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Culturing murine embryonic organs: Pros, cons, tips and tricks

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ABSTRACT

There are three established techniques described for *ex vivo* culture of the early embryonic organs: filter culture, agar block culture and hanging drop culture. Each of these protocols has advantages and disadvantages; here we assess the merits of each approach. Agar block culture has a long history and has been well described. This method results in good embryonic organ morphology. Filter culture has been used to culture a number of different embryonic organs and there are a variety of filter choices available. The key disadvantage of agar-block and filter based culture is that the large amount of media required can make the approach expensive, especially if biologicals such as growth factors are necessary; in addition, using these methods it can be difficult to track particular samples. Hanging drop culture is most commonly used to enable the aggregation of embryonic stem cells into embryoid bodies but it has also been employed for *ex vivo* organ culture. This method requires only 40 μL of media per drop and isolates every organ to a trackable unit. We describe each of these methods and the use of different medias and provide the user with a matrix to help determine the optimal culture method for their needs. Glass-based culture methods required for live imaging are not discussed here.

Contents

Abbreviations: PBS, Phosphate-buffered saline; KO, knock-out; NAC, N-acetyl-L-cysteine; MEM, minimum essential media

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1. Introduction: pros and cons

Ex vivo culture of embryonic organs has been a cornerstone of many important advances in the field of embryology. Studying organogenesis *ex vivo* allows the researcher to investigate basic cell and biological processes in the growing organ in an easily manipulated system. The key advantage of organ culture over primary cell culture is that it preserves tissue architecture and maintains cell-cell contacts and signaling relationships. The addition of exogenous factors such as inhibitors, growth factors and morpholinos to media for *ex vivo* culture has provided many insights into mechanisms of organogenesis (Bowles et al., 2010; 2006; Brennan et al., 2003; Colvin et al., 2001; Cool et al., 2011; Dean et al., 2005; Hartwig et al., 2010; Martineau et al., 1997; McClelland et al., 2015; Mork et al., 2011; Quaggin et al., 1998). In addition, culturing organs from complex genetic crosses and knockouts provides a opportunity to study organogenesis even when genotypes are embryonic lethal (Chaboissier et al., 2004) and to investigate the impact of gene ablation in the context of additional exogenous factors (Taya et al., 1999). Here, we highlight the advantages and limitations of three major approaches to organ culture: filter culture, agar block culture and hanging drop culture (Table 1). These different methodologies serve as a platform for the investigation of organogenesis and signaling pathways in genetic mouse models or in response to exogenous factors, which can be directly added to the culture medium. *Ex vivo* organ culture for the purpose of imaging developmental changes in real time is an important tool for researchers to begin to understand tissue morphogenesis and the signaling pathways that regulate organogenesis. Imaging organs during culture constitutes a complex organ-specific experiment and will not be discussed here.

In many organ systems, there are established filter culture techniques or agar mold-based systems. The key disadvantage of these methods is that they typically require a minimum of 200–600 μL of media. When doing high throughput experiments, and adding expensive growth factors, such a large volume of culture media quickly makes culture impractical or prohibitively expensive. In addition, in such large volume systems any factors secreted by the organ will become highly diluted in the media and will, presumably, be unable to impact on further organ development. These limitations have been partially overcome by co-culturing protein coated beads next to organs such as limb buds; however this approach is not always practical/possible (Berge et al., 2008). Key advantages of the hanging drop culture system include the isolation of each embryo or organ into an easily trackable unit, the small (typically 40 μL) culture volume required, allowing use of smaller quantities of added factors, and the creation of a microenvironment encapsulating a single tissue and any factors it produces during culture (see Table 1).

1.1. Culturing tissues by filter culture methods

Filter culture systems are the most common method used to culture embryonic organs. Most organs can be successfully grown on a Transwell or floating filter for several days (Table 1). Filter culture is particularly good for culturing organs, such as the pancreas, which expand in size outwards and need a substrate to grow out over the course of up to 8 days (Gittes et al., 1996). However, using filter culture for complex crosses, where genotypes will only be determined post-dissection, necessitates the use of many, often expensive, filters. While this is not inevitably problematic when the 200–600 uL of media needed per well is standard media, if exogenous factors are to be added to the media this system can quickly become prohibitively expensive.

In our experience, there is no great advantage to culturing organs such as the pancreas on more expensive Transwell culture systems. These organs grow well on floating filters (such as 5 uM polycarbonate filters); these have advantages in that they are less expensive and the filter does not need to be cut out of the Transwell cassette making downstream processing of the organ much easier (Table 3). The key experimental problem with floating filters is that they occasionally sink, 'drowning' the organs. This

Table 1
Advantages and disadvantages of culture techniques. This table outlines the pros and cons of different culture approaches with relevant sources to help the experimentalist make decisions about the best culture method to suit their needs.

Culture Type		Advantages	Disadvantages	Sources
Filter culture	Transwell	• Most organs culture successfully	• Expensive • Large media volume	Chaboissier et al. (2004), Lee et al. (1999)
	Floating	• Multiple organs per filter • Most organs culture successfully • Easier to process than Transwell • Less expensive than Transwell	• Large media volume	Bowles et al. (2010), Carraro et al. (2010), McClelland et al. (2015)
Agar block culture		• Allows construction of co-cultures so as to assess interactions between heterologous tissues (*e.g.* signaling and migration studies) • Provides physical boundaries to support organ during development	• Customized mold design and production is required (one time investment) • Large media volume • Fiddly to set up • Prone to contamination	Brennan et al. (2003, 2002), Capel and Batchvarov (2008), Martineau et al. (1997), Tilmann and Capel (1999)
Hanging Drop culture		• Small media volume • Efficient for culture of tissues from individual embryos prior to availability of genotype information • Limits loss of any factors produced by the tissue	• Some organs culture poorly • Dehydrates if not well-humidified	McClelland et al. (2015), Ryan et al. (2011), Szczepny et al. (2009)
Glass-based culture		• Useful for live imaging • Low volume culture is advantageous for kidneys	• Single organ culture • Leakage problems • Dehydrates if not humidified	Petzold and Spagnoli (2012), Sebinger et al. (2010)