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Synthetic human embryology: towards a quantitative future

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Study of early human embryo development is essential for advancing reproductive and regenerative medicine. Traditional human embryological studies rely on embryonic tissue specimens, which are difficult to acquire due to technical challenges and ethical restrictions. The availability of human stem cells with developmental potentials comparable to pre-implantation and peri-implantation human embryonic and extraembryonic cells, together with properly engineered *in vitro* culture environments, allow for the first time researchers to generate self-organized multicellular structures *in vitro* that mimic the structural and molecular features of their *in vivo* counterparts. The development of these stem cell-based, synthetic human embryo models offers a paradigm-shifting experimental system for quantitative measurements and perturbations of multicellular development, critical for advancing human embryology and reproductive and regenerative medicine without using intact human embryos.

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Introduction

For centuries, the development of the human embryo has attracted great interest, especially on how it could give rise to the shape, size, function, and intelligence that make humans unique in the *Mammalia* class. Human embryo development also elicits significant clinical interest, as it is not only linked to reproductive health, but is also key to tissue engineering and regenerative medicine. Despite its scientific and clinical significance, human embryo development remains largely mysterious. Most of our knowledge about human development comes from limited histological specimens and medical images, preventing detailed understanding of human development.

Although recent success in prolonging *in vitro* culture of human embryo has generated new insights on human development [1–3], it is limited to a narrow time window due to ethical restrictions, namely, the 14-day rule [4], prohibiting studies of post-implantation developmental stages such as gastrulation, neurulation, and beyond. While *in vitro* culture of mouse and non-human primate embryos are not subject to this ethical restriction and thereby have enabled studies of peri-gastrulation development [5–9], clear evidence of interspecies divergence between humans and mice [10,11] or non-human primates [3,12] calls for caution when extrapolating knowledge from other mammals to humans. Furthermore, the limited availability of mammalian embryos, especially from non-human primates, prevents large-scale genetic studies or screens.

Recently, several groups, ours included, have successfully reconstructed human embryogenic events *in vitro* using human pluripotent stem cells (hPSC), an unlimited cell source with developmental potential matching that of the pluripotent epiblast in the peri-implantation human embryo. By creating properly engineered culture environments, hPSC have now been applied to create multicellular structures to model the human amniotic sac formation, gastrulation, and neurulation. These stem cell-based human embryo models form the basis of the emerging field of synthetic human embryology, wherein different aspects of human embryo development are recreated and studied *in vitro* in a controllable and quantitative manner. Besides as an integrative area combining stem cell and developmental biology and human embryology, the advances in synthetic human embryology now have a new edge. By merging with designer bioengineering technologies and computational modeling, it is now possible to quantitatively control dynamic *in vitro* developmental niche and recapitulate intricate developmental patterning *in silico*. In this review, we summarize the progress of this emerging field and its rapid advancement towards a quantitative future.

Models of human amniotic sac development

Upon implantation, the first developmental milestone of the human embryo is the formation of the asymmetric, lumenal amniotic sac from the epiblast of the human blastocyst. The amniotic sac is enveloped by the squamous amniotic ectoderm at the dorsal pole and the columnar epiblast at the ventral pole, respectively. The development of the amniotic sac is initiated by an apical lumen formation in the initial ball of pluripotent epiblast, followed by patterning of the amniotic ectoderm at its dorsal pole [13] (Figure 1). Being an embryogenic event too early to detect, the development of the amniotic sac in humans has been inaccessible for study until very recently. Two recent studies report that hPSC have an intrinsic lumenogenic property consistent with that of the peri-implantation epiblast *in vivo* [2,14]. A hierarchical lumenogenic program in hPSC was further revealed, mediated by actomyosin tension and driven by fusion of multiple pre-formed extracellular lumens or intracellular apicosomes [15]. Importantly, such a 'vesicle-fusion' mechanism of lumenogenesis was recently shown in both mouse and human embryos [16,17]. Lumenal, pluripotent epithelial sacs of different shapes have also been generated by culturing hPSC onto 2D culture surfaces containing microscale grooves or trenches [14].

