Stem Cell Terminology and ‘Synthetic’ Embryos: A New Debate on Totipotency, Omnipotency, and Pluripotency and How It Relates to Recent Experimental Data

Hans-Werner Denker

Institut für Anatomie, Universität Duisburg-Essen, Essen, Germany

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Stem cell terminology · Potentiality · Pluripotency · Omnipotency · Totipotency · Development · Embryo · Ethics

Abstract

The rapid progress in the stem cell field, in particular in cell reprogramming, combined with certain recent observations from experimental embryology, has ignited a new discussion on stem cell terminology. The current use of terms describing stem cell potentiality is inconsistent and can be confusing, in particular the widely used term *pluripotency* and its distinction from *totipotency*. For cells possessing a complete differentiation potential (but lacking an autonomous embryo-structuring capacity) the term *omnipotency* (or, as recently proposed, *plenipotency*) has been coined. The present commentary takes up this discussion and confronts it with recent reports

on ‘engineering’ viable fish embryos or gastrulating human germ disc models using ‘pluripotent’/omnipotent cells, as well as on symmetry breaking in aggregates of mouse embryonic stem cells. It is concluded that we should start contemplating not only the terminology but also, even more urgently, the ethical implications of the perspective of constructing embryonic anlagen in humans.

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A Recent Proposal for a More Specific Stem Cell Terminology

The rapid progress in the stem cell field, in particular in cell reprogramming, combined with certain recent observations from experimental embryology, has ignited a new discussion on stem cell terminology. Many active researchers in the field might find it boring to discuss terminology, but interesting facts behind the arguments should indeed attract their attention. The current use of terms describing stem cell potentiality is inconsistent and can be confusing, in particular the widely used term *pluripotency* and its distinction from *totipotency*. Some authors use these terms interchangeably, with no clear distinction at all. Other authors (whose number appears to be increasing), however, insist on labeling embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) as *totipotent*, referring to the fact that apparently *all* types of dif-

Abbreviations used in this paper

BMP4	bone morphogenetic protein 4
EB	embryoid body
ESC	embryonic stem cell
hESC	human embryonic stem cell
iPSC	induced pluripotent stem cell
IVF-ET	in vitro fertilization and embryo transfer
PS	primitive streak

differentiated cells can be derived from them (and not just many, as the Latin word *plures* suggests). Again other authors would restrict the use of *totipotency* for cells that have the capacity to form not only all cell types but even a whole embryo, i.e. an organism (e.g. the zygote and early blastomeres). However, the majority of authors still stick to referring to ESCs and iPSCs as *pluripotent*.

In a recent article published in *Stem Cells and Development*, Maureen L. Condic [2014] takes a new and critical look at this inconsistent terminology, with a focus on connotations associated with the term *totipotency*, and she discusses the experimental evidence on which this term and others like *pluripotency* and *multipotency* are based. She emphasizes that, according to what we know by now about the differentiation potential of various types of stem cells, the denomination *pluripotent* is in many cases indeed misleading: accumulating data document that most of the ESC and iPSC lines in use seem to have the potential to produce not only *many* (as the Latin word *plures* suggests) but *all* cell types, depending on the testing conditions. This is particularly obvious when tetraploid complementation is performed, yielding viable embryos consisting entirely of the derivatives of the used stem cells (for this reason, some authors talk about ‘complete’ or ‘full’ pluripotency in such cases). Condic [2014] correctly notes that this may be one reason for the differences in use of terminology between laboratories, which can be confusing, in particular since some groups prefer to refer to ESCs and iPSCs as ‘totipotent’ rather than ‘pluripotent’. In countries with restrictive legislation (e.g. Germany) this use of wording can create ethical and legal confusion because research on ‘totipotent’ cells is explicitly illegal there. When taking a closer look at early mammalian development and at the specific meaning implied when referring to the zygote and early blastomeres as *totipotent*, it becomes clear that the major aspect here is that these early embryonic cells have the potential to orchestrate the development of a *basic body plan*, i.e. to give rise to a real embryo, an organismic whole, in addition to possessing the capacity to produce all differentiated cell types. In culture, ‘pluripotent’ stem cells (ESCs and iPSCs) typically do not show any self-organization potential going that far, i.e. they do not go on to produce complete embryos autonomously. Condic [2014] concludes that for clarity it would be desirable to have an *additional term* available for cells that (1) have the potential to produce (not only many but) *all cell types*, but which at the same time (2) *lack an early embryonic self-organizing potential*. This combination of properties, characterizing a potentiality level below totipotency but above multipo-

