This is not the version of record. The published version is available at https://doi.org/10.1016/j.ijpara.2020.12.009

Succinctus

Further evaluation and validation of HybridoMed Diff 1000 and its comparison to Basch medium for the cell-free culture of *Schistosoma mansoni* juvenile worm stages

Shradha Maharjan [a], Ruth S Kirk [a], Scott P Lawton [a,b], Anthony J Walker [a,*]

[a] Molecular Parasitology Laboratory, School of Life Sciences Pharmacy and Chemistry, Kingston University, Penrhyn Road, Kingston upon Thames, KT1 2EE, UK.

[b] Epidemiology Research Unit, Department of Veterinary and Animal Sciences, Scotland's Rural College (SRUC), An Lòchran, 10 Inverness Campus, Inverness, IV2 5NA, UK.

*Corresponding author.

E-mail address: t.walker@kingston.ac.uk (A.J. Walker)

Abstract

Schistosomules of the human parasite *Schistosoma mansoni* are vital for research focusing on the fundamental functional/developmental biology of schistosomes and many anti-schistosomal drug discovery programmes. Through the further evaluation and validation of a recently tested media, HybridoMed Diff 1000 (HM), for the cell-free culture of juvenile schistosomules, we show that while Basch medium was superior to HM for the survival/development of schistosomules, HM represents a viable and attractive alternative for somule culture, particularly to the early liver stage. Adoption of HM for schistosomule culture could facilitate more standardised approaches, which for drug screening should enable improved multi-centre target-hit evaluation.

Keywords: Basch; HybridoMed; Schistosomulae; schistosome culture; *Schistosoma mansoni*

Human schistosomiasis, caused by *Schistosoma* blood parasites, is a debilitating neglected tropical disease affecting over 200 million people; ~779 million people are at risk of infection (Steinmann et al., 2006; McManus et al., 2018). To cause infection, larvae (cercariae) released from freshwater snail intermediate hosts seek out a human definitive host and invade the skin (Ressurreição et al., 2015). They then transform into biochemically distinct schistosomules (Gobert et al., 2010) that develop a specialised syncytial tegument that facilitates parasite survival within the host. These 'skin somules' enter the circulation and migrate with the blood flow to the lungs ('lung somules') and then the liver ('liver somules') where they grow into adolescent male or female worms (Walker, 2011). Thereafter, males pair with females, which stimulates reproductive maturation (Clough, 1981; Basch and Rhine, 1983; Doenhoff et al., 2019), and the couples relocate to the mesenteric veins of the bowel (for *S. mansoni* or *S. japonicum*) or the pelvic venous plexus (*S. haematobium*). Fertilised eggs deposited by the female worms are destined for release in the excreta but a significant proportion are swept away in the blood flow and become entrapped in tissues/organs causing inflammatory immune reactions that result in hepato-intestinal, or urogenital schistosomiasis (McManus et al., 2018).

Studies on schistosomes continue to focus on somules for a variety of reasons. Firstly, there is paucity in the understanding of the fundamental biology of the somule, its biochemistry, molecular regulation, interactions with the host, and its development (Ressurreição et al., 2016). Secondly, praziquantel, the drug currently used to kill schistosomes is largely ineffective against somules (Xiao et al., 1985; Pica-Mattoccia and Cioli, 2004); therefore, skin somules are employed in drug susceptibility assays using candidate compounds. Thirdly, somules (at their various stages of development) could represent the most targetable for novel drugs and vaccines as they are crucial

for parasite establishment in the host, growth, and ultimately maturation of worms. Finally, somules can be produced *in vitro* by transforming cercariae that have been released from patent snails, removing the need to infect mice specifically to generate large numbers of adult parasites for research. These mechanically transformed somules are biochemically and structurally similar to those that passage through skin (Brink et al., 1977; Protasio et al., 2013).

Various media have been employed for the culture of somules including, for short term maintenance, BME, RPMI 1640, and Medium 199 (Marxer et al., 2012; Patocka et al., 2014; Chan et al., 2016; Ressurreição et al., 2016; Pasche et al., 2019). To culture somules for longer and support their development to liver somules and beyond, Basch Medium 169 (Basch, 1981) or variations thereof is needed (Mann et al., 2010; Stefanić et al., 2010; Paveley et al., 2012; Rojo-Arreola et al., 2014). Basch Medium 169 contains BME, lactalbumin hydrolysate, glucose, hypoxanthine, serotonin, insulin, hydrocortisone, triiodothyronine, vitamins, Schneider's medium, HEPES, serum (human or foetal calf) and antibiotics/antimycotics; erythrocytes are often also included to support the parasite's development. A significant limitation of Basch Medium 169 lies in its complexity and non-commercial availability making it prone to variability, compounded further by the fact that different laboratories formulate different variants thereof. The need to simplify culture conditions and generate *in vitro* grown schistosomes for drug screening prompted Frahm et al. (2019) to investigate the utility of a previously untested medium, HybridoMed Diff 1000 (HM), for somule culture; excellent survival and development to the lung stage in serum- and cell-free conditions were demonstrated, with early and late liver stage somules produced in the presence of human serum. Given the important implications of this work, particularly for schistosome culture and drug discovery, we attempted to independently verify and

extend the previous study; however, herein we report some important discrepancies in the utility of HM for somule culture, including when compared to Basch Medium 169. Notwithstanding, our results corroborate that HM is a valuable alternative medium for the culture of skin to early liver stage *S. mansoni* somules when used with human serum, and we now propose that, where possible and appropriate, investigators move towards employing standard media such as HM for work involving such parasites.

Biomphalaria glabrata snails were infected with miracidia released from eggs derived from livers of experimentally infected mice kept to maintain the *S. mansoni* (Puerto Rican strain) life cycle at the Wellcome Sanger Institute, UK (courtesy of Dr Gabriel Rinaldi). Mouse infection protocols were approved by the Animal Welfare and Ethical Review Body of the Wellcome Sanger Institute, and in accordance with the UK Home Office approved project license P77E8A062. Cercariae were shed from patent snails under light and were then collected and mechanically transformed into skin somules as described previously (Hirst et al., 2016). The somules were next transferred to individual wells of 96-well flat-bottomed culture plates (Nunc, tissue culture treated; 100 somules/well) in 100 µl of media supplemented with 200 U/ml penicillin, 200 µg/ml streptomycin and 500 ng amphotericin B (A5955; Sigma) and were maintained at 37°C and 5% CO_2 for the duration of the assay. HM (F8055; Biochrom) was compared with Basch Medium 169 (Basch; produced with components as detailed in Basch (1981) per 1L, but made fresh in smaller 40 ml batches each week; Supplementary Table S1) with and without human serum (HS; AB converted heat-inactivated, S-102D-HI-US; Life Science Production) or foetal calf serum (FCS; A3160801; Gibco) and media were changed weekly.

