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<td><strong>Author(s)</strong></td>
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<td><strong>Issued Date</strong></td>
<td>2008</td>
</tr>
<tr>
<td><strong>URL</strong></td>
<td><a href="http://hdl.handle.net/10722/126999">http://hdl.handle.net/10722/126999</a></td>
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DIFFERENTIAL EFFECTS ON ENDOTHELIAL CELLS AND SMOOTH MUSCLE CELLS 
AFTER FREEZING ANDREWARMING.

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Introduction: Cryoplasty, a technique which combines conventional 
an 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 confirmed 
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 -10°C with 
or without freezing for 0, 30 or 60 seconds using a custom designed 
conduction cooling stage and then rewarmed to 37°C 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 than 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.