Title: Testing Novel Culturing Systems to Promote the Proliferation *in vitro* of a marine invertebrate species, *Botryllus schlosseri*

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Final Abstract:

Immortalized cell lines may be applicable to further research on stem cell regeneration, allopathic medicine and the beauty industry. Research to establish an immortalized marine invertebrate cell line has been under development since the 1970's. Marine invertebrates are a major source of biomaterials and bioactive natural products that can find applications in various areas of study. *Botryllus schlosseri* is of particular interest for its regenerative capabilities. *B. schlosseri* is a colonial ascidian tunicate, composed of zooids that form colonies with shared vasculature. Stem cells from a zooid or vasculature can regenerate an entire colony. Despite decades of effort, no immortalized cell lines for marine invertebrates like *B. schlosseri* have been created. To better understand the conditions needed to promote the survival and prolong the continuous proliferation of *B. schlosseri* stem cells, we isolated zooids using microdissection techniques and cultured these cells in five different media types with varied composition. Through various qualitative criteria, we found that zooids were healthiest when grown in L-amino acid optimized media that was less synthetic, and adherence to the culture dish was noted when grown in minimal volumes of media. Additionally, while these results showed zooids were stable and adhered over a 48-hour period, parasitic contamination was observed in all five media conditions. Contamination hindered the survival and advancement of cell proliferation as zooid death resulted shortly afterward. Further understanding of optimal culturing conditions for *B. schlosseri* and preventative measures for contaminants still needs to be discovered for establishing and maintaining an immortalized cell line.