

GENERAL STYLE

Follow *Hart's Rules* for style and *Concise Oxford Dictionary* for spelling (for British authors) and *Chicago Manual of Style* and the *Merriam Webster Dictionary* (for American authors):

- 1 -ize, -yse spellings: organize, analyse (exceptions are those words containing -mis-, -cis-, -vis- such as *compromise, exercise, televise*);
- 2 hyphenate compound adjectives (e.g. long-lost sister not long lost sister);
- 3 sixteenth century (*not* 16th century), 1960s (*not* sixties or 60s);
- 4 spell out numbers up to ninety-nine; use Arabic numbers as measurements (e.g. 10 mm but ten people; also 10 per cent, *not* 10% or 10 percent); insert commas in numbers over 1,000 (e.g. 1,595 and 10,000, *not* 1595 and 10000); this, of course, does not apply to dates or ranges of pages in the Reference section (e.g. 1001–12, *not* 1,001–12); use shortest range of figures (e.g. 351–2, 26–7, 211–12, *not* 351–52 or 351–352, 26–27, 211–212 or 211–2; note the special use of teen numbers!); do not use serial comma (e.g. Tom, Dick and Harry);
- 5 titles of books, journals, newspapers, ships, spacecraft, paintings, plays, long poems, genus, species are to be set in italics;
- 6 the order for Prelims should be:

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On first recto at end of the book (if the working or half-working allows it) should take a list of all other books in the current series)

- 7 It is important that copy-editors ensure that there is a call-out in the text for each piece of art and each table and that all call-outs to References are checked; for example, check that (Smith 1998) is in fact in the Reference list.
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- 9 All **artwork** should, if possible, be provided electronically as .eps, .tif, .jpg files (black and white photographs [greyscale] should be high-res at no less than 300 dpi; please supply hard copy of these files; items downloaded from the Internet will not reproduce well, as they will originally have been scanned at 72 dpi). Artwork may also be provided as hard copy, ready for scanning at the typesetter's. **Line art** should be scanned at no less than 800 dpi (as 1-bit .tif files). Any tints in line art will not reproduce at all well on conventional scanners, but will on specialist copydot scanners (like those at the typesetter). Text labels on greyscale art will be bitmapped and should be removed, allowing the typesetter's software to label on top of the photo. **Colour scans** must be supplied as CMYK (avoid the use of .eps with .jpg compression). Min. scan resolution is calculated as 1.5 × screen ruling at output (if screen is 133 lpi [lines per inch] then scanning resolution at same size would be 200 dpi [min]). **Document** files can be in almost any format (Word, WordPerfect, etc. or even as ASCII or text files) but should not be in a specialist application software (e.g. Quark, 3B2, Ventura, etc.; if you have used such software, then simply provide a text file with no coding). Please note that artwork embedded in the document files may not reproduce well as a consequence of the need to convert it to .tif formats).

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[BL]	<i>Bullet list</i>
[NL]	<i>Numbered list</i>
[TT]	<i>Table title</i>
[TAB]	<i>Table body</i>
[FN]	<i>Footnotes</i>
[EQ]	<i>Equation</i>
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1

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Echinoderm cells and tissues

[AU]

C. Moss, C. D. Bavington and J. D. McKenzie

[A]

1.1 THE ECHINODERMS

[BODYF]

A wholly marine phylum, echinoderms are abundantly distributed throughout the oceans at all depths. There are approximately 6,000 extant species which fall into five major classes: Asteroidea (the sea stars), Echinoidea (the sea urchins and sand dollars), Ophiuroidea (the brittle stars and basket stars), Holothuroidea (sea cucumbers) and Crinoidea (the feather stars and sea lilies).