Scoring (0–3) for somule viability, performed under a BMC stereo microscope, was based on standard procedures for compound screening at the WHO-TDR (Ramirez et al., 2007; Manneck et al., 2010; Frahm et al., 2019) whereby: 0 = dead parasites, severe granulation, no contractile movement; 1 = medium granulation, limited contractile movement; 2 = slight granulation, slowed contractile movements; 3 = totally vital, no granulation, normal regular contractile movements (Supplementary Fig. S1). Skin somules were identified as those parasites that retained the shape of the cercarial head, whereas lung somules were characteristically more elongated; liver somules were those larger in diameter/length with a clearly observable gut, whereas the late liver somules possessed clearly visible oral and ventral suckers with increased body length posterior to the ventral sucker (Basch, 1981) (Supplementary Fig. S1). Thirty somules were scored per well at each time point for each treatment and independent replicate assays performed. All images of somules were captured using a Moticam 1080 digital camera coupled to a Motic SMZ171 stereomicroscope. To evaluate the impact of the different media on the size of liver somules, images of early/late somules were captured at weekly intervals from five weeks after initial transformation and somule area determined using ImageJ (Schneider et al., 2012). Finally, to assess any possible effects of electroporation on somule health/viability and development in the different media, newly transformed skin somules were electroporated (square-wave 20 ms pulse, 120V) in Eagle's Basal Medium (BME; Gibco) in 4 mm electroporation cuvettes (VWR; 732-1137) using a BioRad GenePulser XCell and were scored/developmentally evaluated over 21 days. Statistical comparisons (using SPSS Statistics, version 26) of viability and somule developmental stage were performed by Kruskal-Wallis test and Mann-Whitney U test;

somule area analysis was done by Analysis of Variance (ANOVA) with Fisher's post-hoc multiple pairwise comparison.

We first evaluated the ability of HM and Basch to support the viability and development of newly transformed somules over 28 days with and without serum. Despite numerous independent attempts, we were unable to sustain good levels of somule survival in HM alone (Fig. 1). The overall viability score in HM declined by 43% and 92% on days 7 and 28, respectively, with concomitant somule death rates of 31% and 83% (Fig. 1A, B). This contrasted the work of Frahm et al. (2019) who reported no significant loss of viability over the same duration. Furthermore, although the viability score for somules in Basch alone was similar to that in HM at day 7, after 28 days the mean viability score was significantly greater ($p \leq 0.001$; Fig 1A) with considerably fewer somules dead ($p \leq 0.01$; Fig. 1B), implying that the somules were able to better 'adjust' to the Basch media. For either medium the mean viability score was greater throughout the assay when serum was incorporated, although overall, HS outperformed FCS, particularly when incorporated with HM (Fig. 1A). Also, with FCS, Basch supported enhanced somule viability when compared to HM after 14 days culture ($p \leq 0.001$; Fig. 1A). In the context of *S. mansoni* development, lung somules were present under all culture conditions on day 7, however, there were significantly more lung somules in Basch with HS than in other media ($p \leq 0.05$; Fig. 1B). At this time, the first early liver somules also appeared, but only in Basch containing 20% HS. After two further weeks (day 21), 54% of the surviving somules cultured in Basch with 20% HS were early liver stage, which was more (28%; $p \leq 0.001$) than those in HM with the same HS concentration (Fig. 1B). Moreover, at days 14 and 21, there was a marked restriction of development from the skin stage in all media when compared to Basch with HS (Fig. 1B). Thus, a certain component(s) of the considerably more

complex Basch media seems to drive development beyond the lung stage, but only in the presence of HS and not FCS; this interdependency of components to support somule development requires further investigation. Although the mean number of early liver somules increased in HM plus HS between days 21 and 28, there remained fewer than in Basch plus HS, which in turn supported the first development of late liver somules (Fig. 1B). Concomitant with this, somules in Basch plus HS appeared to be larger than their counterparts in HM with HS and, generally, somules grown in FCS instead of HS (with either media) were of a lesser quality (Fig. 1C).

Electroporation is an important technique enabling functional genomic studies in schistosomes; somules can be electroporated with double stranded (ds)RNA, small interfering (si)RNA, transposons and retroviruses with relative ease (reviewed by Mann et al. (2010)) and the effects of gene manipulation/silencing on somule form and function evaluated. However, electroporation can be detrimental to somule survival. We therefore evaluated the ability of HM and Basch to support the culture of newly transformed somules that were electroporated by square-wave 20 ms pulse. Over 21 days culture, there were no significant differences in the viability scores for somules that had been electroporated when compared to control somules for either HM or Basch media with 10% HS (Supplementary Fig. S2). Furthermore, electroporation was not detrimental to the development of somules over this duration with broadly similar proportions of skin, lung, and liver somules present in control and electroporated groups for each culture condition (Supplementary Fig. S2). Thus, HM is suitable for the culture of somules through to lung and early liver forms after electroporation, permitting functional genomic studies to be performed using a simple medium in these parasite stages.

We next evaluated the ability of HM to support the longer-term survival of somules and their development to the late liver stage. Between day 35 and day 56 there was a slight reduction in the overall mean viability score for somules in each culture media, irrespective of HS concentration (Fig. 2A). However, Basch supplemented with 20% HS consistently outperformed HM plus 20% HS over the three-week period ($p \leq 0.001$), while in the presence of 10% HS this difference was less marked and only evident at weeks 7 and 8 ($p \leq 0.001$; Fig. 2A). Strikingly, however, and in contrast to the recently published work of Frahm et al. (2019), HM supplemented with 20% HS failed to support the development of somules from the early to late liver stage over 56 days in several independent experiments. Whereas Frahm et al. (2019) observed late liver somules at week 4, which increased in number thereafter, we only observed late liver somules in Basch medium containing either 10% or 20% HS (Fig. 2B) and these first appeared at week 3 using 20% HS (Fig. 1B). To better determine the differences in growth seen between the various culture conditions, the size (based on area) of individuals in mixed populations of early and late liver stage somules was determined using ImageJ. Although there was no significant difference in size between liver somules cultured in HM with different concentrations of HS, somules grew noticeably larger in Basch with 20% HS compared to with 10% HS at all time points ($p \leq 0.001$), with a ~2.1-fold size increase seen on day 56 (Fig. 2C). Furthermore, at day 35, somules grown in Basch incorporating 20% HS were 4-fold larger ($p \leq 0.001$) than those in HM with 20% HS and this differential persisted through to the end of the experiment (3.8-fold difference on day 56; $p \leq 0.001$) (Fig. 2C-D). The effects seen with the lower concentration of HS were less marked, but somules were still significantly larger (≥1.5-fold) from day 42 onwards when 10% HS was incorporated into Basch rather than into HM ($p \leq 0.01$) (Fig. 2C).

