Improved embryoid bodies for studying development, embryotoxicity, and placental function *in vitro*

Marlon Schneider
Developmental biology – pre-implantational stages


Late blastocyst
>100 cells
E4.5
Developmental biology – post-implantational development

Harrison et al., Science (2017)
Background

Basic and applied research

- Identification of molecular mechanisms and principles of self-organization during embryonic development
- ~20-50% early pregnancy failure (mostly embryo-uterus communication defects) → time of implantation is a developmental “black box”
- Largely based on animal studies (rodents, rabbits)
Background

Embryotoxicity

Prenatal development toxicity study (OECD TG 414)

• test substance is administered to pregnant animals (time span: from implantation to as close as possible to the normal day of delivery)
• females are killed before delivery and foetuses are evaluated for soft tissue and skeletal changes

1. Whole embryo culture (WEC) assay
• embryos at different stages are dissected from maternal tissue
• less expensive and more rapid compared to *in vivo* testing
• does not involve experimentation on adult animals

2. Limb bud micromass (MM) assay
• *ex vivo* culture of limb bud cells of mid-organogenesis embryos
• single cell suspension → differentiation into chondrocytes and neurons
• observation of alcian blue staining (cartilage)
• does not involve experimentation on adult animals

3. Embryonic stem cell test (EST)
• employs embryonic stem cells and 3T3 cells
• composed of two procedures: cytotoxicity (3T3 and D3) and differentiation (D3) assays
• less expensive, no animals needed
• biological relevance?

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Drawbacks of embryoid bodies as an experimental tool

Unphysiological gradients of cell proliferation, viability and metabolism

Lack of inductive signals

Hirschhäuser et al., J Biotechnol (2010)
**Approach - Self-assembly of all three cell types**

- **Cell number**
  - 650 cells/embryoid
  - 36 cells/embryoid

- **Cell ratio**
  - 50% ES, 50% TS
  - 30% ES, 70% TS
  - 8% ES, 8% XEN, 83% TS

- **Coating vs. non-coating**

**Cell culture format**

- 96-Well
- Matrigel
- Hanging Drop
- Aggrewell™

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Results – Successful self-assembly

Non-Coating  Coating

- Non-Coating: 64.2%
- Coating: 67.4%

Successful formation of embryoids

Approach: Coating, 50/50 Medium, 3 ES/ 3 XEN /30 TS

Gata6  Oct4  Tfap2c

Synthetic embryos are a hot topic

**ETS (ES + TS) embryos**
- Combination of ES and TS cells (50%/50%)
- Similar to mouse embryos at 5-6 days after fertilization
- Formation rate: 22%

**Blastoids**
- Combination of ES and TS cells (29%/71%)
- Delayed addition of TS cells to the ES cell aggregate
- Similar to mouse embryos at 3.5 days after fertilization
- Formation rate: 70%

**ETX (ES + TS + XEN) embryos**
- Combination of ES, TS and XEN cells (23%, 60%, 17%)
- Similar to mouse embryos at 5.5 days after fertilization
- Formation rate: 70% → 29.8% specific morphology
Potential applications

Basic research
- Testing of chemicals for health effects
- Lineage specification and embryo patterning
- Epigenetic influences on developmental processes
- Embryo-maternal communication and implantation

Outlook


Modi et al., Front Biosci (2012)
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Thank you for your attention

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References