[BODY]

Echinoderms possess a number of unique features, definitive of their class and phylum. These include a triploblastic body plan organized around an oral-aboral axis with a calcareous endoskeleton consisting of plates or ossicles. They have a unique tubular system, which consists of a network of canals and appendages of the body wall. This system is divided into the water vascular, haemal and perihemal canals which are associated with tube-foot action/locomotion, coelomocyte production and vascular function respectively (Hyman, 1955). Another unique feature is the possession of a decentralized nervous system consisting of a nerve ring, radial nerves and a nerve net.

[A]

1.2 ECHINODERM CELLS AND TISSUES

[B]

1.2.1 Tissue layout and cell culture

[BODYF]

Electron microscopy has provided extensive details of the cell types found in echinoderms and the tissues they comprise (for a recent review see Harrison and Chia, 1994), building on a large body of earlier data (reviewed by Hyman, 1955). There is some variation in tissue make-up and cellular population between species and classes, although simple structures and cell classifications, such as the nature of coelomic epithelium, are similar throughout echinoderms with few obvious unique cell types (Holland, 1984; McKenzie, 1994).

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4 Echinoderm cells and tissues

[Ch. 1

Certain cell types found throughout echinoderm tissues are therefore likely to be common constituents of primary cultures. For example, all the commonly recognized epithelial forms (including columnar, squamous, stratified and pseudostratified) are found throughout echinoderms and epithelial cell types will be found in most, if not all, primary cultures of complex tissues. These cells are often joined apicolaterally by a zonula adhaerens leaving a comparatively 'loose' structure on the basal side of the epithelia (Holland, 1984). They form structures such as the epidermis and body wall, plus body-wall appendages. For example, dissociation of tube feet results in a typical mixed primary culture of sensory, support, secretory and muscle cells (Fig. 1.1A). The coelomic cavity and its extensions are also lined by a pseudostratified layer of epithelial cell types, which cover the interior of the body wall, the outer surfaces of the digestive and reproductive systems and the inner surfaces of the water vascular system. Well-defined features characterize specific cell types within these epithelia and such details have provided new insights into the range of cell types found in echinoderm tissues.

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Fig. 1.1. A, Cells from the tube feet of *Marthasterias glacialis* after 10 days *in vitro*. Cells showing different morphologies are present. Spindle-shaped cells are particularly common (arrow). B, An explant from *M. glacialis* tube foot. After 2 days *in vitro* there is a halo of cells around the explant. (Culture conditions as detailed in Fig. 11.4.) C, Cells with pseudopodia and coelomocyte-like appearance extend from an explant of *Ophiura ophiura* nerve cord. D, An explant of regenerating *Asterias rubens* nerve cord. Cells moving out of the explant may be coelomocytes which have moved into the regenerating area after nerve ablation (see Moss *et al.*, 1998b). Bars A, C and D have a width of 25 μm and B a width of 150 μm .

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In summary, echinoderms show a unique arrangement of closely interdigitated cells of various types, with few areas of homogenous tissue. These tissues consist of thin layers of cells associated with connective tissue and the calcite skeleton, and cell numbers are comparatively low in terms of overall body mass. These features may indicate the need for a novel approach to cell-culture conditions, especially in terms of the choice of starting tissues, use of mixed-cell populations and the need for specific physical factors and environments.

1.2.2 Reproductive and other proliferative tissues

[C] *Reproductive tissue*

Initiation of cell-culture techniques in vertebrates has often focused on a known tissue type that exhibits higher levels of proliferation *in vivo*. This has led to the use of reproductive tissues because of their predisposition to divide and differentiate. Such an approach has been adopted for *in vitro* studies both on invertebrates and vertebrates, and is especially important in the use of cells, such as mammalian neurons, which rarely show growth in adult tissues. In echinoderms reproductive tissue which could potentially be used for cell-culture studies includes the gonads, embryos and larvae, adult rudiment and juvenile:

- wound healing and regeneration;
- tissues involved in wound healing;
- normal adult echinoderm tissues;
- holothurian intestinal regeneration.