We conducted the above experiments using our standard somule culture approaches. These incorporate the use of 100 µl medium/well, Nunc 96-well tissue culture treated plates, AB converted heat-inactivated HS, and antibiotics/antimycotics (200 U/ml penicillin, 200 µg/ml streptomycin and 500 ng amphotericin B). However, Frahm et al. (2019) employed 150 µl medium/well, Falcon (353075, Corning Incorporated) tissue culture treated plates, off-the-clot non-heat-inactivated HS (OC-HS), and 200 U/ml penicillin/200 µg/ml streptomycin but without amphotericin B (P4333; Sigma). We therefore conducted a further set of experiments, employing identical components to Frahm et al. (2019), to ascertain whether such subtle differences could be responsible for the disparity between studies. Neither the brand of culture plate nor the volume of medium used significantly affected the viability or development of somules over two weeks when using either HM or Basch with or without OC-HS (mixed gender, S-106D-EU, Life Science Production) (data not shown). Interestingly, however, in the absence of serum, somules displayed improved viability (Fig. 3A c.f. Fig 1A) and thus development (Fig. 3B c.f. Fig 1B) over 28 days when amphotericin B was excluded, with somules developing to the lung stage and early liver stage for HM and Basch, respectively. Nevertheless, either with or without serum, viability remained greater in Basch than HM at day 7 and thereafter ($p \leq 0.01$; Fig. 3A). Serum (either FCS or HS) also seems to protect cultures against potential amphotericin B toxicity (Fig. 1A). Inclusion of OC-HS instead of HS markedly improved somule development (Fig. 1 c.f. Fig. 3). However, despite this and as found previously (Fig. 1B) Basch remained considerably superior to HM driving stronger growth and development throughout (Fig. 3B). Although a good proportion (~53% of surviving somules) of early liver somules were observed with HM from day 14 onwards, no late liver stage somules were observed by 28 days. In contrast, with Basch and OC-HS,

41% of somules had progressed to the late liver stage by day 28 (Fig. 3B). Also, from day 21, significantly fewer (~50%) somules died in Basch than in HM when OC-HS was employed ($p \leq 0.01$). The considerable difference in somule growth and development observed between the two media is highlighted by somule size analysis. On day 28, somules cultured in Basch and OC-HS were a striking 6.0-fold larger than those cultured in HM and OC-HS ($p \leq 0.001$) (Fig. 3C, 3D). Also, OC-HS considerably enhanced the growth of somules in Basch when compared to HS, with somules reaching a similar size (~20,000 pixels) two weeks earlier (day 28 instead of day 42) (Fig 3C c.f. Fig. 2C). Although we cannot exclude the possibility that some of this difference may be due to lack of amphotericin B in the OC-HS experiments, that such growth enhancement was not observed with HM suggests that it is more likely a consequence of the OC-HS in combination with Basch rather than a suppressive effect of the antimycotic itself. Collectively, our results show that (i) Basch considerably outperformed HM media for somule growth and development; and (ii) unlike Basch, HM did not support parasite development beyond the early somule stage. However, our data do further validate HM as a useful defined medium for the generation of lung *S. mansoni* somules, and early liver somules when supplemented with human serum.

The disparity in *S. mansoni* somule development when using HM discovered between the work detailed here and that of Frahm et al. (2019) is difficult to explain. Despite several attempts we found that HM alone was unable to support the satisfactory maintenance and early development of somules over 28 days. However, the incorporation of serum (human or FCS) supported the growth and development of somules to the early liver stage, albeit less effectively than Basch with serum, which in turn supported development to late liver stage over the same duration. While Frahm et al. (2019) found considerable variation in the survival and growth of somules to liver

stages between individual experiments for HM with 20% HS, we did not observe such variability in separate HM cultures (albeit only comparable at 10% HS, e.g. day 21 (Fig. 1B and Supplementary Fig. S2)). The possibility that *S. mansoni* strain differences (Puerto Rican used here, NMRI/Brazilian strains used by Frahm et al. (2019)) might have influenced the different experimental outcomes cannot be ruled out. Nevertheless, HM seems to be a very good alternative to Basch for studies with somules up to and including the early liver stage and, importantly, HM has the principal advantage over of Basch of being a defined and commercially available medium. Good growth/development is also achieved without the need for human erythrocytes which are commonly incorporated in media by investigators. Schistosome research could benefit from a greater use of 'standardised protocols' enabling better comparison of findings and this may well be very important in the context of drug screening, where the use of complex Basch media (that can even differ between laboratories) is often less desirable. Even in cases where simple basal media (e.g. BME) are used to maintain skin stage somules for short-term (~1-3 day) drug assays, different types of media are often employed by different workers, complicating comparison. Furthermore, as we move towards better understanding the functional biology of the developing somule through the application of techniques such as transcriptomics, proteomics and metabolomics, the impact of culture conditions need to be better considered. For example, it is likely that the global proteome of the somule surface membranes (outer tegument layer), as investigated by Sotillo et al. (2015), would differ according to culture conditions, as might the expression of surface receptors that influence downstream signalling proteins such as extracellular signal-regulated kinase (Ressurreição et al., 2016) and Akt (McKenzie et al., 2018). Thus, it would be valuable

to try to standardise culture conditions enabling synergy across laboratories, and HM (or other standard media) may be important towards helping achieve this goal.

Acknowledgements

We are extremely grateful to Dr Gabriel Rinaldi of the Wellcome Sanger institute (UK) for supplying *S. mansoni* infected mouse livers.

References

Basch, P.F., 1981. Cultivation of *Schistosoma mansoni* in vitro. I. Establishment of cultures from cercariae and development until pairing. J. Parasitol. 67, 179–85. https://doi.org/10.2307/3280632.

Basch P.F., Rhine W.D., 1983. Schistosoma mansoni: reproductive potential of male and female worms cultured in vitro. J. Parasitol. 69, 567-569. https://doi.org/10.2307/3281371.

