
Sponge Hybridoma Technology: Enabling Continuous Cell Division in Non-Dividing, Compound Producing Species

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Abstract

In vitro (cell culture) production of sponge-derived compounds has become increasingly feasible with the development of nutrient media optimized for amino acids (M1 medium), growth factors, vitamins, lipids, and trace elements (OpM1 medium) resulting in the establishment of primary cell lines from several sponge species and the creation of the first continuous sponge cell line from *Geodia barretti*. However, many sponge species producing pharmaceutically relevant bioactive compounds do not divide in either M1 or OpM1 (e.g., *Axinella corrugata*, stevensine producer). To address this, hybridoma technology, the fusion of dividing sponge cells lacking compound production with non-dividing, compound-producing cells, offers a potential solution. Preliminary studies have demonstrated the feasibility of sponge cell fusion, detecting and monitoring fused cells over 48 hours using an automated cell counter. In this study, we optimized and compared cell fusion of *G. barretti* and *A. corrugata* using polyethylene glycol (PEG) and electrofusion. Under optimal conditions, the viability of the fused and non-fused cells remained above 70% for both techniques, demonstrating their suitability for sponge hybridoma applications. Based on flow cytometry analysis, there is 15% fusion efficiency for both techniques. Fluorescence microscopy was used to confirm fusion of cells of the two sponge species. Results of this study demonstrate the feasibility and potential of sponge hybridoma cell lines for production of bioactive compounds.

Keywords: Sponge cell culture, hybridoma, cell fusion, polyethylene glycol fusion, electrofusion, bioactive compound production

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