DIFFERENTIAL EFFECTS ON ENDOTHELIAL CELLS AND SMOOTH MUSCLE CELLS AFTER FREEZING AND REWARMING.

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Introduction: Cryoplasty, a technique which combines conventional angioplasty with the delivery of cold thermal energy, has recently emerged as a treatment for peripheral vascular disease with improved late results, achieving greater long term patency rates when compared to conventional angioplasty. The mechanisms behind these improved results remain unclear. Smooth muscle cells (SMC) play key roles in restenosis and neointimal hyperplasia. Endothelial cells (EC) play an important role in the modulation of healing after angioplasty. Our previous studies confi rmed supercooling induces greater rates of apoptosis in SMCs compared to ECs. Furthermore, morphological data indicate that the portion of the vessel wall directly in contact with the cryoplasty balloon is frozen transiently. This study compares the effects of supercooling versus freezing on the SMC as well as examines the viability of ECs and SMCs when frozen and re-warmed. Method: Bovine aortic ECs and SMCs were supercooled to approximately ◆10C with or without freezing for 0, 30 or 60 seconds using a custom designed conduction cooling stage and then rewarmed to 37C for 24 hrs. TUNEL assay was used to measure the degree of apoptosis. Viability was assessed via Trypan Blue exclusion. Results: Rates as high as 19.8% of cells in apoptosis as assessed by the TUNEL assay were observed when SMC's were frozen. SMC supercooling yielded rates reaching only 10.4% (Fig.1). The rate of apoptosis also increased with increasing frozen time, climbing from 5.6% with 0 second frozen time to 19.8 % after 60 seconds frozen time (Fig. 1). We also observed an increasing number of detached cells with increasing frozen time (50% detachment after 60 seconds of frozen time). Viability of SMC's declined as much as 44% with freezing compared to supercooling (Fig.2). Higher viability rates were maintained by ECs with as much as 80% viable cells after 60 seconds frozen time. SMC viability declined to 41% after 60 seconds of frozen time. Viability was affected by increasing frozen time. ECs experienced a modest decline in viability by 4% (from 84% at 0 seconds to 80 % at 60 seconds frozen time). SMCs experienced a greater decline of 31% (from 70 % at 0 seconds frozen time to 41% at 60 seconds frozen time). Also noted was an increased in detachment rate in SMCs compared to ECs most notably with increasing frozen time (60% SMC detachment vs 10.4% EC detachment with 60 seconds of frozen time). Conclusion: Freezing induces SMC apoptosis. The effect of freezing on SMC apoptosis is greater then with supercooling alone. The rate of apoptosis increases with longer SMC frozen time. Furthermore, longer frozen times result in increasing number of dead cells (detached cells) which include cells that have completed apoptosis, contributing to an even higher apoptosis rate. Freezing results in decreased viability in ECs and SMCs, however this was more drastic in SMCs. A contributing factor was that the detachment rate was greater in SMCs compared to ECs, resulting in a more pronounced reduction in SMC viability. Further examination behind the differential effect freezing has on vascular cell lines may elucidate the mechanisms behind the greater long term patency rates associated with Cryoplasty.

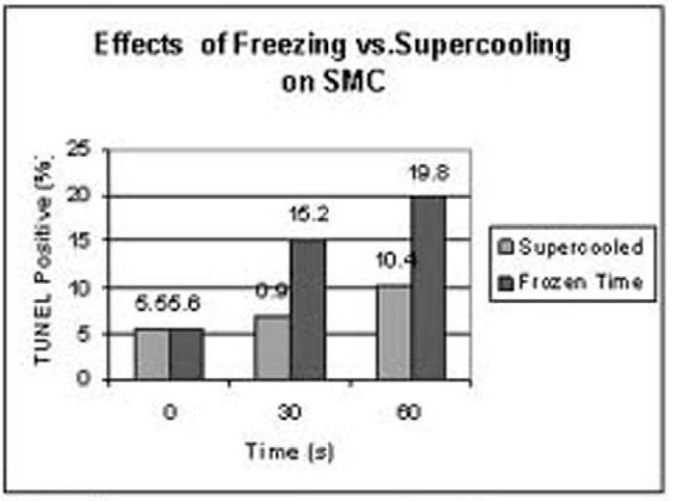

Figure 1

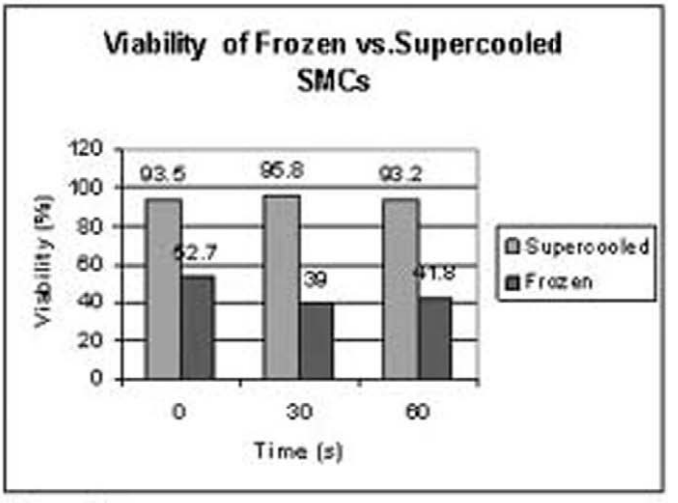

Figure 2