Brink, L.H., McLaren, D.J., Smithers, S.R., 1977. *Schistosoma mansoni*: a comparative study of artificially transformed schistosomula and schistosomula recovered after cercarial penetration of isolated skin. Parasitology 74, 73–86. https://doi.org/10.1017/S0031182000047545

Clough. E.R. 1981. Morphology and reproductive organs and oogenesis in bisexual and unisexual translpants of mature *Schistosoma mansoni* females. J. Parasitol. 67, 535-539. https://doi.org/10.2307/3280487

Chan, J.D., McCorvy, J.D., Acharya, S., Johns, M.E., Day, T.A., Roth, B.L.,

Marchant, J.S., 2016. A miniaturized screen of a *Schistosoma mansoni* serotonergic G protein-coupled receptor identifies novel classes of parasite-selective inhibitors. PLoS Path. 12, e1005651. https://doi.org/10.1371/journal.ppat.1005651

Doenhoff, M.J., Modha, J., Walker, A.J., 2019. Failure of in vitro-cultured schistosomes to produce eggs: how does the parasite meet its needs for host-derived cytokines such as TGF-β? Int. J. Parasitol. 49, 747–757. https://doi.org/10.1016/j.ijpara.2019.05.004

Frahm, S., Anisuzzaman, A., Prodjinotho, U.F., Vejzagić, N., Verschoor, A., Prazeres da Costa, C., 2019. A novel cell-free method to culture *Schistosoma mansoni* from cercariae to juvenile worm stages for in vitro drug testing. PLoS Negl. Trop. Dis. 13, e0006590. https://doi.org/10.1371/journal.pntd.0006590

Gobert, G.N., Tran, M.H., Moertel, L., Mulvenna, J., Jones, M.K., McManus, D.P., Loukas, A., 2010. Transcriptional changes in *Schistosoma mansoni* during early schistosomula development and in the presence of erythrocytes. PLoS Negl. Trop. Dis. 4, e600. https://doi.org/10.1371/journal.pntd.0000600

Hirst, N.L., Lawton, S.P., Walker, A.J., 2016. Protein kinase A signalling in *Schistosoma mansoni* cercariae and schistosomules. Int. J. Parasitol. 46, 425–437. https://doi.org/10.1016/j.ijpara.2015.12.001

Mann, V.H., Morales, M.E., Rinaldi, G., Brindley, P.J., 2010. Culture for genetic manipulation of developmental stages of *Schistosoma mansoni*. Parasitology 137, 451–62. https://doi.org/10.1017/S0031182009991211

Manneck, T., Haggenmüller, Y., Keiser, J., 2010. Morphological effects and tegumental alterations induced by mefloquine on schistosomula and adult flukes of *Schistosoma mansoni*. Parasitology 137, 85–98.

https://doi.org/10.1017/S0031182009990965

Marxer, M., Ingram, K., Keiser, J., 2012. Development of an in vitro drug screening assay using *Schistosoma haematobium* schistosomula. Parasit. Vect. 5, 1. https://doi.org/10.1186/1756-3305-5-165

McKenzie, M., Kirk, R.S., Walker, A.J., 2018. Glucose uptake in the human pathogen *Schistosoma mansoni* is regulated through Akt/protein kinase B signaling. The J. Infect. Dis. 218, 152–164. https://doi.org/10.1093/infdis/jix654

McManus, D.P., Dunne, D.W., Sacko, M., Utzinger, J., Vennervald, B.J., Zhou, X.-N., 2018. Schistosomiasis. Nat. Rev. Dis. Primers 4, 13. https://doi.org/10.1038/s41572-018-0013-8

Pasche, V., Laleu, B., Keiser, J., 2019. Early antischistosomal leads identified from in vitro and in vivo screening of the medicines for malaria venture pathogen box. ACS Infect. Dis. 5, 102-110. https://doi.org/10.1021/acsinfecdis.8b00220

Patocka, N., Sharma, N., Rashid, M., Ribeiro, P., 2014. Serotonin signaling in *Schistosoma mansoni*: a serotonin–activated G protein-coupled receptor controls parasite movement. PLoS Path. 10, e1003878. https://doi.org/10.1371/journal.ppat.1003878

Paveley, R.A., Mansour, N.R., Hallyburton, I., Bleicher, L.S., Benn, A.E., Mikic, I., Guidi, A., Gilbert, I.H., Hopkins, A.L., Bickle, Q.D., 2012. Whole organism high-content screening by label-free, image-based bayesian classification for parasitic diseases PLoS Neg. Trop. Dis. 6, e1762. https://doi.org/10.1371/journal.pntd.0001762

Pica-Mattoccia, L., Cioli, D., 2004. Sex- and stage-related sensitivity of *Schistosoma mansoni* to in vivo and in vitro praziquantel treatment. Int. J. Parasitol. 34, 527–533. https://doi.org/10.1016/j.ijpara.2003.12.003

Protasio, A. V, Dunne, D.W., Berriman, M., 2013. Comparative study of transcriptome profiles of mechanical- and skin-transformed *Schistosoma mansoni* schistosomula. PLoS Neg. Trop. Dis. 7, e2091. https://doi.org/10.1371/journal.pntd.0002091

Ramirez, B., Bickle, Q., Yousif, F., Fakorede, F., Mouries, M., Nwaka, S., 2007. Schistosomes: challenges in compound screening. Exp. Opin. Drug Discov. 2, S53–S61.

Ressurreição, M., Elbeyioglu, F., Kirk, R.S., Rollinson, D., Emery, A.M., Page, N.M., Walker, A.J., 2016. Molecular characterization of host-parasite cell signalling in *Schistosoma mansoni* during early development. Sci. Reports 6, 35614. https://doi.org/10.1038/srep35614

Ressurreição, M., Kirk, R.S., Rollinson, D., Emery, A.M., Page, N.M., Walker, A.J., 2015. Sensory protein kinase signaling in *Schistosoma mansoni* cercariae: host location and invasion. J. Infect. Dis. 212, 1787-1797. https://doi.org/10.1093/infdis/jiv464

Rojo-Arreola, L., Long, T., Asarnow, D., Suzuki, B.M., Singh, R., Caffrey, C.R., 2014. Chemical and genetic validation of the statin drug target to treat the helminth disease, schistosomiasis. PloS One 9, e87594. https://doi.org/10.1371/journal.pone.0087594

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–5. https://doi.org/10.1038/nmeth.2089

Sotillo, J., Pearson, M., Becker, L., Mulvenna, J., Loukas, A., 2015. A quantitative proteomic analysis of the tegumental proteins from *Schistosoma mansoni* schistosomula reveals novel potential therapeutic targets. Int. J. Parasitol. 45,

505–516. https://doi.org/10.1016/j.ijpara.2015.03.004

Stefanić, S., Dvořák, J., Horn, M., Braschi, S., Sojka, D., Ruelas, D.S., Suzuki, B., Lim, K.-C., Hopkins, S.D., McKerrow, J.H., Caffrey, C.R., 2010. RNA interference in *Schistosoma mansoni* schistosomula: selectivity, sensitivity and operation for larger-scale screening. PLoS Negl. Trop. Dis. 4, e850. https://doi.org/10.1371/journal.pntd.0000850

Steinmann, P., Keiser, J., Bos, R., Tanner, M., Utzinger, J., 2006. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect. Dis. 6, 411–25. https://doi.org/10.1016/S1473-3099(06)70521-7