To recapitulate amniotic ectoderm development, two culture systems were developed recently with tunable mechanical stiffness of the culture environment [18•,19•]. Using both systems, a threshold of mechanical stiffness of the culture environment was shown to control symmetry breaking of spherical lumenal hPSC tissues, resulting in spontaneous differentiation of hPSC into squamous amniotic cells and the formation of an asymmetric amniotic sac-like structure. Such a mechanical cell fate switch for amniotic differentiation of hPSC provides a potential explanation for the absence of extraembryonic BMP sources adjacent to the prospective amniotic ectoderm *in vivo* [13]. Amniogenic differentiation of hPSC requires endogenous BMP-SMAD signaling [18•]. By adjusting the initial cell number within each hPSC colony, synthetic human amniotic sac model was created that not only resemble post-implantation amniotic sac development, but sequentially exhibit gastrulation-like features [19•]. To date, this synthetic amniotic sac model remains the only system that can recapitulate spontaneous symmetry breaking and differentiation and expansion of the amniotic ectoderm as seen in the peri-implantation human embryo.

Recently, a microfluidic device has been reported to achieve controllable and programmable patterning of the synthetic amniotic sac model by exogenous inductions and inhibitions [20••]. Excitingly, this microfluidic amniotic sac model has successfully recapitulated the progressive development of all cell lineages in the amniotic sac of the peri-implantation human embryo, including the specification of primordial germ cells. The microfluidic amniotic sac model is compatible with live imaging, thus allowing tracking developmental signaling dynamics. Given its controllability and scalability, the microfluidic amniotic sac model could assist in high-throughput drug and toxicity screens. Leveraging bioengineering tools for scalable tissue patterning, another amniotic microtissue array system was recently reported [21], providing another promising platform for high-throughput toxicity screens.

