Developmental biology of *Fasciola hepatica*: 3D co-culture using HepG2 spheroids to create mini-livers allows investigation of host-pathogen interactions

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ABSTRACT

The helminth parasite *Fasciola hepatica* is a significant cause of animal and human morbidity worldwide. Investigations of the parasite's development biology are hampered by our inability to culture and propagate juvenile worms *in vitro*. HepG2 is a human non-tumorigenic liver cell line with high proliferation rates and epithelial-like morphology. We have shown that co-culture with three-dimensional HepG2 cell aggregates (3D spheroids) promotes the survival, growth and development of the infective stage of the parasite, the newly excysted juvenile (NEJ) *in vitro*. Parasites grown in the presence of HepG2 spheroids, mini-livers, were observed regularly interacting with the spheroids, invading the tissue, indicating the importance of tactile stimuli. Parasites actively feed on and ingest the peripheral cells of the spheroids. We investigated parasite development using immunohistochemistry and scanning electron microscopy (SEM). The parasites exhibited not only a rapid increase in size and temporal expression of developmental genes, but also extensive development of the gut caecum, musculature, and surface sensory system. Parasites grown with 3D mini-livers mimic *in vivo* parasite-host liver interactions, greatly improving our ability to investigate and understand *F. hepatica*-host biology. This co-culture system has the potential to facilitate the development of new parasite control methods. Therefore, we are continuing to improve culture conditions to favour parasite growth and development.