Einführung in die Stammzell- und Embryonenforschung II (ESF-II/9) WS2022/23

Zur Herstellen von Lebewesen aus einer Stammzelle

Biologische Grundlagen – Stand der Forschung – Gesellschaftliche Auswirkungen

3. Doppelstunde

Teil 2 Herstellung von Lebewesen - Stand der Forschung (3. bis 6. Doppelstunde)

2.1. Ex vivo Embryonen aus einer pluripotenten Stammzellen

- 2.1.1. Blastoide - Herstellung von Blastozysten aus Stammzellen
- 2.1.2. Gastruloide – Gastrulation in Stammzellaggregaten
- 2.1.3. Embryoids - Synthetische Embryonen

2.2. Ex vivo Keimzellen aus pluripotenten Stammzellen

- 2.2.1. Der weibliche und männliche Reproduktionszyklus ex vivo
- 2.2.2. Herstellung von Zygoten - In vitro Fertilisation und Klonen
- 2.2.3. Herstellung von künstlichen Plazenten aus Stammzellen
- 2.2.4. Herstellung von Mäusen aus Stammzellen in Leihmüttern

Teil 2 Herstellung von Lebewesen - Stand der Forschung (3. bis 6. Doppelstunde)

2.1. Ex vivo Embryonen aus einer pluripotenten Stammzellen

2.1.1. Blastoide - Herstellung von Blastozysten aus Stammzellen

2.1.2. Gastruloide – Gastrulation in Stammzellaggregaten

2.1.3. Embryoids - Synthetische Embryonen

Für dritte DSt

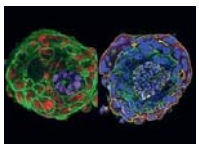
Teil 2 Herstellung von Lebewesen - Stand der Forschung (3. bis 6. Doppelstunde)

2.1. Ex vivo Embryonen aus einer pluripotenten Stammzellen

2.1.1. Blastoide - Herstellung von Blastozysten aus Stammzellen

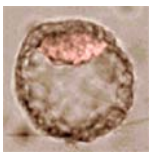
ESCs / iPSCs →

Spontan nicht möglich,
Weil TE Festlegung bereits
im Blastomeren Stadium
stattfindet.



Blastoids

? ↔



Blastocyst, day 3.5

Maus: Clemens A. van Blitterswijk & Niels Geijsen, 2018
Mensch: Nicolas Rivron, 2022

2.1.1. Blastoide - Herstellung von Mäuse Blastozysten aus Stammzellen

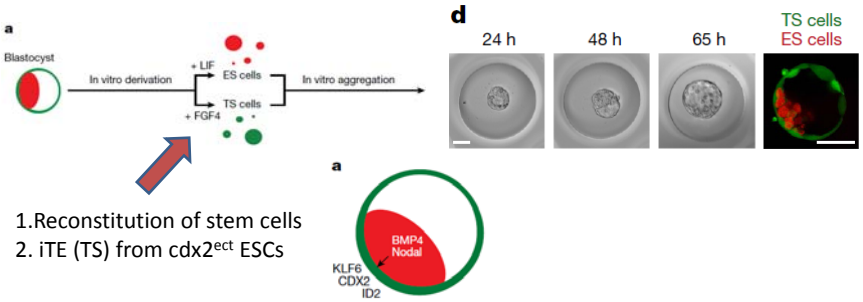
2018

LETTER

https://doi.org/10.1038/s41586-018-0051-0

Blastocyst-like structures generated solely from stem cells

Nicolas C. Rivron[1,2]*, Javier Frias-Aldeguer[1,2], Erik J. Vrij[1], Jean-Charles Boisset[2], Jeroen Korving[2], Judith Vivié[2,3], Roman K. Truckenmüller[1], Alexander van Oudenaarden[2], Clemens A. van Blitterswijk[1,5] & Niels Geijsen[2,4,5]