tency, would be typical for most of the stem cells so far usually referred to as ‘pluripotent’ in the literature. Condic [2014] proposes abandoning the custom of labeling these cells as ‘pluripotent’ and rather replacing this term for the described types of cells with a new word she created: *plenipotent*.

In developing her argument, Condic [2014] correctly acknowledges that essentially the same biological facts were already discussed in exactly this context at an earlier time point, arriving at fairly the same proposal to redefine and amend the current terminology [Denker 2002, 2004; Condic, 2014, p. 797]. At that earlier time point another term, *omnipotency*, had been proposed to describe this combination of cellular properties which she now suggests labeling ‘*plenipotency*’ (potential to form all cell types but lack of an autonomous basic body plan formation potential). The term *omnipotency* did already exist in the embryological literature before the boom of stem cell research began, although it was rarely used. Apart from the semantic question of which of the two terms may be more appropriate, one can easily agree with most parts of the analysis of biological facts which Condic [2014] presents [for an updated version of the terminology, including e.g. the important and heavily researched distinction between ‘naïve’ and ‘primed’ states of stem cells, see table 1 in Denker, 2012]. I will address further below some conflicting biological aspects and new experimental findings.

Preference for any of the two proposed terms, *omnipotency* or *plenipotency*, is not intended to be a major point of the present commentary. However, it may be worth mentioning that I personally still prefer *omnipotency*. Condic [2014, p. 797] rejects the term *omnipotency* only for a very peculiar reason, i.e. because ‘... the strong connotation of this word outside the field of science compromises its utility’. My answer would be that terminological concerns of circles outside the scientific community cannot provide a particularly convincing argument in our community. On the other hand, Condic [2014] and I agree that it would be helpful to have some additional term at hand to label the discussed type of ‘complete pluripotency’ as different from totipotency, if only for the purpose of discussing more deeply whether or not this distinction does indeed reflect a real biological difference, and/or what it may be at the molecular level. It should be remembered that it is by no means clear whether any sharp line can be drawn between totipotency (narrow definition, see above, i.e. including the embry structuring capacity) and *omnipotency/plenipotency* (this is questioned by Denker [2004; 2012, footnote to ta-

ble 1]). As far as *totipotency* is concerned, I prefer the narrow definition (including the embryo-structuring capacity [Denker, 2012]) as Condic [2014] also does, and I will use the term in this specific sense hereafter. As a better term for ‘pluripotency’, I would propose sticking to the use of *omnipotency* for linguistic reasons: *omnia* (Latin for *all*) clearly points to the fact that the complete spectrum of differentiated cell types can be derived from such cells. The term *omnipotency* can thus be expected to be relatively resistant to inaccurate use. In contrast, *plenus* (full) does not serve this need equally well (definition of ‘full’ in Webster’s New World Dictionary: ‘having in it all there is space for’ [Neufeldt and Guralnik, 1988]).