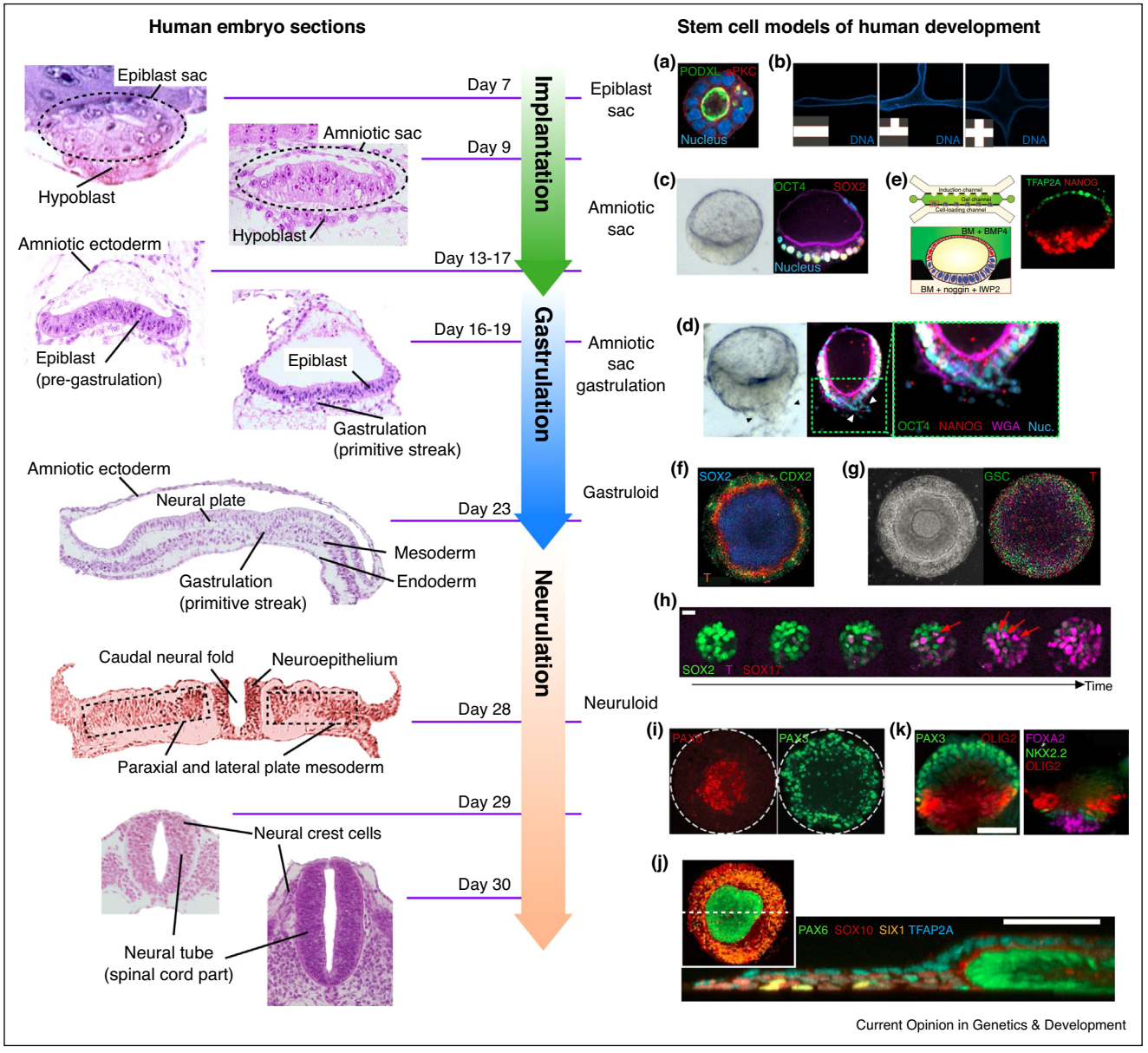
Models of human gastrulation

After the amniotic sac formation, gastrulation is the next developmental milestone. Gastrulation is initiated by the formation of the primitive streak, culminating in the delineation of the three germ layers. The first human gastrulation model, termed 'gastruloid', was developed by culturing hPSC on 2D adhesive micropatterns of various sizes. Treatments of 2D hPSC colonies with BMP4 resulted in the emergence of concentric regions expressing SOX2, T, SOX17, and CDX2, from colony center to edge, mimicking the development of the three germ layers as well as the trophectoderm [22••,23]. However, the gastruloid bypasses a few essential steps of gastrulation, namely, the formation of the primitive streak and its organizer. By replacing exogenous BMP4 with WNT3A, a morphologically distinguishable primitive streak-like structure develops next to SOX2+ cells in the gastruloid [24••]. Modulation of ACTIVIN-NODAL signaling in this streak model showed biased induction of posterior versus anterior primitive streak-like structures, as reflected by differential SNAIL expression. Importantly, anterior primitive streak-like structure induced in the model is GSC+, an organizer marker. When such anterior primitive streak-like structure was grafted into host chick embryos, it induced a secondary axis and neural tissue, confirming its role as an organizer [24••].

Although 2D human gastruloid models provide trackable systems to study human gastrulation, they still deviate significantly from the actual human embryo in term of 3D topology. To address this limitation, lumenal, pluripotent epithelial tissues formed by hPSC have recently been utilized for modeling human gastrulation [25•]. With precisely controlled, uniform BMP4 treatment, the 3D human gastruloid model exhibited asymmetric cell fate patterning, mimicking anterior-posterior symmetry breaking of the epiblast at the onset of gastrulation. Significant future efforts will be needed to further develop the 3D human gastruloid model, to achieve greater structural fidelity of the organizer, the ingressing cells through the primitive streak, and the germ layer organization.

The 2D gastruloid model allows precise, independent controls of colony shape, size, and cell density and is compatible with live imaging [22••,26–28]. Thus, the 2D

Figure 1



Modeling early human development with stem cell models.
Human embryo sections from different gestational days throughout the first month are shown on the left with key developmental structures labeled, including the epiblast sac, amniotic sac, primitive streak, neural plate, mesoderm, endoderm, neural fold, neural tube, and neural crest. All section images are from the Virtual Human Embryo project. **(a–k)** Using hPSC, various synthetic models of human development have been generated, with morphological and molecular features resembling those identified in human embryo sections on the left. These models include the synthetic epiblast sac model (a and b) [14,15], the synthetic amniotic sac model (c and d) [19•], the microfluidic amniotic sac model (e) [20••], 2D gastruloid (f) [23], 2D human organizer model (g) [24••], 3D human gastruloid (h) [25•], 2D synthetic neural plate model (i) [34•], 2D neuruloid (j) [38••], and 3D NT model (k) [42••]. Reproduced with permission.

gastruloid model has become a popular experimental system to elucidate mechanisms underlying germ layer lineage diversification and organization. It was shown that autonomous patterning in the 2D gastruloid model involves a BMP-WNT-NODAL signaling axis [24••]. Two interacting patterning mechanisms were also revealed, involving an edge-sensing response [28,29] and inhibitory feedback loops [26,28–32] in response to BMP and WNT ligands. The edge-sensing response to BMP or WNT ligands is resulted from cell density-dependent, edge-sensitive intercellular organization and presentation of ligand receptors at apical

versus basolateral surfaces of hPSC. Furthermore, exogenous BMP or WNT signals induce BMP antagonist NOGGIN or WNT antagonist DKK1, respectively. NOGGIN and DKK1 in turn diffuse throughout the 2D colony to form traveling waves, activation-inhibition boundaries, or elicit sustained signaling. Different mathematical models have been developed as well to simulate patterning in the 2D gastruloid model [26,28–30]. These simulations further predict patterning within irregularly shaped cell colonies and reveal diverse patterning features that lie both within and outside of the Turing instability [33]. Computational modeling of the 3D gastruloid system is an important future direction. Continuous development of lineage and signaling reporter hPSC lines, coupled with rapid advances in in toto imaging tools and single-cell methods, will greatly aid in quantitative descriptions of the 3D gastruloid model down to the single-cell resolution, facilitating such computational efforts.