1.3 STUDIES ON ECHINODERM CELLS

Experiments involving the establishment of echinoderm cells and tissues *in vitro* stretch back to at least the early part of the twentieth century (for early review see Rannou, 1971). Much of this work focused on studies of dissociated embryos, as echinoderm models were increasingly used as tools by the developmental biologist (Okazaki, 1965). Early experiments on coelomocytes were also carried out *in vitro*, making it easier to examine their physiological roles, such as phagocytosis and humoral immunity. It was found that it was possible to maintain these cells in simple culture conditions long enough to observe aggregation and formation of syncytia (Bertheussen and Seljelid, 1978).

Development of invertebrate tissue-culture methodology is currently aimed at optimizing four main factors:

- (1) a suitable starting tissue and a means to dissociate it without cell damage;
- (2) the nature of the media in terms of available nutrients, pH and antimicrobial compounds;
- (3) the need for supplementation of the media with conditioning factors such as addition of FCS, native growth factors or haemal extracts;

[TT]

Table 1.1. Potential models for echinoderm cell-culture investigations.

[TAB]

Class	Genus/Species	Model tissue type
Astroidea	<i>Asterias</i>	Tube feet – mixed cultures
Echinoidea	<i>Strongylocentrotus</i>	Gastrules (mesenchyme cells), gonad, coelomic fluid
Holothuroidea	<i>Leptosynapta</i> , <i>Holothuria</i>	Longitudinal body-wall muscles
Ophiuroidea	<i>Ophiura</i>	Radial nerve cord – neurons
Crinoidea	<i>Antedon</i>	Regenerating arms
Astroidea	<i>Coscinasterias calamaria</i> <i>Stephanasterias albula</i>	Asexual reproduction by fissiparity, resulting in clones. All tissue types, especially regenerating section of body
Ophiuroidea	<i>Ophiactis savignyi</i> <i>Ophiocomella ophiactoides</i>	
Holothuroidea	<i>Holothuria parvula</i>	
Astroidea	<i>Linckia multiforma</i>	Autotomy resulting in clones

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(4) the nature of the substrate which the cells are in contact with, possibly promoting or inhibiting adherence, aggregation or outgrowth.

Techniques for the culture of echinoderm tissues have also been based on these factors, and on the conditions determined for other marine invertebrates and vertebrates (Tables 1.1–1.2) (Wong *et al.*, 1984; Machii and Wada, 1989; Raftos *et al.*, 1990). It is generally regarded that a balanced salt solution or sea water, with additional nutrients from a commercial media, are the essential starting point of most marine invertebrate cell cultures (Machii and Wada, 1989).* However, a host of conditions have now been tested on echinoderm cells and some of the more recent and routinely used examples are detailed in Table 1.2. From the results of these and current studies, basic protocols have been determined and these protocols have produced some cultures which have been suitable for their intended end use (Kaneko *et al.*, 1990, 1995, 1997; Odintsova *et al.*, 1994, 1999). Despite the success of these and other studies in maintaining primary cultures of echinoderm cells for several weeks, it is clear that some vital components are still missing, or protocols are inappropriate (Rinkevich, 1999). We still find ourselves little progressed from the original aims, stated in early studies, to establish monolayers or cell lines to use for assays and screening (Cecil and Nigrelli, 1972).

1.3.1 The development of methodology towards long-term echinoderm cultures

An initial step in improvements in echinoderm cell-culture methodology may be to identify model species and tissues, rather than the ad hoc approach that has existed to date. For some research purposes, long-term cultures with proliferating cells are

[FN]

*Supplementation of the media with effective conditioning factors to enhance cell activity is still limited by our lack of knowledge of native invertebrate growth factors, with substrates and many supplements being based on mammalian substances.

Table 1.2. Basic media constituents in echinoderm cell-culture studies.