1.Reconstitution of stem cells
2. iTE (TS) from cdx2^{ect} ESCs

2.1.1. Blastoide - Herstellung von Blastozysten aus Stammzellen

2018

LETTER

Blastocyst-like structures generated solely from stem cells

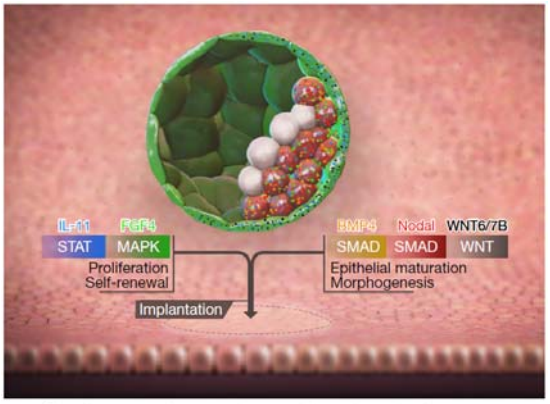


Fig. 6 | Embryonic inductions drive trophectoderm development and implantation. Left, trophectoderm proliferation and self-renewal.

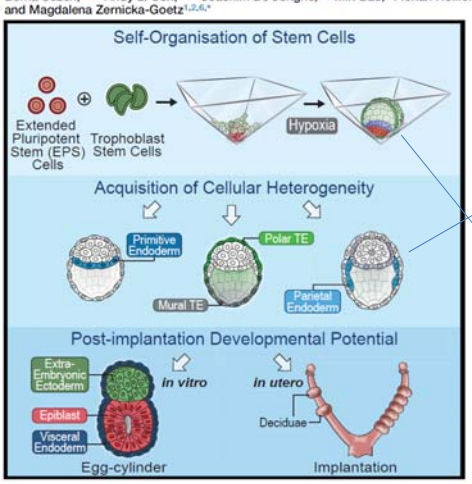
2.1.1. Blastoide - Herstellung von Blastozysten aus Stammzellen

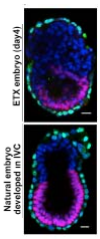
2019

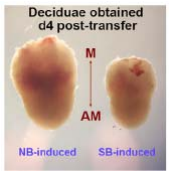
Self-Organization of Mouse Stem Cells into an Extended Potential Blastoid

Developmental Cell
Article

Bema Sozen,[1,2,5] Andy L. Cox,[1,2,5] Joachim De Jonghe,[3,5] Min Bao,[1] Florian Hollfelder,[3] David M. Glover,[2,4] and Magdalena Zernicka-Goetz[1,2,6,*]







Blastozyst Blastoid

https://doi.org/10.1016/j.devcel.2019.11.014

2.1.1. Blastoide - Herstellung von humanen Blastozysten aus Stammzellen

2022

Human blastoids 3.0

Cell Stem Cell
Previews

Viviane S. Rosa[1] and Marta N. Shahbazi[1,*]

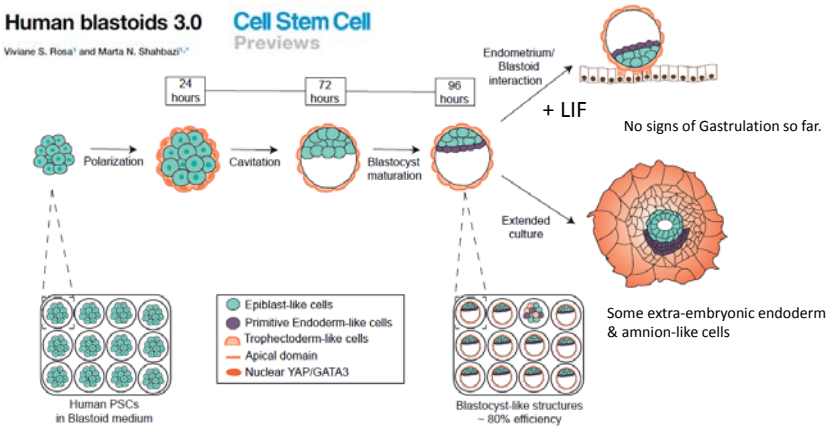
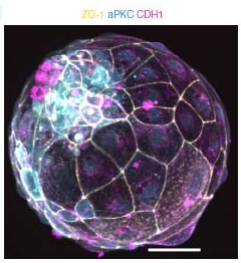


Figure 1. Schematic summary of the sequential events that drive human blastoid formation

Human PSCs are aggregated in non-adherent microwells and, within 24 h, outer cells acquire an apical domain leading to YAP activation and initiation of trophectoderm-like cell specification. Cavity formation denotes the formation of an early blastocyst-like structure. Upon subsequent maturation, inner cells segregate to form epiblast- and primitive endoderm-like cells. Blastoids composed of three main tissues (epiblast-, primitive endoderm-, and trophectoderm-like cells) interact with human endometrial cells and can therefore be used to model peri-implantation stages. https://doi.org/10.1016/j.stem.2021.12.006



ZO-1 Zona occludens 1

aPCK apical Phosphokinase C

CDH1 Cadherin 1

https://doi.org/10.1038/s41586-021-04267-8

Menschen Blastoid von Nicolas Rivrons Arbeitsgruppe

Implantation ?

