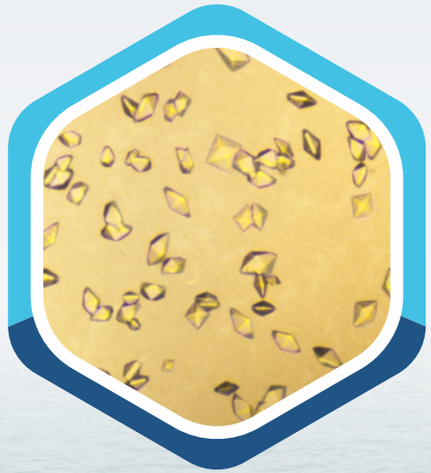
Treatment	Ca(%)	C(%)	O(%)
Lustrous Nacre coated bead	**87.09**	2.69	5.64
Lustrous Nacre coated bead – Cross Section	75.60	4.03	20.37
Uncoated bead (control)	**21.57**	13.43	34.56

Mineral composition of biomineralized beads

- Lustrous hue developed on several nuclear bead surfaces owing to the thickness of nacre coating intensification by rhombohedral calcium carbonate crystals involved in nacre biomineralization. This is the first time that formation of a pearly nacreous material on a bead surface was achieved under *in-vitro* conditions using cultured mantle epithelial cells. The repeatability and success rate of in-vitro lustrous pearl formation was 71%.

Lustrous prototypes of *in-vitro* marine pearl



Rhombohedral Calcium Carbonate Crystals

- The scientific advancement shows that *in-vitro* cultured mantle epithelial cells retain the ability to biomieralize nacre forming lustrous marine pearl prototype marching towards a high end technology of developing laboratory pearl. The technology also could help make pearl production more sustainable and ethical by reducing the environmental impact of traditional farming methods by offering more consistent and controlled way to produce high quality pearls which could make them more affordable and accessible.

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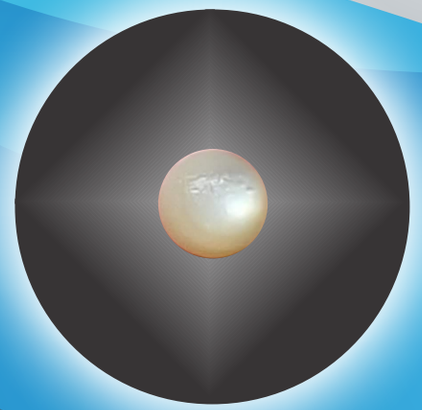
PROTOTYPE DEVELOPMENT OF MARINE BLACK PEARL THROUGH *IN-VITRO* MANTLE EPITHELIAL CELL CULTURE TECHNOLOGY IN BLACK LIP PEARL OYSTER *PINCTADA MARGARITIFERA*



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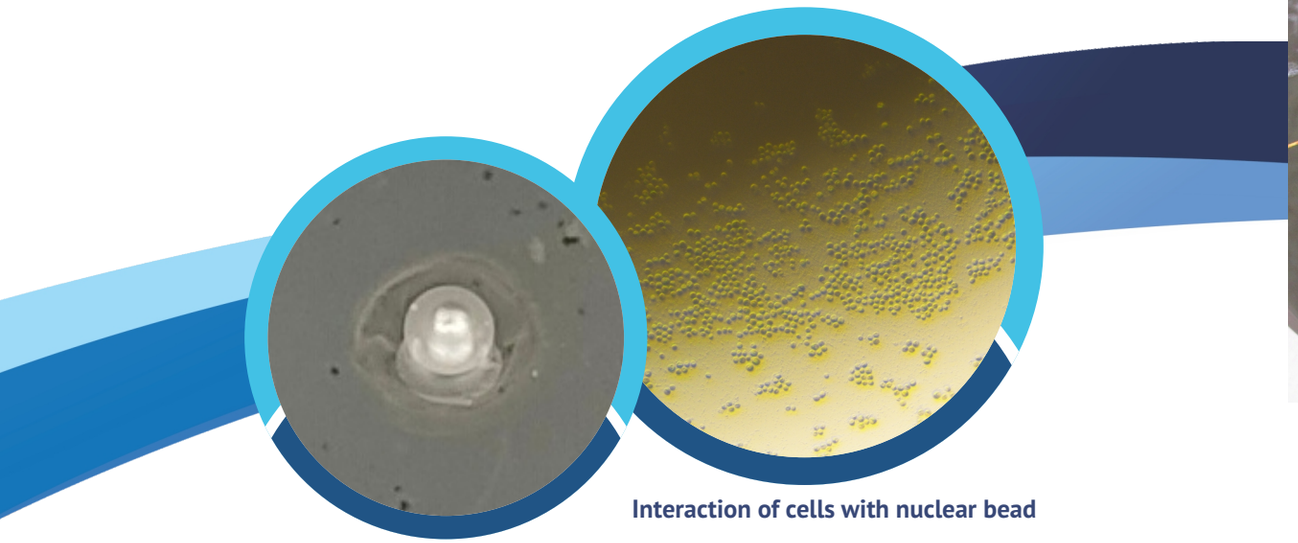