Walker, A.J., 2011. Insights into the functional biology of schistosomes. Parasit. Vect. 4, 203. https://doi.org/10.1186/1756-3305-4-203

Xiao, S.H., Catto, B.A., Webster, L.T., 1985. Effects of praziquantel on different developmental stages of *Schistosoma mansoni* in vitro and in vivo. J. Infect. Dis. 151, 1130–7. https://doi.org/10.1093/infdis/151.6.1130

Figure Legends

Fig.1. Basch Medium 169 (Basch) outperforms HybridoMed DIF-1000 (HM) for the *in vitro* culture of *Schistosoma mansoni* somules over 28 days. Newly transformed somules were maintained in Basch or HM supplemented with antibiotic/antimycotic and with or without foetal calf serum (FCS) or human serum (HS). (A) Viability and (B) somule development were evaluated by scoring and staging 30 parasites in each replicate/treatment, respectively; mean values (±SEM; n≥360) are from at least three independent experiments each with a minimum of four biological replicates. (C) Photomicrographs of representative somules were taken on day 28 post-transformation; scale bar = 100 µm, arrow heads indicate dead somules.

Fig. 2. Basch Medium 169 (Basch) outperforms HybridoMed DIF-1000 (HM) for the *in vitro* culture of *Schistosoma mansoni* somules to the late liver stage. Newly transformed somules were maintained in Basch or HM supplemented with antibiotic/antimycotic and 10% or 20% human serum (HS). (A) Viability and (B) somule development were evaluated between days 35 and 56 post-transformation by scoring and staging 30 parasites in each replicate/treatment, respectively; mean values (±SEM; n≥360) are from at least three independent experiments each with a minimum of four biological replicates. (C) The mean (±SEM; n≥45) size of early liver/late liver somules (on days 35-56) was evaluated using ImageJ. (D) Photomicrographs of representative somules were taken on day 56 post transformation; scale bar = 100 µm, arrow heads indicate dead somules. $^{xx}p\leq0.01$, $^{xxx}p\leq0.001$ when comparing between media with 10% HS; ***p≤0.001 for samples with 20% HS.

Fig. 3. Off-the-clot human serum improves performance of Basch Medium 169 (Basch) and HybridoMed DIF-1000 (HM) for the *in vitro* culture of *Schistosoma mansoni* somules. Newly transformed somules were maintained in Basch or HM supplemented with antibiotic (no antimycotic) and 20% off-the-clot non heat-inactivated human serum (OC-HS) for 28 days. (A) Viability and (B) somule development were evaluated by scoring and staging 30 parasites in each replicate/treatment, respectively; mean values (±SEM; n≥330) are from at least three independent experiments each with a minimum of three biological replicates. (C) The mean (±SEM; n≥45) size of early liver/late liver somules at day 28 was evaluated using ImageJ. (D) Photomicrographs of representative somules were taken on day 28 post transformation; scale bar = 100 µm, arrow heads indicate dead somules. $^{xx}p \leq 0.01$, $^{xxx}p \leq 0.001$ when comparing between media with 20% OC-HS; **p≤0.01, ***p≤ 0.001 for samples without OC-HS.

Supplementary Fig. S1. Viability scoring and staging of *Schistosoma mansoni* somules cultured *in vitro*. (A) Representative images of skin somules with associated viability score points (0-3) based on morphology and granularity; contractile behaviour was also considered. (B) Individual somules within each culture were allocated a score. (C) Representative images of somules at different developmental stages. Scale bar = 100 µm.

Supplementary Fig. S2. Electroporation of *Schistosoma mansoni* somules does not affect somule viability or development in Basch Medium 169 (Basch) or HybridoMed DIF-1000 (HM). Newly transformed somules were electroporated (square-wave 20 ms pulse, 120V) and were then maintained in Basch or HM supplemented with

antibiotic/antimycotic and 10% human serum (HS) for 21 days. (A) Viability and (B) somule development were evaluated by scoring and staging a minimum of 30 parasites in each replicate/treatment, respectively; mean values (±SEM; n≥450) are from at least three independent experiments each with five biological replicates. (C) Photomicrographs of representative somules were taken on day 21 post transformation; scale bar = 100 μm and arrow heads indicate dead somules.

Supplementary Table S1. Composition of Basch 169 Medium (Basch, 1981) used in this study.

Component	Working conc.	Product ID	Supplier
Lactalbumin hydrolysate	1 g/L	L9010	Sigma Aldrich
Glucose	1 g/L	G7021	Sigma Aldrich
Hypoxanthine	0.5 µM	H9377	Sigma Aldrich
Serotonin hydrochloride	1 µM	H9523	Sigma Aldrich
Insulin (human)	8 µg/ml	3435	Tocris
Hydrocortisone	1 µM	H0888	Sigma Aldrich
Triiodothyronine	0.2 µM	T6397	Sigma Aldrich
MEM Vitamins (100x)	0.5 x	M6895	Sigma Aldrich
Schneider's Insect Medium	5%	S0146	Sigma Aldrich
HEPES buffer (1M)	10 mM	10041703	Fisher BioReagents
Basal Medium Eagle (BME)	To final volume (1 L)	41010026	Gibco (Thermo Fisher)

