Isolation and Characterization of Primary VIC Subpopulations Based on Adhesion Strength

Kent E Coombs[1], Matthew N. Rush[1], Elizabeth Dirk[1,2].
Center for Biomedical Engineering[1], Chemical and Nuclear Engineering[2], University of New Mexico, Albuquerque, NM.

Statement of Purpose: Valvular interstitial cells (VICs) are a heterogeneous population in the aortic heart valve. Distinct populations have been difficult to identify and isolate in culture. In this work, we use a simple preferential detachment technique to isolate unique populations of VICs – strongly attached (SLOW3) and weakly attached (FAST3) [1]. Previous studies have demonstrated that the SLOW3 are an enriched population of myofibroblastic cells (activated, extracellular matrix forming) that might be useful for tissue engineering. We hypothesized that these more adherent cells (SLOW3) are a population of pre-osteoblastic cells, activated and differentiating toward an osteoblastic, diseased state. To examine the two cell populations, proliferation, presentation of activation marker alpha smooth muscle actin (aSMA), and early and late osteoblastic differentiation markers alkaline phosphatase (ALP) and osteocalcin (BGLAP) were measured.

Methods: VICs were isolated from porcine hearts (Hormel Inc.) using standard collagenase digestion methods [2]. Magnetic anti-CD31 dynabeads were used to remove valvular endothelial cells. VICs were cultured under normal culture conditions. During passages 1-3 VICs at 80% confluence were incubated with a series of low trypsin solutions (0.025%) for 2 min. After each 2 min interval, lifted cells were collected. The first 25% to detach and the last 25% to detach were then cultured to 80% confluence when the trypsin process was repeated (Fig. 1).

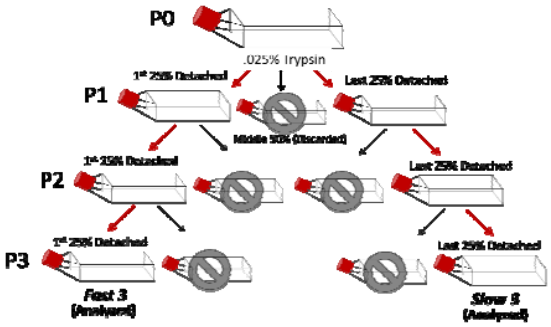

Figure 1. Diagram of trypsin treatments and collected VIC.

Cells for growth studies and ALP were used at passage 3 (P3), genetic analysis was done on passage 4 cells (P4) in 10% FBS media or 10% FBS osteoblastic media (10mM β-glycerophosphate, 10^{-6} M ascorbic acid, & 10^{-7} dexamethasone). Proliferation was determined over 14 days using a Beckman Coulter counter. TaqMan probes for aSMA, BGLAP/osteocalcin, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) an endogenous control, were used for qPCR along with Promega GoScript RT PCR system. Finally, to determine ALP protein content, cell lysate was collected and ALP activity measured based on its ability to cleave 4-NPP to a fluorescent form 4-NP measured at 450nm. Standard MTT was used to determine cell number at each time point for the ALP assay.

Results: Growth curves show less adhered FAST3 proliferate faster with a doubling time of 1.8 days compared to SLOW3 at 2.8 days (Fig. 2A). Published immunocytochemistry studies have shown more adherent SLOW3 cells express higher levels of aSMA [1]. Our results corroborate this finding, with a ~8.5 fold higher expression of aSMA in our SLOW3 at 3 days (Fig. 2B).

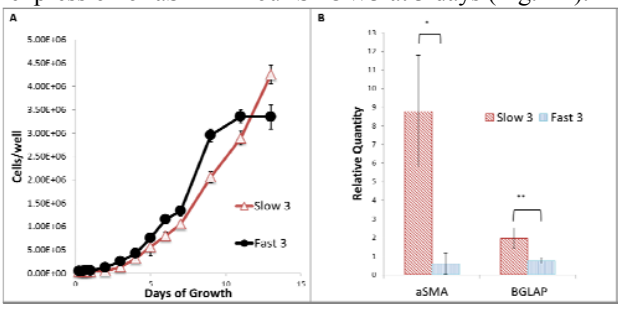

Figure 2. A) Growth curves with cell proliferation calculated during exponential growth. B) qPCR comparing Slow 3 cells and Fast 3 cells after 3 days in normal culture conditions. Slow 3 aSMA and Fast 3 aSMA expression differs significantly (*p=.05) and Slow 3 BGLAP differs significantly from Fast 3 BGLAP (**p=0.05).

qPCR results show that SLOW3 have significantly higher expression of BGLAP/osteocalcin suggesting that SLOW3 are moving down the pre-osteoblastic differentiation pathway (Fig. 2B). Furthermore, SLOW3 have higher levels of ALP activity compared to less adherent cells (FAST3) at day 5. Interestingly literature has associated faster proliferation, increased aSMA, BGLAP, and increased ALP activity with osteoblastic VICs (OB VICs) [3]. However our data indicate that SLOW3 exhibit high aSMA, BGLAP, and ALP activity like OB VICs, but proliferate slower. These results suggest that perhaps the increased proliferation rate of OB VICs in current literature is an artifact of using non-separated VIC populations, which include faster proliferating subpopulations like FAST3.

Conclusions: Results from our ALP assay and qPCR show that more adherent SLOW3 cells are more likely to progress towards diseased OB VICs when cultured in normal conditions *in vitro*, as compared to less adherent FAST3 cells. However, SLOW3 cells proliferate more slowly than FAST3, contradicting the current understanding of OB VICs in literature. These results suggest the need to further subdivide VICs into more appropriate subpopulations. In conclusion, SLOW3 cells should be removed from the study of healthy VICs as they are prone to developing diseased markers and are slower to proliferate. However, SLOW3 may be a more relevant subpopulation to study disease valve propagation.

References: *1*) Belvins TL. J Heart Valv Dis. 2006;15:815-822 *2*) Johnson, C.M, J Mol Cell Card, 1987:19:1185-1193. 3) Monzack EL, J Heart Valve Dis., 2011, 20:449-463.