Interaction of cells with nuclear bead

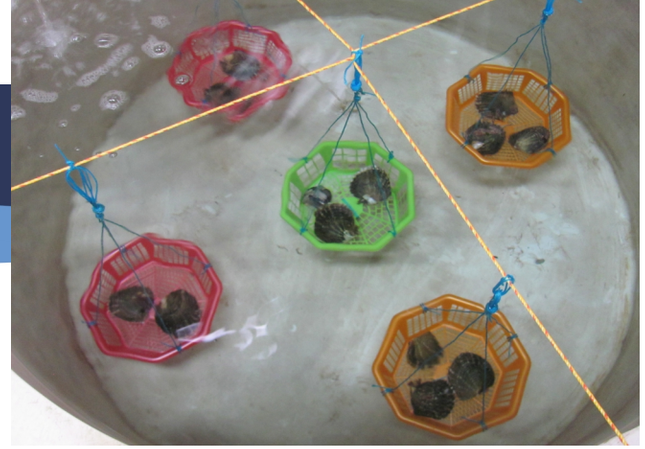
Prototype in semi-solid substrate

- The marine pearls in nature are formed as a result of protective and defense mechanism when a foreign object enters between the shells of a pearl producing mollusc. The mantle epithelial layer in turn starts secreting a nacreous material which covers the foreign object with several layers of nacre, in the form of brick and mortar which is a composite material consisting of calcium carbonate present in the form of aragonite tablets and a proteinaceous organic matrix resulting in the formation of a lustrous pearl.

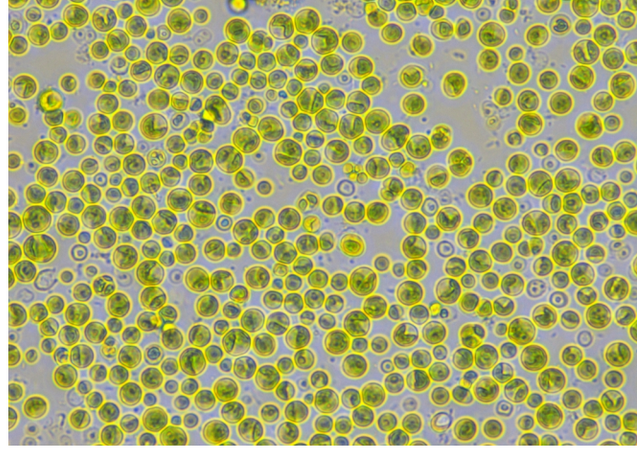
- Pearl production under natural condition is affected by various environmental factors such as seawater pH, salinity, ocean acidification and water temperature. Therefore, development of an *in-vitro* cell culture system with live, viable mantle cells is the need of hour for producing natural pearls under controlled conditions. The success of the *in-vitro* marine pearl production technology necessitates in keeping the mantle epithelial cells in a continuously proliferating condition and ensuring that they retain their biomineralization functions for a prolonged period.

- Live adult animals of black-lip pearl oyster, *Pinctada margaritifera,* were collected from the Andaman and Nicobar Islands. The animals were maintained at Kovalam field laboratory and fed with live algal feed.