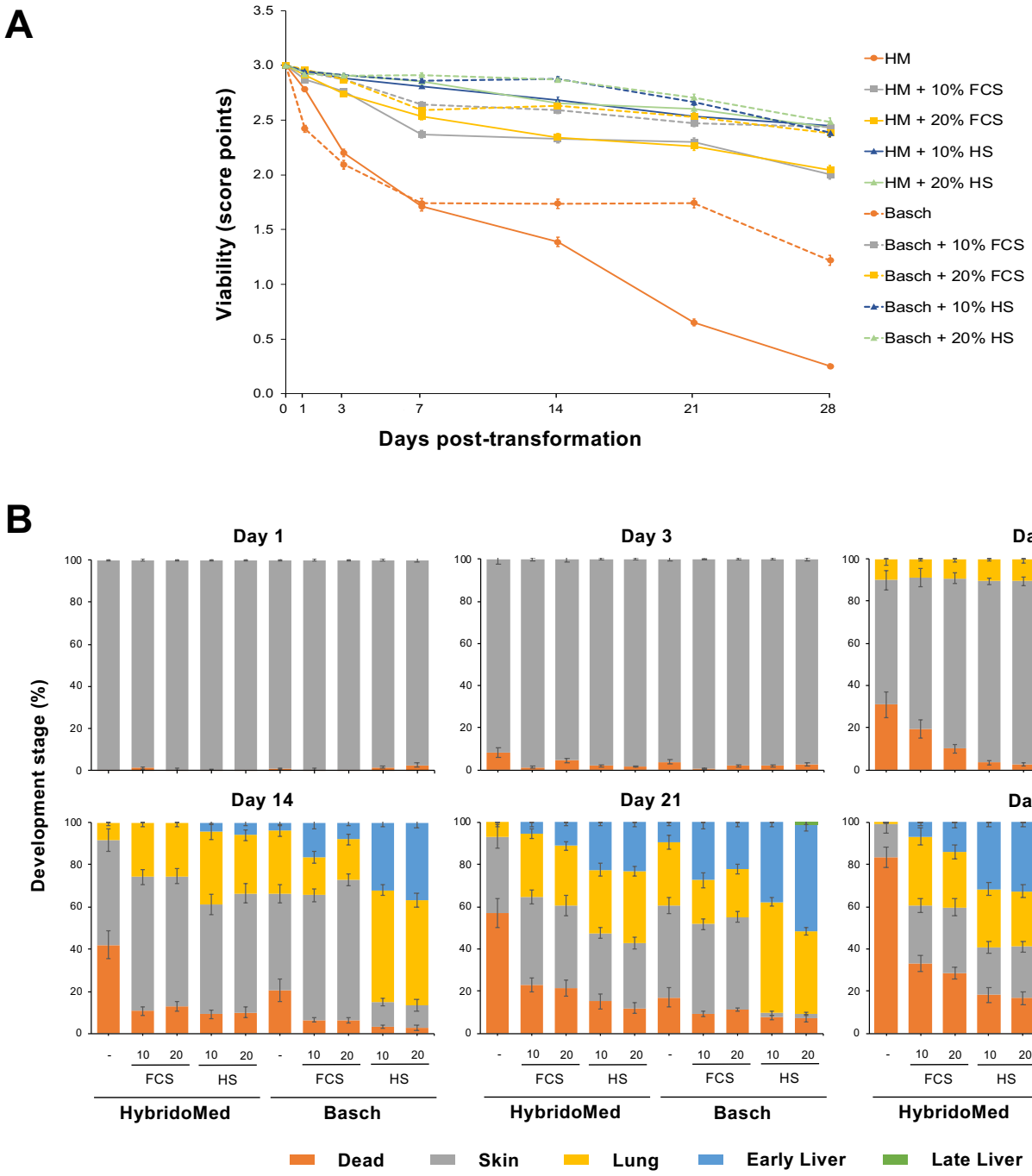

A
Viability (score points)
Days post-transformation
HM
HM + 10% FCS
HM + 20% FCS
HM + 10% HS
HM + 20% HS
Basch
Basch + 10% FCS
Basch + 20% FCS
Basch + 10% HS
Basch + 20% HS
B
Development stage (%)
Day 1
Day 3
Day 7
Day 14
Day 21
Day 28
FCS
HS
HybridoMed
Basch
Dead
Skin
Lung
Early Liver
Late Liver
C
HM -Serum
HM + 10% FCS
HM + 20% FCS
HM + 10% HS
HM + 20% HS
Basch -Serum
Basch + 10% FCS
Basch + 20% FCS
Basch + 10% HS
Basch + 20% HS

Fig. 1

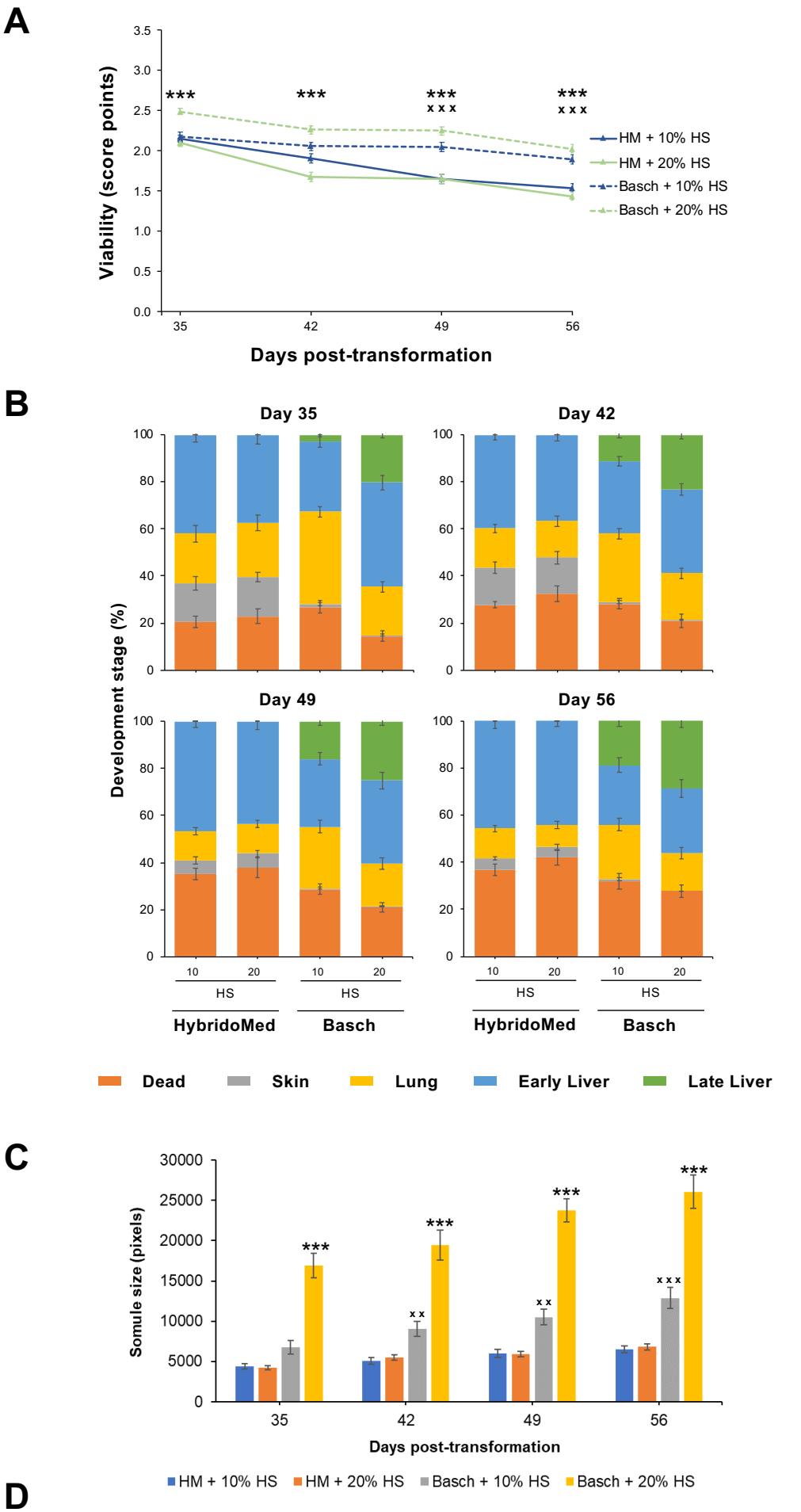

A
Viability (score points)
Days post-transformation
HM + 10% HS
HM + 20% HS
Basch + 10% HS
Basch + 20% HS
B
Day 35
Day 42
Day 49
Day 56
Development stage (%)
HS
HybridoMed
Basch
Dead
Skin
Lung
Early Liver
Late Liver
C
Somule size (pixels)
Days post-transformation
D
HM + 10% HS
HM + 20% HS
Basch + 10% HS
Basch + 20% HS