Models of human neurulation

Following gastrulation, neurulation heralds the development of the central nervous system. Neurulation is initiated by induction of the neural plate (NP) in the dorsal ectoderm, leading to the development of a neuroepithelium layer demarcated by neural plate border cells that separate the neuroepithelium from the prospective epidermis. Using microcontact printing to generate 2D hPSC colonies with defined sizes and shapes, a human NP patterning model was developed [34•]. Dual SMAD inhibition with brief WNT induction [35,36] was sufficient to drive circular hPSC colonies to self-organize and pattern, with PAX6+ neuroepithelium at the colony center and PAX3+/SOX10+ neural plate border/neural crest cells at the colony border. Using dual SMAD inhibition for neuroepithelium induction and exogenous BMP4 for neural crest induction [37], a neurulation model, termed 'neuruloid', was recently developed using micropatterned 2D hPSC colonies. Importantly, the neuruloid recapitulated the 3D morphology and relative spatial organization of PAX6+ neuroepithelium, SOX10+ neural crest, SIX1+ placode, and KRT18+ surface ectoderm as seen *in vivo* [38••]. A signaling logic based on balanced BMP-WNT signaling was suggested to control patterning of the neuruloid [39]. The neuruloid was further applied to study developmental aspects of Huntington's disease using a transgenic hPSC line carrying mutated human Huntingtin proteins. Large-scale, disease-relevant phenotypical profiling is now possible with mass-produced neuruloids coupled with machine learning via deep convolutional neural networks [38••].

After neural induction, the NP folds toward the dorsal side of the embryo and fuses to form the neural tube (NT). NT development continues with differentiation of distinct classes of neuronal progenitor cells located at defined positions within the NT. 3D cultures of hPSC have also been utilized for modeling the NT development and its dorsal-ventral (DV) patterning. Cultured in a 3D native hydrogel with low mechanical stiffness, hPSC treated with dual SMAD inhibition followed by caudalization and ventralization with retinoic acid and smoothened agonist or sonic hedgehog [40,41] self-organize and develop into DV patterned lumenal neuroepithelial cysts [42••]. DV patterning of neuroepithelial cysts features sequential emergence of the ventral floor plate, P3, and pMN domains in discrete, adjacent regions and a dorsal territory progressively restricted to the opposite dorsal pole [42••], consistent with DV patterned NT [43]. Similar DV patterned NT models have previously been reported with mouse embryonic stem cells [44,45]. DV patterned human NT model offers an experimental platform for understanding the emergent self-organizing principles and patterning mechanisms involved in regional patterning of the NT.

Conclusions

The past several years has witnessed the rapid emergence of the field of synthetic human embryology. All current stem cell models of human development have leveraged the developmental potential and self-organizing properties of hPSC. In the future, other stem cell types, such as extended pluripotent stem cells [46], trophoblast stem cells [47], or extraembryonic endoderm stem cells [48], will become available and be further utilized to develop new models or be incorporated into existing models. These new stem cells will allow the development of synthetic models of extraembryonic tissues (such as the placenta [49]) or more complete embryo-like structures that recapitulate higher-order processes such as embryonic-extraembryonic interaction and formation of multiple body axes [5,50], further broadening the technological scope of synthetic human embryology. Although synthetic blastocyst models capable of forming decidua and implantation have been developed with mouse cells [51–54], the development of synthetic human embryo models and their applications must remain in the ethically permitted realm encoded by internationally recognized guidelines [55]. Moreover, we are now at the point where the field is becoming more quantitative, leveraging technological advances in bioengineering tools and in toto imaging and single-cell methods. In addition to fundamental studies, translational applications of synthetic human embryo models to clinically relevant problems will be a future area of growth in this exciting field. Equipped with emerging technologies such as single-cell tools, multi-omics and optogenetics, synthetic human embryo models will open new avenues for understanding human development and reproduction and providing insights to guide developments in tissue engineering and regenerative therapeutics.

Conflict of interest statement

Nothing declared.

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