Basic media constituents	References	Comments
Filtered sea water (FSW) with serum	Kaneko <i>et al.</i> (1990), (1995), (1997); Benson and Chuppa (1990); Dan-Sohkawa <i>et al.</i> (1993)	Cells successfully cultured for 5 to 15 days – other media not tested
FSW plus serum with 2 mM flutamine, 0.1% glucose, 15 mM HEPES	Poccia (1988)	Media sustained cells for 5 weeks. Tests without serum, glutamine and glucose unsuccessful
FSW, 10% Eagles medium, 10 mM HEPES, adenosine and sodium pyruvate	Bertheussen and Seljelid (1978)	Cells survived for longer periods in this medium, plus additives, than in sea water with HEPES
Eagles minimum essential medium 853 mg l ⁻¹ L-glutamine, 15 mg l ⁻¹ phenol red	Brillouet <i>et al.</i> (1981)	Short-term (up to 72 h) suspension cultures of axial organ cells
Osmotically balanced L-15 plus 50 mg l ⁻¹ glucosamine, 100 mg l ⁻¹ glutamine, 25 mg l ⁻¹ taurine	Odintsova <i>et al.</i> (1994), (1999)	Embryonic cells survive for over 10 days
25% L-15, 75% FSW, 10 mM HEPES, 2 g l ⁻¹ glucose, 300 mg l ⁻¹ glutamine	Moss <i>et al.</i> (1998b)	Cells and explants survive for 4 weeks
RPMI 500 mg l ⁻¹ FSW	Moss <i>et al.</i> (1998b)	Cells and explants survived for 4 days

not essential. However, a focus on specific tissues is more likely to result in the standardization of conditions, and improvements in techniques leading to cell lines. That is, the use of easily obtained possibly large species, with the tissue type in question easily dissected and where the numbers of a specific cell type are high. Table 11.1 indicates some suggested species and tissues that could be developed as model systems.

Supplementation of media

A major obstacle in all marine invertebrate cell culture has been the lack of availability of suitable factors to stimulate and maintain cell proliferation. This is one of the reasons for the failure to produce a primary cell line, and in most cases even to achieve cell proliferation. Many studies have used mammalian sera, growth factors and hormones (see Table 11.3) with variable results. Indeed, FCS or similar has been

routinely added to echinoderm cultures and is still present in most currently used media, despite known problems with its use in some long-term culture systems (Goodwin, 1991; Ferkovich and Oberlander, 1991):

$$[\text{EQ}] \quad \sum_{n=1}^{\infty} \frac{\Delta V_s}{V_{e,if}} = - \int_{M_f+M_0}^{M_0} \frac{dM_s}{M_s} e^{-1} \quad (1.1)$$

The addition of serum fulfils many functions in mammalian cell culture including the provision of nutrients, hormones and growth factors, attachment and spreading factors, and protease inhibitors. There may be problems in marine invertebrate culture, which arise from the lack of one or more of these factors.

[DIS] Certain echinoderms are recognized pest species. For example, several species have appeared out of their normal range, often as a result of carriage of larvae in the ballast water of ships. A particularly well-known example is that of the Japanese starfish *Asterias amurensis* which has become extremely common in Tasmania, to the detriment of local species (Byrne *et al.*, 1997). Some asteroids are also problematic for shellfish growers, eating a large percentage of the crop every year (Clark *et al.*, 1997). It would be extremely useful to develop highly specific biological controls to remove unwanted species. Cell-culture technology using cultures of pest species may be one way to create the stringent conditions necessary to investigate and test potential control methods.

1.6 REFERENCES

- [REF] Battaglione, S. C., Seymour, J. E. and Ramofafia, C. (1999) Survival and growth of cultured juvenile sea cucumbers, *Holothuria scabra*. *Aquaculture* **178**, 293–322.
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- Wong, R. G. (1984) Nerve-growth-promoting factor produced in culture media conditions by specific CNS tissues of the snail *Heliosoma*. MSc thesis, University of Tokyo, Japan.

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