Fig. 2

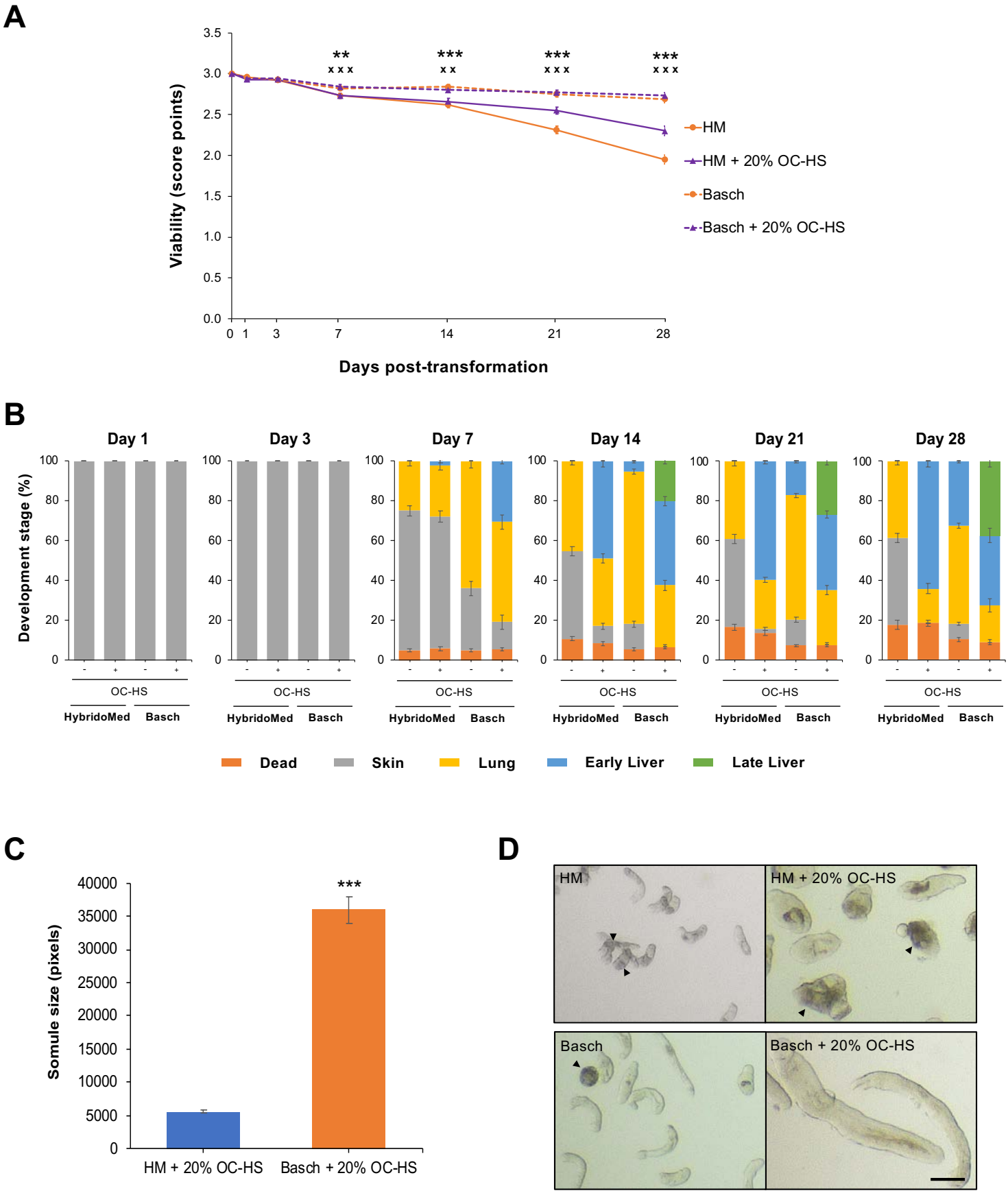
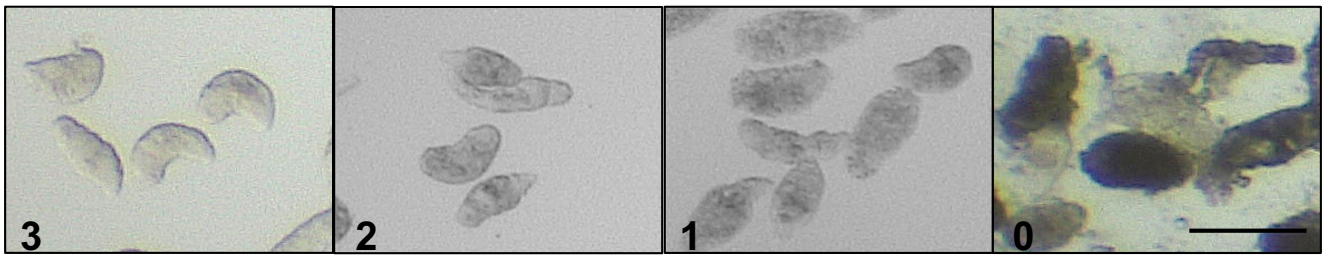
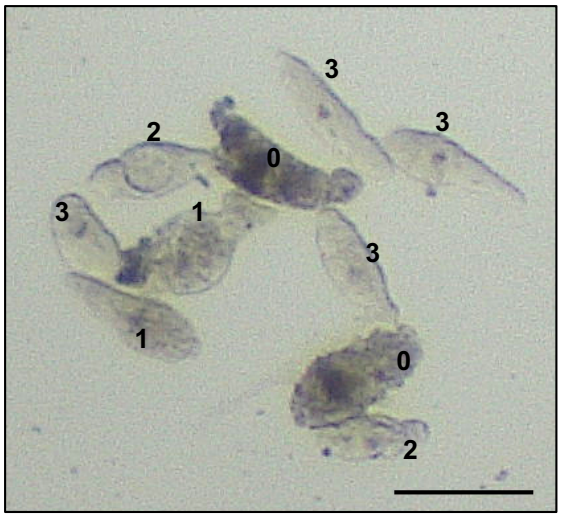

