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## Vascular smooth muscle cells exhibit a progressive loss of rigidity with serial culture passaging

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### Abstract

One drawback of in vitro cell culturing is the dedifferentiation process that cells experience. Smooth muscle cells (SMC) also change molecularly and morphologically with long term culture. The main objective of this study was to evaluate if culture passages interfere in vascular SMC mechanical behavior. SMC were obtained from five different porcine arterial beds. Optical magnetic twisting cytometry (OMTC) was used to characterize mechanically vascular SMC from different cultures in distinct passages and confocal microscopy/western blotting, to evaluate cytoskeleton and extracellular matrix proteins. We found that vascular SMC rigidity or viscoelastic complex modulus (G) decreases with progression of passages. A statistically significant negative correlation between G and passage was found in four of our five cultures studied. Phalloidin-stained SMC from higher passages exhibited lower mean signal intensity per cell (confocal microscopy) and quantitative western blotting analysis showed a decrease in collagen I content throughout passages. We concluded that vascular SMC progressively lose their stiffness with serial culture passaging. Thus, limiting the number of passages is essential for any experiment measuring viscoelastic properties of SMC in culture.

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