One controversial point in the argument of Condic [2014] is that she reserves the term *totipotency* for *single cells* only, in contrast to *groups of cells*. In addition, she assumes that totipotency (in the narrow sense, i.e. including a basic body plan formation capacity) of cells specifically relies on cytoplasmic asymmetry cues (axis-determining factors) which are derived from the oocyte (and are possibly further processed at fertilization) and which are then segregated to blastomeres during cleavage (typical examples of this type of totipotent cell are the zygote and early blastomeres). This would imply that other types of cells, not possessing such cytoplasmic determinants directly segregated from an oocyte, or cell lines (like ESCs) processed for a long time in vitro (so that they must be expected to have lost those determinants) would not be able to show, or develop, totipotency. As discussed earlier [Denker, 2004, 2012], many data from experimental embryology give good reason to believe, however, that groups (clusters, colonies) of ‘pluripotent’/omnipotent cells can gain totipotency. Under appropriate experimental conditions, they seem to need no more than relatively simple asymmetry cues, such as may even be provided by stochastically arising inhomogeneities of their environment in culture, in order to start the typical gene activation cascades which can lead to early embryonic pattern formation (in the sense of gastrulation, and perhaps even culminating in the formation of a basic body plan). Not only the initiation of this morphogenetic process but also the degree of order finally gained are strongly dependent on environmental conditions (I will come back to this point below). This implies that, if these asymmetry cues are provided, such clusters of omnipotent cells may develop an embryo-structuring capacity, i.e. totipotency (in the narrow sense). The resulting potentiality of such a cell cluster would indeed conform to the definition of totipotency of Condic [2014], except that she would not include this case because she reserves totipotency for single cells, not cell clusters.

New Experimental Data: Prospects for Creating ‘Synthetic’ Embryos?

Of great interest in this context are some recent data from experimental embryology that shed new light on the respective validity of these controversial views. Xu et al. [2014] reported on the construction of a vertebrate embryo using artificially created morphogen gradients in groups of nontotipotent (but omnipotent) cells that had been isolated from zebrafish embryos. According to these findings, it must be concluded that induction of totipotency in clusters of omnipotent cells is possible using this type of strategy, thus questioning whether it is reasonable to restrict the use of the term totipotency to single cells as proposed by Condic [2014]. The publication by Xu et al. [2014] is already receiving quite a bit of attention from developmental biologists and deserves to elicit also the interest of stem cell researchers, ethicists, philosophers, and politicians. Specifically, in order to generate viable zebrafish embryos from groups of ‘naïve’ uncommitted embryonic cells that would normally not show totipotency, the authors employed a trick by providing the cell cluster with engineered gradients of appropriate morphogens, i.e. an experimental design close to what had been discussed before with regard to induced morphogenesis in colonies of ‘pluripotent’ cells [Denker, 2004]. Xu et al. [2014] showed that the creation of two opposing gradients of bone morphogenetic protein (BMP) and Nodal, morphogens known to play key roles in determining the anterior-posterior axis of the embryo, ‘is sufficient to initiate the principal molecular and cellular processes necessary to organize a complete embryonic axis. All other signaling pathways required to achieve full embryonic development are induced and regulated in response to the two initial, experimentally engineered signals. Therefore, our findings establish a baseline for the minimal signaling requirements for early embryonic development and provide a framework for future studies in the field of regenerative medicine that are aimed at constructing tissues and organs in vitro from populations of cultured pluripotent cells’ [Xu et al., 2014].

In an interview, one of these authors indicated that they will test the applicability of their strategy to mammalian (instead of fish) cells, i.e. ‘pluripotent’/omnipotent mouse cells (e.g. ESCs), and that they are confident they will be successful in constructing an embryo also in that case [Barney, 2014]. Does this mean that ‘synthetic’ mammalian embryos, constructed from ESCs or iPSCs in vitro, are what we must expect to be presented with next? It can be argued that the animal cap cells which Xu et al.

[2014] used in their experiments are different from stem cell lines in that they had not been propagated and passaged in vitro, and that these cells, albeit incapable of forming an embryo if cultured without artificial BMP/Nodal gradients, might still carry with them some cryptic positional cues (an idea that would be somewhat related to the speculative interpretation of embryo dissociation and cell reaggregation experiments as presented by Condic [2014]). But Xu et al. [2014] claim they can rule this out on the basis of their experience with these cells. Do we have reason to be concerned about the perspective of creating a gastrulating embryonic anlage and in this way a basic body plan using 'pluripotent'/omnipotent mammalian (and possibly human) stem cells in vitro? Is this at all a realistic perspective in spite of the fact that not even viable 'synthetic' blastocysts (e.g. constructed from appropriate stem cells like ESCs plus trophoblast and primitive endoderm stem cells) have been reported so far?

