Development of two reproducible haemocyte culture systems for application in crustacean immunity studies

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Keywords: *Crustacean Immunity; Haemocyte cultures; Penaeus vannamei*

For studying shrimp immunity, *in vitro* haemocyte cultures are essential. Despite the considerable amount of work that has been done in this field, well-described and reproducible culture techniques are lacking. The current work aimed to establish two *in vitro* culture systems for haemocytes of *Penaeus vannamei*, with cells either in attachment or in suspension, using Nunc® Nunclon™Δ Surface and Nunc® Hydrocell Surface cell culture plates, respectively. Quantified haemocyte suspensions were seeded in modified L-15 (Leibovitz) medium. Furthermore, the survival performance of haemocytes was evaluated in medium supplemented with L-glutathione (GSH) and EDTA-free protease inhibitor cocktail.
Haemocytes cultured in attachment for 1h could be separated in adherent and non-adherent cell fractions. For the first time, attachment of shrimp haemocytes to the cell culture substrate was successfully prevented by the Nunc® Hydrocell Surface. The clustering of haemocytes kept in suspension was recorded by cell live imaging. Haemocytes cultured under both systems could be kept up to 5 days. Supplementation with GSH significantly improved the cell survival and delayed formation and melanisation of clusters. On the other hand, addition of protease inhibitors did not improve cell survival.
In order to prove the suitability of these models for the *in vitro* study of shrimp immunity, the phagocytic and antibacterial activities of adherent haemocytes towards *V. campbellii* were evaluated after 1h of co-culture. Phagocytosis was detected in 11.5±0.14% of haemocytes, with an average of 2.4±0.1 bacteria per haemocyte. Furthermore, haemocytes clearly demonstrated an antibacterial activity.
It was concluded that these models could keep haemocytes functionally active during the time required for the study of innate immune responses of shrimp towards pathogens in a reproducible way.