A
Viability (score points)
Days post-transformation
HM
HM + 20% OC-HS
Basch
Basch + 20% OC-HS
B
Day 1
Day 3
Day 7
Day 14
Day 21
Day 28
Development stage (%)
OC-HS
HybridoMed
Basch
Dead
Skin
Lung
Early Liver
Late Liver
C
Somule size (pixels)
HM + 20% OC-HS
Basch + 20% OC-HS
D
HM
HM + 20% OC-HS
Basch
Basch + 20% OC-HS

Fig. 3

A



B



C

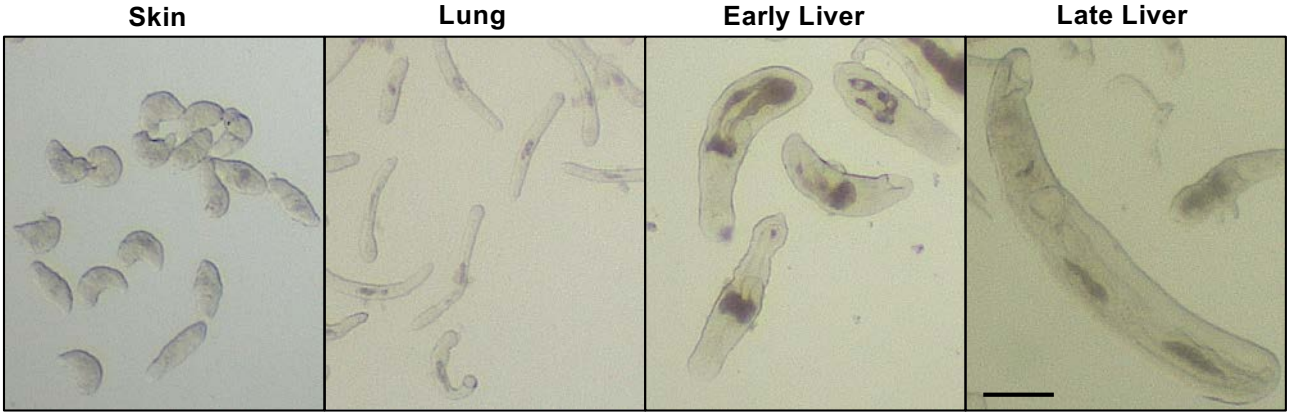


Fig. S1

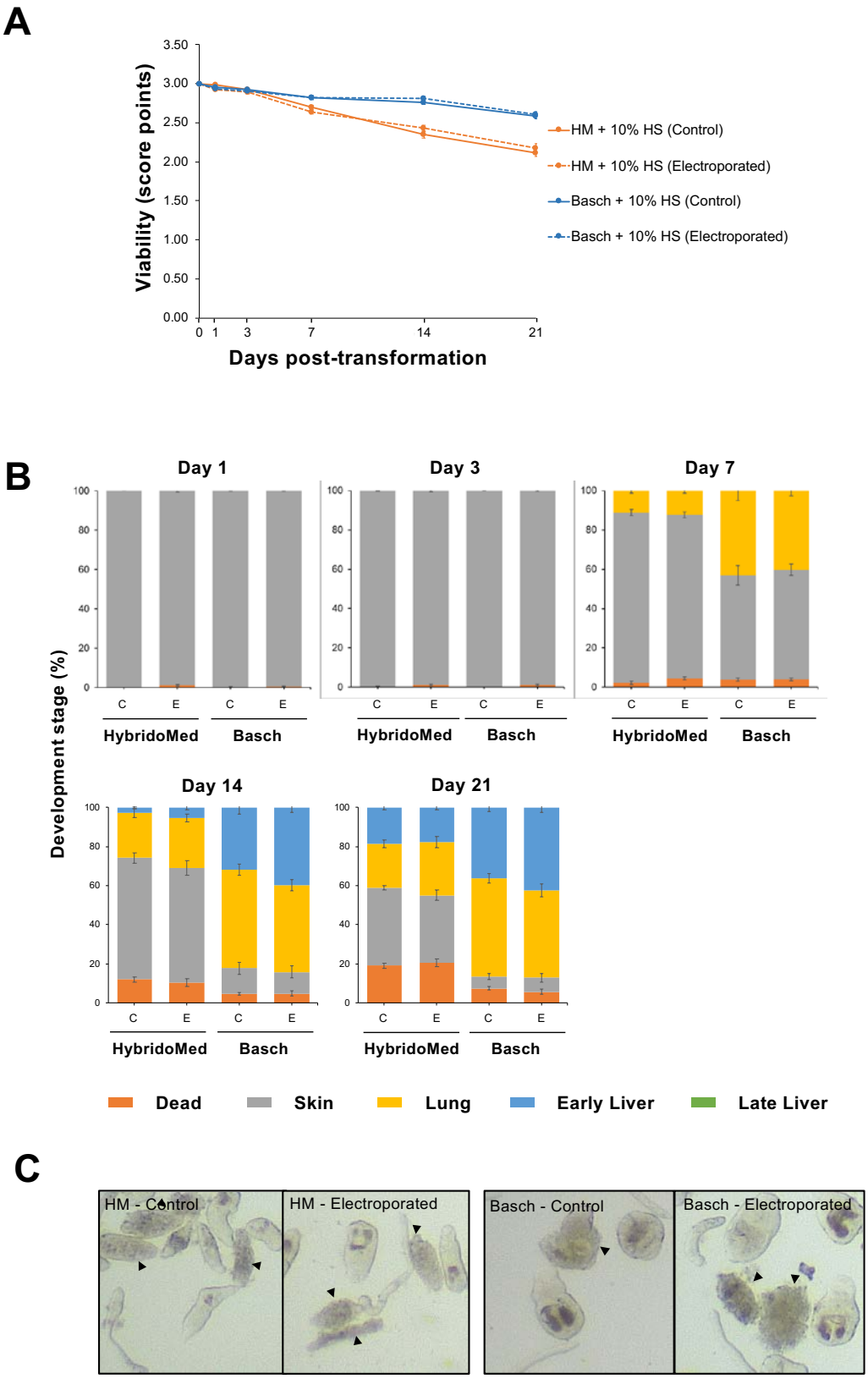

A
Viability (score points)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0 1 3 7 14 21
Days post-transformation
HM + 10% HS (Control)
HM + 10% HS (Electroporated)
Basch + 10% HS (Control)
Basch + 10% HS (Electroporated)
B
Day 1
Day 3
Day 7
Day 14
Day 21
Development stage (%)
100
80
60
40
20
0
C E C E
HybridoMed Basch
Dead
Skin
Lung
Early Liver
Late Liver
C
HM - Control
HM - Electroporated
Basch - Control
Basch - Electroporated

Fig. S2