ABSTRACT

Sponge cultures are identified as alternative for optimal utilization of sponge derived bioactives without disturbing biodiversity. Among different sponge culture methods, *in vitro* culture methods attain significance primarily for identification of factors promoting sponge survival or growth and secondarily to evaluate the potency of obtaining bioactives from cultured sponges.

Several demosponges are reported from Palk Bay region of India. Two demosponges *Axinella donnani* and *Haliclona pigmentifera* were selected in the present study as model sponges for developing *in vitro* cultures; based on their acclimatization, short term survival rates and initial bioactivity screening. Incubator methods were effectively utilized for identification of optimal dissolved oxygen (DO), temperature and salinity essential for enhancing survival of sponges. A novel medium is developed for sponge *in vitro* culture using cells and fragments of *A. donnani*. The novel medium enhanced survival rates of aggregates and fragments of *A. donnani*. The fragments maintained in novel medium also retained the bioactive, hexane soluble fatty acid and sterol components even after 180 days of culture. These hexane fractions exhibited anti-brine shrimp, antibacterial, mosquito larvicidal and cytotoxicities at lower concentrations than natural sponge extracts. The model sponge *H. pigmentifera* exhibited survival and adhesive growth for 42±2 days under hypoxic conditions without external nutritional

supplementation. TEM studies revealed the presence of archaeocytes, collenocytes, spherulous cells and collagenous extracellular matrix (ECM) in hypoxia surviving *H. pigmentifera*. Western blot studies also revealed the presence of human hypoxia inducible factor (HIF-1α) like protein from these sponges. The decellularized ECM (DECM) from hypoxia surviving sponges exhibited biocompatibility under *in vitro* and *in vivo* conditions. Proliferation and adhesive growth of mouse fibroblast like cell line (L929) was noticed on incubation of these cells with DECM for 48 h. Resorption of DECM within 40 days and normal wound healing was also observed on sub-cutaneous implantation of the DECM in Wistar rats.

Thus, the present study encompassed successful selection of model demosponges for developing *in vitro* culture methods, optimization of novel medium for enhancing the survival of sponge, retention of bioactive substances in the cultured sponge, evaluation of sponge survival under hypoxic conditions, detection of HIF-1α like protein from sponge and evaluation of biocompatibility of collagenous DECM obtained from *in vitro* surviving sponges. The survival rates of sponges in the present study were comparatively lower than some previous studies on sponge cultures. However, the utilization of serum or cell free medium for enhancing the survival of sponges for '180 days' and the identification of survival of sponges under hypoxia without nutrient supplementations are novel findings which indicate the significance of serum free media and DO level optimization of

sponge culture. In addition, the potential of *A. donnani* and *H. pigmentifera* is also identified in this study, as they are shown to possess bioactive metabolites and biocompatible ECM.

The incubator culture methods and medium developed in the present study can be further evaluated for developing *in vitro* culture for other sponge species. The sponge species available in India (other than studied in the present study) can also be further evaluated for their biotech potential using the methods developed in this study.