Experiments in this direction, using human ESCs, were indeed recently reported by Warmflash et al. [2014]. These authors presented a method that applies geometric constraints for manipulating differentiation and pattern formation in ESC colonies in vitro, and with appropriate variants of their methodology they detected an astonishing regularity in the spatial arrangement of the differentiating cells. In response to BMP4, colonies showed not only pronounced trophoblast differentiation (as expected due to earlier work by other groups) but also a remarkable spatial arrangement of these extraembryonic cells as well as of germ layers, specifically showing 'an outer trophoderm-like ring, an inner ectodermal circle and a ring of mesendoderm expressing primitive-streak markers in between'. The resulting geometry was dependent on the boundary, i.e. on physical constraints as demonstrated impressively by culturing on various micropatterned surfaces. The authors concluded that their observations demonstrated 'an intrinsic tendency of stem cells to make patterns', that this 'can be harnessed by controlling colony geometries', and that 'geometric confinement ... [is] sufficient to trigger self-organized patterning in hESCs' under their culturing conditions.

The article is meant as a proof of principle showing how the development of a gastrulating embryonic disc can be engineered in vitro. The resulting colonies which these authors describe do not really resemble the morphology of early postimplantation stage human embryos, however, and they also are, for example, by far less embryo-like than a highly structured 'embryoid body' (EB) found in cultures of marmoset monkey ESCs and studied histologically by Thomson et al. [1996]. In the colonies that were

engineered by Warmflash et al. [2014], the outer ring of trophoblast cells, for example, was geometrically different from the trophoblast shell present at the outside of an early postimplantation stage human embryo. Still, these authors may be correct in assuming that this pile of trophoblast cells may likewise be able to serve functions of localized signal exchange between extraembryonic and intraembryonic cells, as is known to be significant in structuring the embryonic disc and laying down embryonic axes in vivo. The germ layers formed in the engineered colonies in vitro were also not quite as ordered as they are in vivo: they were not elongated or pear shaped, and the area of cells undergoing epithelial-mesenchymal transition (as in gastrulation) in their culture models was likewise not elongated and not eccentrically located like a primitive streak (PS) would be in a real embryo. However, Warmflash et al. [2014] expect that such differences between the models and real embryos could possibly be corrected in future experiments using improved variants of their technique, specifically other shapes of the micropatterned matrix/substratum (not round).

Warmflash et al. [2014] point out that a wide application of this methodology appears possible, and that it offers a possibility to model processes leading to basic body plan development in humans in order to allow large-scale experimentation that could never be feasible (and would have to be considered ethically unacceptable) with real human embryos. According to what is known from other studies about the influence of physical constraints and extracellular matrix on pattern formation, it may indeed be a realistic perspective that modification of the geometry of the microengineered substratum/matrix could lead to formation of a gastrulation area that comes closer to the morphology of a PS. Many observations demonstrate that, when early embryonic cells or ESCs are cultivated in vitro, the degree and the kind of order attained strongly depend on physical conditions of culture. Culture conditions can on one hand determine what cell type will dominate in the cultures of 'pluripotent' cells: 'ground state (naïve)' or 'primed' type [Theunissen et al., 2014], possibly depending on selection for or against a subpopulation of naïve-like cells in these populations [Wang et al., 2014]. On the other hand, culture conditions may also more directly influence pattern formation/morphogenesis during differentiation of the cells, in particular how close these processes may come to ordered gastrulation [Behr et al., 2005; Maranca-Hüwel and Denker, 2010; Poh et al., 2014]. Particularly important seem to be the extracellular matrix provided as a substratum, the surfaces of other cells to which the cells can attach, and their geometric arrangement.

In addition, Warmflash et al. [2014] address specific signaling through morphogens and they emphasize that their in vitro system allows investigation in humans of details of how BMP4 signaling and its regulation control the gradients of Activin-Nodal that pattern mesendodermal fates during gastrulation. Remarkably, BMP and Nodal are exactly the molecules found to be instrumental in the experiments on creating ‘engineered’ fish embryos [Xu et al., 2014] (see above). Since these morphogens are known to play key roles in determining the anterior-posterior axis of the embryo, the two approaches of Xu et al. [2014] and Warmflash et al. [2014] will most probably be combined in future experiments along these lines, studying the effects of local sources of morphogens and of micropatterned matrix-coated surfaces in stem cell cultures. Thus, we can expect that the strategies presented by Warmflash et al. [2014] as well as Xu et al. [2014] will open avenues to how engineering of a basic body plan (or even complete embryos) can be pushed towards higher degrees of normality.

