Epithelial cell primary cultures isolated from buds of Botryllus schlosseri

PREAMBULE: Botrylloides are colonial ascidians useful for basic understanding of self and non-self recognition, regulated apoptosis, and whole body regeneration.

1. Laboratory-bred colonies at sizes of up to 200 zooids are first starved for 24 h by incubation in 0.2 µm filtered seawater (FSW) supplemented with 50 µg/ml gentamycin, then washed for 30 s in 70% ethanol, and immersed in FSW with 1% mixed antibiotic solution (PSA) containing 10^4 U/ml penicillin, 10 mg/ml streptomycin and 25 µg/ml amphotericin B.
2. Blastogenic stage D buds at size of ~300 µm are excised under a stereomicroscope using a pair of 1-ml disposable syringes equipped with 28-G size needles. By means of the needle tip, the tunic is removed and buds are dissected out, transferred into a sterile 100-µm pore-size nylon cell strainer immersed in FSW–PSA solution. Extensive rinsing of buds with FSW (200–300 ml) through the filter is performed.
3. Groups of up to 5 buds are transferred into a petri dish and excess of FSW is removed and substituted with 2 ml of tissue culture medium and incubated in a cell culture chamber.
4. Medium is renewed 24 h after expiration of the buds and supplemented with 1 ng/ml of recombinant human FGF. After this procedure, ~90% of isolated buds that attached to the substrate by day 9 also developed epithelial monolayers. Explants are grown either after developing epithelial monolayer or at earlier culture stages when still in sphere forms. Cells and spheres in culture extended their lifespan from a few days, which is the normal situation in the colony, to months in the *in vitro* culture situation. Some cells lying atop of the epithelial sheets and cells of the external thickened epithelial envelop of the dissected bud start to express *piwi* (Rabinowitz and Rinkevich 2011).

Attention: Cell culture conditions (e.g. temperature) should probably be optimized for each laboratory given that different populations of botryllid ascidians have different temperature requirements depending on where they normally live.

Botryllid piwi can be identified by western blot and immunocytochemistry using a Bl-Piwi polyclonal antibody (Rinkevich et al., 2010).

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Animals: Botryllus schlosseri colonies are reared on tilted glass slides in tanks with running sea water and fed with enriched rotifers (lyophilized) and/or combinations of algae/commercial invertebrates feeds.

Apparatus: Humidified cell incubator set to 20°C.

Tissue culture medium: L-15 synthetic medium made iso-osmotic to seawater and supplemented with 4 mM L-glutamine, 20 mM HEPES, 3% heat-inactivated fetal calf serum and 1% PSA.

Lab ware: 35-mm Petri dishes and serologic pipettes (Cell Star, Greiner-Bio-One); 1-ml disposable syringes equipped with 28-G size needles and sterile 100-µm pore-size nylon cell strainer (Falcon, Becton Dickenson)

Additional information:

Rabinowitz C, Rinkevich B *(2011) De novo emerged stemness signatures in epithelial monolayers developed from extirpated palleal buds. In Vitro Cell Dev Biol Anim 47:26-31*

Rinkevich Y; Rosner A; Rabinowitz C; Lapidot Z; Moiseeva E; Rinkevich B *(2010) Piwi positive cells that line the vasculature epithelium, underlie whole body regeneration in a basal chordate. Dev Biol 345:94-104*

Laird DJ, Chang W-T, Weissman IL, Lauzon RJ *(2005) Identification of a novel gene involved in asexual organogenesis in a budding ascidian. Dev Dyn 234:997-1005*

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