Reviewer's report

**Title:** Manipulation of Valve Composition to Elucidate the Role of Collagen in Aortic Valve Calcification

**Version:** 1  **Date:** 20 December 2013

**Reviewer:** Elena Aikawa

**Reviewer's report:**

This is an interesting study of the effects of collagen deficiency in modulating VIC phenotype. The authors propose that collagen disruption precedes changes in the myofibroblastic and osteogenic changes in VIC phenotype that drives CAVD. Overall, the manuscript is well-written and the results are clear and compelling. The most interesting observation is that the collagen loss leads to both an increase in new collagen synthesis as well as VIC phenotypic changes. Traditionally, the fibrotic response in CAVD was thought to precede the calcific response; however, this study indicates that the two may be completely interrelated.

**Specific Comments:**

1. Previous studies have used excised leaflets in bioreactors to study the effects of strain (along with various biochemical stimuli) on leaflet changes. These studies should be mentioned in the present manuscript with special emphasis placed on the difference between static and strained leaflets in these previous studies. Could the lack of strain lead to changes in the leaflet composition (i.e., the apparent drop in HA content) over time in the control samples in the current study? Adding a dynamically strained culture condition is probably beyond the scope of the current study, but the authors should at least address this limitation.

2. Figure 4 is missing a significance marker.

3. The authors mention that the changes in VIC phenotype were not found “to occur when ECM components other than HA or collagen are depleted.” This certainly increases the strengths of the current results, but it would be nice to mention the ECM components that have been tested and show these results in this study.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
No conflict of interests.