Zu beachten ist, dass alle bisher vorgestellten Daten und auch die weiteren immer nur mittels einer Methode erbracht wurden. Grundlage waren entweder morphologische Aussagen mittels Immunfluoreszenzmikroskopie und Reprotegenexpression (zB. GFP), oder Analysen der „molecular signatures“ von single cell sequencing (zB. U-maps von principal component analysis of n-dimensionalen Datensätzen, oder ganz vereinzelt durch wirkliche funktionelle Beweise, wie „live cell imaging“ in Embryonen (zB. Zebrafisch, siehe unten stehendes Zitat (link).

Wünschenswert und aussagekräftiger wäre eine derzeit aber nicht mögliche Kombination von zwei oder drei dieser erwähnten Methoden.

Pang et al. *Cell Discovery* (2020)6:74
https://doi.org/10.1038/s41421-020-00204-7

Cell Discovery
www.nature.com/celldisc

ARTICLE Open Access

Light-sheet fluorescence imaging charts the gastrula origin of vascular endothelial cells in early zebrafish embryos

Meijun Pang[1], Linlu Bai[1,2], Weijian Zong[1], Xu Wang[1], Ye Bu[1], Connie Xiong[1], Jiyuan Zheng[1], Jieyi Li[1], Weizheng Gao[3], Zhiheng Feng[3], Liangyi Chen[1], Jue Zhang[3], Heping Cheng[1], Xiaojun Zhu[1] and Jing-Wei Xiong[1,2]

Abstract
It remains challenging to construct a complete cell lineage map of the origin of vascular endothelial cells in any vertebrate embryo. Here, we report the application of in toto light-sheet fluorescence imaging of embryos to trace the origin of vascular endothelial cells (ECs) at single-cell resolution in zebrafish. We first adapted a previously reported method to embryo mounting and light-sheet imaging, created an alignment, fusion, and extraction all-in-one software (AFEIO) for processing big data, and performed quantitative analysis of cell lineage relationships using commercially available Imaris software. Our data revealed that vascular ECs originated from broad regions of the gastrula along the dorsal–ventral and anterior–posterior axes, of which the dorsal–anterior cells contributed to cerebral ECs, the dorsal–lateral cells to anterior trunk ECs, and the ventral–lateral cells to posterior trunk and tail ECs. Therefore, this work, to our knowledge, charts the first comprehensive map of the gastrula origin of vascular ECs in zebrafish, and has potential applications for studying the origin of any embryonic organs in zebrafish and other model organisms.

https://doi.org/10.1038/s41421-020-00204-7

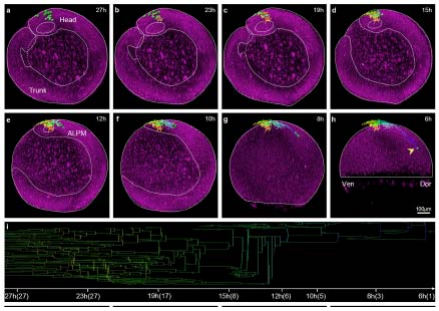


Fig. 3 Retrospective cell-lineage tracking reveals the distinct gastrula origins of vascular ECs in the trunk and head.

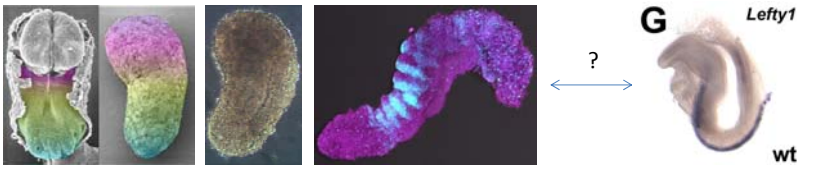
https://doi.org/10.1038/s41421-020-00204-7

