxide synthase (NOS) is well known to inhibit the intimal hyperplasia and promote re-endothelialization. Nitric oxide synthase exists as three isoforms eNOS, iNOS and nNOS. We were specifically interested in examining the gene profile of eNOS transduced human coronary arterial smooth muscles cells (HCASMCs) to understand the mechanism for the eNOS inhibitory effect on smooth muscle cell proliferation. To this aim we performed a whole genome wide analysis on eNOS transduced HCASMCs after 48hours. We also analysed iNOS and nNOS transduced cells for comparison. The identification of an eNOS smooth muscle specific target could aid in further developing gene/drug therapeutic approaches to vascular repair.

Materials and Methods
HCASMCs (Promocell) were transduced with Adenovirus carrying the eNOS gene. Cells were also treated with adenovirus without transgene and the diluent (PBS) as controls respectively. RNA was extracted from cells post 48hours transduction and reverse transcribed into cDNA for Microarray analysis on the Affymetrix 2. platform. Candidate genes were validated by Real-time PCR and function further examined in vitro. Statistical analysis was performed by two tailed t-tests with significance at p<0.05.

Results
Several genes were identified as uniquely up regulated in eNOS transduced cells and were validated by real time PCR. The Hsp70 gene family was highly induced and their importance to eNOS signaling was further investigated. Hsp70A and B transcripts were induced ~2 fold. The human specific gene Hsp70B’(p6) was induced 12fold and 23fold by eNOS and constitutively active form of eNOS (ca.eNOS) respectively. Hsp70B’ protein was elevated as detected by immunoblotting. To examine the overexpression of Hsp70B in HCASMCs an adenovirus carrying the Hsp70B’ gene under control of a CMV promoter was generated.

Discussion and Conclusions
The findings from this study provide evidence to support the further investigation of a unique isoform of the Hsp70 gene family in human atherosclerosis. This could be an alternative target protein to modify by gene therapy or pharmacology in preventing neointimal hyperplasia for vascular repair. One limitation of this study is that these observations were made in vitro and would be strengthened by examination of the target genes in human atherosclerotic tissues.

Fig.1: Heat Map of Ad.NOS transduced HCASMCs showing alterations in gene transcripts between treatments. Gene expression is displayed as higher (red) or lower (blue).

Fig.2: Real-time PCR analysis of Hsp70B’ transcripts elevated in HCASMCs transduced with wild-type and mutant forms of eNOS. (p<0.05).

Acknowledgments
Deirdre O’Connor for reagents. This work has been funded in part by an Enterprise Ireland grant received by TOB.

Disclosures
Authors have nothing to disclose.