Lessons from Self-Organization versus Engineered Morphogenesis: Do They Teach Us Anything about the Appropriate Terminology (Totipotency vs. Omnipotency) and Ethical Implications?

In addition to the large range of differentiated cell types that ‘pluripotent’/omnipotent stem cells can give rise to, the morphogenetic capacity of these cells is currently attracting much interest from stem cell workers. A recent phenomenon is that authors interested in tissue and organ replacement strategies are now often addressing the formation of ‘organoids’ in vitro as a result of ‘*self-organization*’ [see for example Eiraku et al., 2011; Suga et al., 2011; Boucherie et al., 2012; Nakano et al., 2012; Sato and Clevvers, 2013; Takasato et al., 2013; Takebe et al., 2014]. How close may a self-organization capacity come to totipotency? Already early in the history of ESC research, some observations suggested that the morphogenetic potential of primate ESCs may include processes of basic body plan formation [Thomson, 1996]. These early studies were of a descriptive type, reporting observations on ‘EBs’ (or ‘differentiated colonies’) forming spontaneously in culture, without trying to directly influence the patterns formed [Behr et al., 2005; Fuchs et al., 2012; Hong et al., 2012; Poh et al., 2014]. In contrast to Warmflash et al. [2014] and Xu et al. [2014], those earlier investigators did not intend to ‘engineer’ embryonic disc models or even whole embryos. Interestingly, some of the authors pointed out that they

were astonished to find that the autonomously formed (entirely self-organized) EBs showed close similarities with regard to gastrulation-related gene activation cascades as compared to real mouse embryos [ten Berge et al., 2008]. Symmetry breaking is a crucial initial process required for axis development during basic body pattern formation. That symmetry breaking does indeed occur spontaneously in aggregates of ‘pluripotent’/omnipotent stem cells in vitro is now being discovered as a new research focus. In a recent systematic study along these lines, van den Brink et al. [2014] concluded that the formation of such gastrulation stage-like structures (which they termed ‘gastruloids’) in vitro is ‘for the most part autonomous’ but that ‘the culture conditions influence the cell types that develop within them’.

What does this tell us about the potentiality level of those cells? When contemplating the role of self-organization capacity versus pattern engineering, it is important to remember two facts: (1) early embryonic-type cells (totipotent or omnipotent) are very sensitive to their environment, and any pattern formation they initiate depends very much on these environmental conditions. Even complete normal mouse embryos do not express their totipotency if they are not allowed to implant in a uterus but are transferred to extrauterine sites (e.g. under the kidney capsule). In this case, they form a teratoma [for literature, see Andrews, 2002; Denker, 2004]. The observation that stem cells (ESCs and iPSCs) produce teratomas if transferred to such ectopic sites can thus in principle not prove that they are not totipotent, although that argument can be read often. (2) The kind of pattern formed by totipotent or omnipotent cells can be manipulated by extracellular matrix or by the kind of substratum to which they can attach, or by adding other cells (with adhesive or repellent properties, or cells which release morphogens). This fact is well known from the literature on experimental embryology [Denker, 2004] and was not in itself a surprising finding in the study presented by Warmflash et al. [2014].

Whether a clear line can be drawn between *totipotency* (understood as the capacity for autonomous development of an organismic whole) and *omnipotency* (capacity for assisted development under the guidance of added patterning instructions), may be difficult to say, therefore, in the present state of knowledge. The morphogenetic potential which omnipotent or totipotent cells exhibit depends on the environment given to them. It is difficult or even impossible to construct a completely neutral environment in order to exclude such influences in a testing situation (the importance of a neutral environment was discussed by Denker [1976] with regard to the isolation experiment in