Für dritte DSt

Teil 2 Herstellung von Lebewesen - Stand der Forschung (3. bis 6. Doppelstunde)

2.1. Ex vivo Embryonen aus einer pluripotenten Stammzellen

2.1.2. Gastruloide – Gastrulation in Stammzellaggregaten



Embryo

doi: https://doi.org/10.1371/journal.pone.0002511.g002

2.1.2. Gastruloide – Gastrulation in Stammzellaggregaten

2005

Epithelial–Mesenchymal Transition in Colonies of Rhesus Monkey Embryonic Stem Cells: A Model for Processes Involved in Gastrulation

STEM CELLS Original Article

Rüdiger Behr, Carola Heneweer, Christoph Viebahn, Hans-Werner Denker, Michael Thie

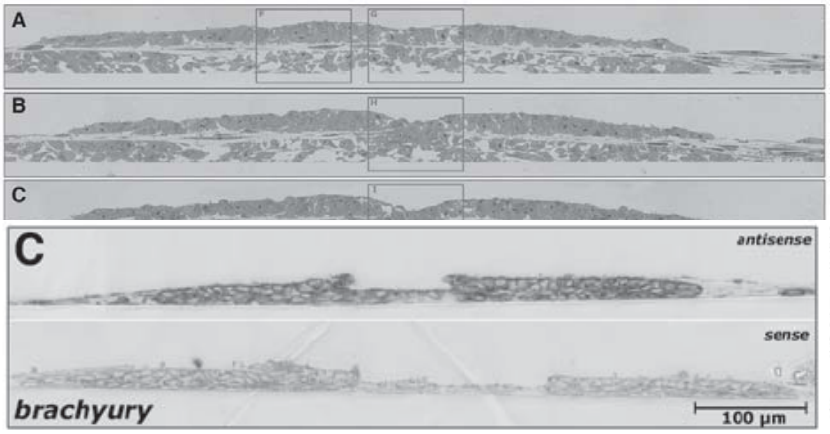


Figure 1. A typical differentiated colony of rhesus monkey embryonic stem cells after 5 days of culture in a series of cross-sections

2.1.2. Gastruloide – Gastrulation in Stammzellaggregaten

2011

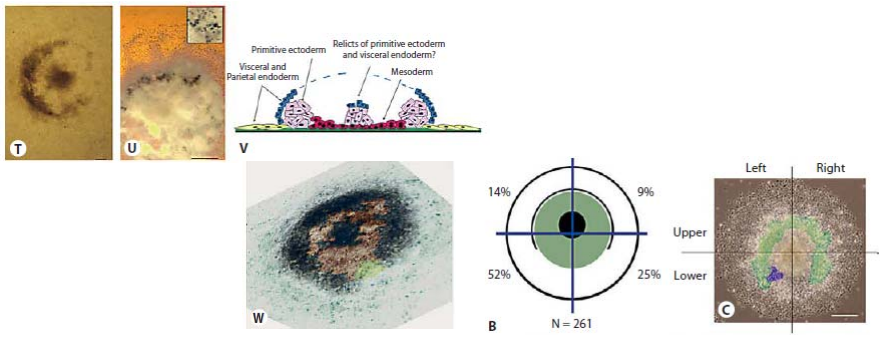
Self-Organization Phenomena in Embryonic Stem Cell-Derived Embryoid Bodies: Axis Formation and Breaking of Symmetry during Cardiomyogenesis

Cells Tissues Organs

DOI: 10.1159/000328712

Christiane Fuchs[a] Matthias Scheinast[a] Waltraud Pasteiner[a] Sabine Lagger[b]
Manuela Hofner[a] Alexandra Hoellrigl[a] Martina Schultheis[a] Georg Weitzer[a]

[a]Max F. Perutz Laboratories, Department of Medical Biochemistry, Division of Molecular Biology and [b]Division of Molecular Genetics, Medical University of Vienna, Vienna, Austria

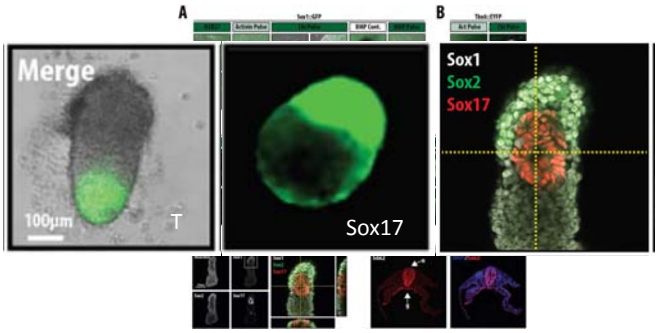


Development

Symmetry breaking, germ layer specification and axial organisation in aggregates of mouse embryonic stem cells 2014