- The growth and proliferation of in-vitro cultured pure cell lines of mantle epithelial cells were carried out using an indigenously developed cost effective medium. The granulated epithelial cells upon maturation released colored granules which in turn induced formation of nacre crystal deposits.

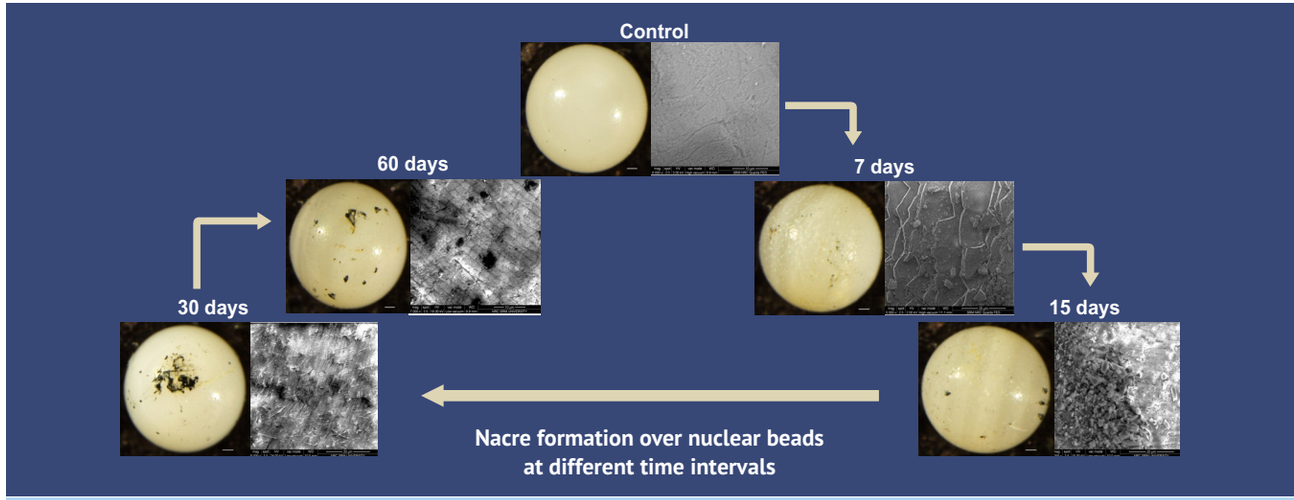


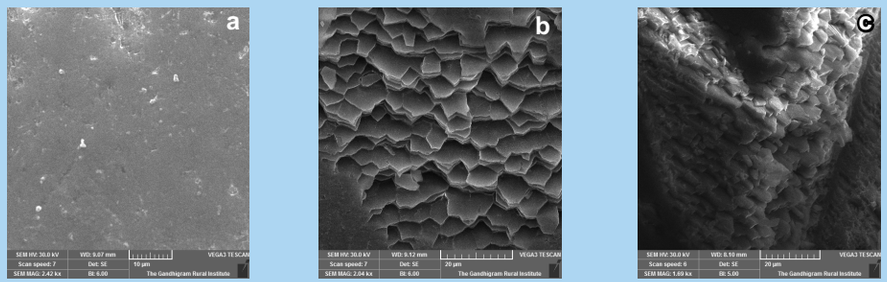
Pinctada margaritifera in Hatchery



In-vitro cultured Granulated Mantle Epithelial Cells

- Granulated epithelial cells and nuclear shell beads were incubated in a semi-solid substrate filled with culture medium to induce nacre formation on the beads. On visual observation, a brown discoloration was observed on the surface of the bead after 7-10 days. Evaluation of the surface of the nuclear beads and its cross section by scanning electron microscopy (SEM) after 60 days of incubation revealed a good brick and mortar pattern, characteristic of nacreous layer formation, comprising of aragonite platelets and matrix proteins similar to that seen in the nacre layer of molluscan shell. Calcium, carbon, and oxygen were the major elements examined.





SEM images of nacre coating on nuclear beads, a: control. b, c: Lustrous bead and its cross-section