developmental biology). This is true not only for stem cell lines as discussed above but also for cells freshly isolated from early embryos. An example is blastomere biopsy in the human (for preimplantation genetic diagnosis, PGD/PID). People arguing in favor of the ethical acceptability of preimplantation genetic diagnosis usually point out that there are no reports in the literature unequivocally demonstrating the totipotency of single blastomeres isolated from 8-cell cleavage stages. While the latter may be true, we need to remember a study by Ziomek et al. [1982] that showed in the mouse that even cells isolated from later (16-cell) stages can form viable embryos if they are recombined with several other, similar cells obtained from the same developmental stage. So these cells, being unable to form an embryo when isolated and left alone, can gain totipotency as a group. This observation on one hand contradicts once more Condic's restriction of the application of the term totipotency to single cells [Condic, 2014]. On the other hand, it should remind us to be careful when judging biopsied 8- (16-) cell blastomeres as ethically nonproblematic (with regard to a suspected totipotency) in the human. Whatever term we like to use when addressing the potentiality of early embryonic-like mammalian cells (freshly isolated from embryos, or ESCs, or iPSCs), we should be aware that terms like totipotency, omnipotency/pluripotency, and 'pluripotency' tend to distract our attention from the fact that mammalian embryos (and their constituent cells) represent the regulatory type of development ('conditional specification' according to Gilbert [2014]). This implies that, in contrast to many other species (representing a mosaic type of development, 'autonomous' specification according to Gilbert [2014]), they respond very sensibly to the environmental conditions provided to them. The signals provided by the environment can be destructive (disturbance of the regular patterning in cases of teratoma formation after the transfer of a normal embryo to an ectopic site) or instructive (engineered pattern formation according to Xu et al. [2014] and Warmflash et al. [2014]). With regard to ethical aspects of working with early embryonic-type human cells, it is very important to keep this peculiar combination of self-organization capacity and plasticity in mind.

In conclusion, any progress with defining a clear and logical terminology is certainly desirable. However, with regard to the reasoning of Condic [2014], I find it mandatory to draw attention to the fact that totipotency can be induced in groups (clusters) of omnipotent cells, as now suggested by the work of Warmflash et al. [2014] and Xu et al. [2014] on 'synthetic' embryos [Warmflash et al., 2014; Xu et al., 2014]. I would advocate giving, in any dis-

course, priority to ethical implications rather than the terminology. It appears that we should be aware of the development of methodologies permitting the creation of embryos from omnipotent cells in mammals. Specifically, with regard to the aim of 'engineering' gastrulation stage embryonic disc models [as in the work presented by Warmflash et al., 2014] we must keep in mind that the endpoint of experiments on gastrulation comes necessarily close to the PS stage that represents the 14-day limit for experimentation on human embryos defined by UK legislation. This should be kept in mind in the context of the perspectives expressed at the end of the paper by Warmflash et al. [2014] ('As the patterns arise in a self-organized manner, micropatterned stem cell culture also provides a novel, controlled platform for studying how signaling generates developmental patterns. We thus propose that geometrically controlled cell culture should become standard practice for embryonic stem cell differentiation.'). I would think that a recommendation like this one should not be understood as a proposal to search for conditions under which the colonies come closest morphologically to a well-structured embryonic disc of a real embryo, at least not when working with human cells. Experiments along these lines may be performed with nonhuman primate cells, and such research could be of great interest to embryologists. This argument is somewhat similar to reasoning published before, concerning the cloning of embryos by tetraploid complementation, with 'pluripotent' nonhuman versus human cells [Denker, 2009]. If experiments along the lines defined by Warmflash et al. [2014] are performed with the intent of finding out which of their methodological variants may be safest to *exclude* any (unintended) formation of a regular PS (and would thus exclude basic body plan formation, i.e. individuation) whenever human omnipotent stem cells are being kept in culture, this may be an important undertaking. However, these experiments would have to be done with nonhuman primate (and not human) cells. To define such culture conditions might indeed be a step forward in dealing with the ethical problems posed by the morphogenetic (self-organization) potential of omnipotent stem cells. Investigating this might, in addition, be helpful in the search for criteria for distinguishing between various levels of potentiality of cells (classification as naïve or primed 'pluripotency', omnipotency, and totipotency). With human 'pluripotent'/omnipotent stem cells, however, performing experiments with the aim of creating models of gastrulating embryonic discs can definitely not be advocated, for ethical reasons, even though such investigations may appear attrac-