Susanne C. van den Brink,Peter Baillie-Johnson,Tina Balayo,Anna-Katerina Hadjantonakis,Sonja Nowotschin,David A. Turner, Alfonso Martinez Arias
Development . 2014 Nov;141(22):4231-42. die: 10.1242/dev.113001.

Fig. 7.



Symmetry breaking, germ layer specification and axial organisation in aggregates of mouse embryonic stem cells

Development

Symmetry breaking, germ layer specification and axial organisation in aggregates of mouse embryonic stem cells

Susanne C. van den Brink,Peter Baillie-Johnson,Tina Balayo,Anna-Katerina Hadjantonakis,Sonja Nowotschin,David A. Turner, Alfonso Martinez Arias

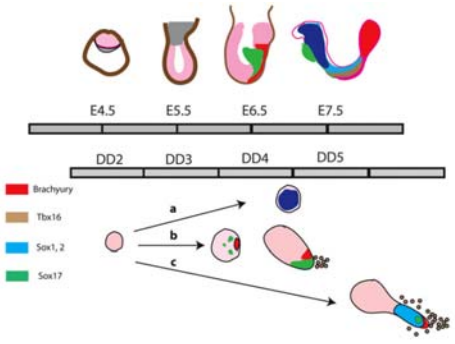


Fig. 9. Comparison of events in embryos and aggregates. (Top) Timeline of embryogenesis, with the illustrated stages acting as landmarks. (Bottom) A representation of the behaviour of aggregates exposed to different signalling environments over the indicated periods of differentiation, as inferred from our experiments labelled here as a, b and c. We propose that the third day of differentiation of the aggregates is equivalent to the E5.5-6.0 postimplantation epiblast. DD, day of aggregate differentiation. The dark blue shading indicates anterior Sox1 expression.

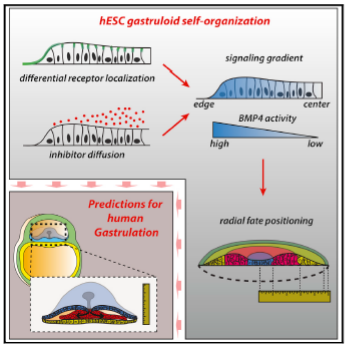
2.1.2. Gastruloide – Gastrulation in Stammzellaggregaten

2016

Developmental Cell

A Balance between Secreted Inhibitors and Edge Sensing Controls Gastruloid Self-Organization

Graphical Abstract



Authors

Fred Etoc, Jakob Metzger, Albert Ruzo, ..., M. Zeeshan Ozair, Ali H. Brivanlou, Eric D. Siggia

Correspondence

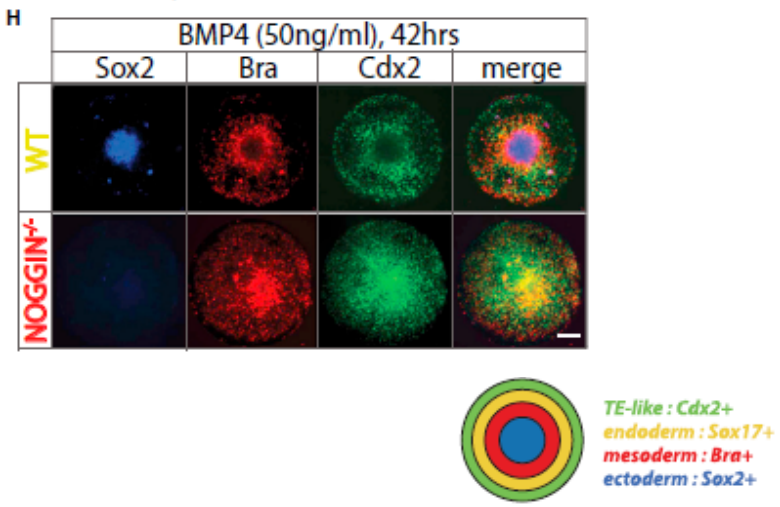
brvnlou@rockefeller.edu (A.H.B.), siggiae@rockefeller.edu (E.D.S.)

In Brief

In the embryo, cell fates are specified by a combination of chemical and physical factors. Using an in vitro model for human gastrulation, Etoc et al. show that a complex developmental transition can be reduced to two independent inhibitory mechanisms linked to differential cell polarization and the diffusion of NOGGIN.

2.1.2. Gastruloide – Gastrulation in Stammzellaggregaten

2016



TE-like : Cdx2+
endoderm : Sox17+
mesoderm : Bra+
ectoderm : Sox2+

2.1.2. Gastruloide – Gastrulation in Stammzellaggregaten

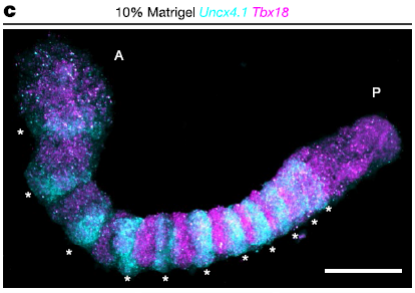
2020

Article

Single-cell and spatial transcriptomics reveal somitogenesis in gastruloids

Susanne C. van den Brink[1,6,12], Anna Alemany[1,6], Vincent van Batenburg[1,6], Naomi Moris[2], Marloes Blotenburg[1], Judith Vivié[1], Peter Baillie-Johnson[2], Jennifer Nichols[3,4], Katharina F. Sonnen[5], Alfonso Martinez Arias[2] & Alexander van Oudenaarden[1,6]

Nature | Vol 582 | 18 June 2020 | **405**



https://doi.org/10.1038/s41586-020-2024-3

2.1.2. Gastruloide – Gastrulation in Stammzellaggregaten

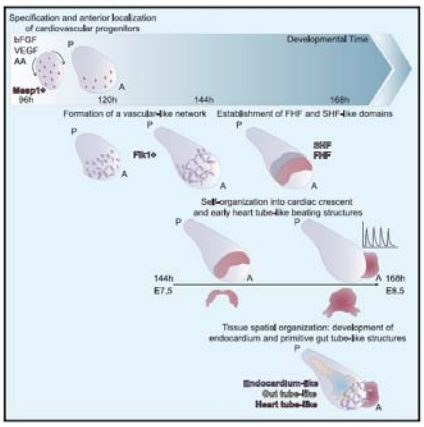
Cell Stem Cell

Article

2020

Capturing Cardiogenesis in Gastruloids

Graphical Abstract



Authors

Giuliana Rossi, Nicolas Broguiere, Matthew Miyamoto, ..., Robert G. Kelly, Chulan Kwon, Matthias P. Lutolf

Correspondence

matthias.lutolf@epfl.ch

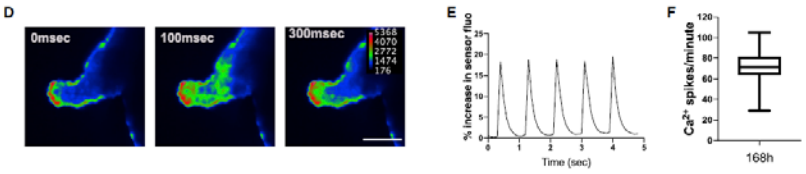
In Brief

Rossi et al. describe an embryonic organoid model that mimics the early development of the heart, from the generation of cardiovascular precursor cells to the specification of the first and second heart fields. These axially patterned organoids support the formation of cardiac crescent and early cardiac tube-like structures while reproducing the cell diversity and tissue-tissue interactions typical of embryos.

2.1.2. Gastruloide – Gastrulation in Stammzellaggregaten

Cell Stem Cell Article 2020

Capturing Cardiogenesis in Gastruloids



Herzmuskelzellen kontrahieren auch!

Zusammenfassung

2.1.1. - 2. Blastoide und Gastruloide

Blastoide können aus ESCs / iPSCs nicht spontan entstehen, weil Trophektoderm-Schicksal bereits in frühen Blastomeren festgelegt wird. Herstellung von iTSCs aus ESCs ist notwendig.

Gastruloide entstehen spontan aus Embryoid Bodies in Suspensionskultur, bis jetzt wurden immer aber nur kleine Teile des gastrulierenden Embryos nachgebildet.