tive to some authors because they might open possibilities for large-scale experimentation that of course would never be possible with human embryos [e.g. those donated in in vitro fertilization and embryo transfer (IVF-ET) programs]. In case engineering of viable 'synthetic' embryos proves successful with nonhuman primate cells so that the technology may thus appear transferable to the human system, we should develop policies for how to avoid misuse of such cells and technologies in order to ensure that these techniques remain what these authors [Warm-

flash et al., 2014; Xu et al., 2014] are hoping for: a boost for stem cell research and a benefit to mankind. In stem cell generation strategies, reprogramming protocols that bypass 'pluripotency'/omnipotency may offer an escape from the ethical dilemma [Denker, 2012].

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References

- Andrews, P.W. (2002) From teratocarcinomas to embryonic stem cells. *Philos Trans R Soc Lond B Biol Sci* 357: 405–417.
- Barney, J. (2014) U.Va. smashes barrier to growing organs from stem cells. 2014. <https://news.virginia.edu/content/uva-smashes-barrier-growing-organs-stem-cells> (accessed December 20, 2014).
- Behr, R., C. Heneweer, C. Viebahn, H.-W. Denker, M. Thie (2005) Epithelial-mesenchymal transition in colonies of rhesus monkey embryonic stem cells: a model for processes involved in gastrulation. *Stem Cells* 23: 805–816.
- Boucherie, C., S. Mukherjee, E. Henckaerts, A.J. Thrasher, J.C. Sowden, R.R. Ali (2012) Self-organising neuroepithelium from human pluripotent stem cells facilitates derivation of photoreceptors. *Stem Cells* 31: 408–414.
- Condic, M.L. (2014) Totipotency: what it is and what it is not. *Stem Cells Dev* 23: 796–812.
- Denker, H.-W. (1976) Formation of the blastocyst: determination of trophoblast and embryonic knot; in Gropp, A., K. Benirschke (vol.- eds): *Developmental Biology and Pathology*. Curr Top Pathol. Berlin, Springer, vol 62, pp 59–79.
- Denker, H.-W. (2002) Forschung an embryonalen Stammzellen: eine Diskussion der Begriffe Totipotenz und Pluripotenz; in Oduncu, F., U. Schroth, W. Vossenkuhl (eds): *Stammzellenforschung und therapeutisches Klonen*. Göttingen, Vandenhoeck & Ruprecht, pp 19–35.
- Denker, H.-W. (2004) Early human development: new data raise important embryological and ethical questions relevant for stem cell research. *Naturwissenschaften* 91: 1–21.
- Denker, H.-W. (2009) Ethical concerns over use of new cloning technique in humans. *Nature* 461: 341.
- Denker, H.-W. (2012) Time to reconsider stem cell induction strategies. *Cells* 1: 1293–1312.
- Eiraku, M., N. Takata, H. Ishibashi, M. Kawada, E. Sakakura, S. Okuda, K. Sekiguchi, T. Adachi, Y. Sasaki (2011) Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* 472: 51–56.
- Fuchs, C., M. Scheinast, W. Pasteiner, S. Lagger, M. Hofner, A. Hoellrigl, M. Schultheis, G. Weitzer (2012) Self-organization phenomena in embryonic stem cell-derived embryoid bodies: axis formation and breaking of symmetry during cardiomyogenesis. *Cells Tissues Organs* 195: 377–391.
- Gilbert, S.F. (2014) *Developmental Biology*. Sunderland, Sinauer Associates.
- Hong, N., B.P. He, M. Scharlt, Y. Hong (2013) Medaka embryonic stem cells are capable of generating entire organs and embryo-like miniatures. *Stem Cells Dev* 22: 750–757.
- Maranca-Hüwel, B., H.-W. Denker (2010) Epithelial-mesenchymal transition in rhesus monkey embryonic stem cell colonies: the role of culturing conditions. *In Vitro Cell Dev Biol Anim* 46: 516–528.
- Nakano, T., S. Ando, N. Takata, M. Kawada, K. Muguruma, K. Sekiguchi, K. Saito, S. Yonemura, M. Eiraku, Y. Sasaki (2012) Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell* 10: 771–785.
- Neufeldt, V., D.B. Guralnik (eds) (1988) *Webster's New World Dictionary of American English*. Cleveland, Webster's New World Dictionaries.
- Poh, Y.C., J. Chen, Y. Hong, H. Yi, S. Zhang, J. Chen, D.C. Wu, L. Wang, Q. Jia, R. Singh, W. Yao, Y. Tan, A. Tajik, T.S. Tanaka, N. Wang (2014) Generation of organized germ layers from a single mouse embryonic stem cell. *Nat Commun* 5: 4000.
- Sato, T., H. Clevers (2013) Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. *Science* 340: 1190–1194.
- Suga, H., T. Kadoshima, M. Minaguchi, M. Ohgushi, M. Soen, T. Nakano, N. Takata, T. Wataya, K. Muguruma, H. Miyoshi, S. Yonemura, Y. Oiso, Y. Sasaki (2011) Self-formation of functional adenohypophysis in three-dimensional culture. *Nature* 480: 57–62.
- Takasato, M., P.X. Er, M. Becroft, J.M. Vanslambrouck, E.G. Stanley, A.G. Elefanti, M.H. Little (2013) Directing human embryonic stem cell differentiation towards a renal lineage generates a self-organizing kidney. *Nat Cell Biol* 16: 118–126.
- Takebe, T., R.R. Zhang, H. Koike, M. Kimura, E. Yoshizawa, M. Enomura, N. Koike, K. Sekine, H. Taniguchi (2014) Generation of a vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nat Protoc* 9: 396–409.
- ten Berge, D., W. Koole, C. Fuerer, M. Fish, E. Eroglu, R. Nüsse (2008) Wnt signaling mediates self-organization and axis formation in embryoid bodies. *Cell Stem Cell* 3: 508–518.
- Theunissen, T. W., B.E. Powell, H. Wang, M. Mitropova, D.A. Faddah, J. Reddy, Z.P. Fan, D. Maetzel, K. Ganz, L. Shi, T. Lungjangwa, S. Imsoonthornsuksa, Y. Stetzer, S. Rangarajan, A. D'Alessio, J. Zhang, Q. Gao, M.M. Dawlaty, R.A. Young, N.S. Gray, R. Jaenisch (2014) Systematic identification of culture conditions for induction and maintenance of naive human pluripotency. *Cell Stem Cell* 15: 471–487.
- Thomson, J.A., J. Kalishman, T.G. Golos, M. Durning, C.P. Harris, J.P. Hearn (1996) Pluripotent cell lines derived from common marmoset (*Callithrix jacchus*) blastocysts. *Biol Reprod* 55: 254–259.
- van den Brink, S.C., P. Baillie-Johnson, T. Balayo, A.K. Hadjantonakis, S. Nowotschin, D.A. Turner, A. Martinez-Arias (2014) Symmetry breaking, germ layer specification and axial organisation in aggregates of mouse embryonic stem cells. *Development* 141: 4231–4242.
- Wang, J., G. Xie, M. Singh, A.T. Ghanbarian, T. Raskó, A. Szvetnik, H. Cai, D. Besser, A. Prigione, N.V. Fuchs, G.G. Schumann, W. Chen, M.C. Lorincz, Z. Ivicz, L.D. Hurst, Z. Izsvák (2014) Primate-specific endogenous retrovirus-driven transcription defines naive-like stem cells. *Nature* 516:405–409.
- Warmflash, A., B. Sorre, F. Etoc, E.D. Siggia, A.H. Brivanlou (2014) A method to recapitulate early embryonic spatial patterning in human embryonic stem cells. *Nat Methods* 11: 847–854.
- Xu, P.F., N. Houssin, K.F. Ferri-Lagneau, B. Thisse, C. Thisse (2014) Construction of a vertebrate embryo from two opposing morphogen gradients. *Science* 344: 87–89.
- Ziomek, C.A., M.H. Johnson, A.H. Handyside (1982) The developmental potential of mouse 16-cell blastomeres. *J Exp Zool* 221: 345–355.