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Commentary

Stem Cell-Derived Organoids, Embryoids, and Embryos: Advances in Organismic Development In Vitro Force Us to Re-Focus on Ethical and Legal Aspects of Model Choice

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Abstract: While research on stem cell-derived tissues and *organoids* is rapidly expanding, the technically related creation of complex *embryoids* has recently excited a vivid discussion since it raises ethical questions about individuation and the possible gain of viability. The present study focuses on the onset of organismic development and the proposed biological and legal definitions for the terms embryo, embryoid, and organoid. It is concluded that such considerations have become important for investigators' choices of the appropriate in vitro model systems, allowing the formation of organoids vs. complex embryoids.

Keywords: organoids; embryoids; embryos; model choice; definitions; ethics; law

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1. Introduction

Research on stem cell-derived *tissues and organoids* is rapidly expanding, providing not only fascinating insights into basic principles of morphogenesis but also taking steps towards practical applications in drug discovery and toxicity testing, as well as tissue and organ replacements in humans [1–9]. Recently, the technically closely related creation of complex *embryoids* has excited vivid discussions, particularly regarding ethical questions [10–12]. Indeed, when performed with human stem cells, the formation of complex embryoids raises questions that stem cell researchers focussing on tissue and organ replacement may not be familiar with. Discussions have been specifically ignited since the novel artificially constructed complex embryoids touch upon aspects of individuation [13,14]. In order to address this, the International Society for Stem Cell Research (ISSCR) has proposed Guidelines for Stem Cell Research and Clinical Translation [15,16]; these have, in turn, initiated criticism because obviously a number of ethical aspects (e.g., of autonomy, individuation, and informed consent of cell donors) have not been covered sufficiently [12]. While the latter authors have addressed the topic from a reproductive sciences perspective, the present study intends to help researchers in the fields of tissue replacement, organoids, and developmental biology to become aware of the biological background for the ethical problems that are connected with growing pluripotent stem cells in such advanced in vitro systems. A focus is on the special situation when three-dimensional stem cell-derived constructs can gain properties of viable embryos. It is proposed that in light of the recent observations, the constructs of this type should be classified on the basis of their developmental potentiality, specifically depending on whether they should be seen as developing *organisms* rather than *organoids*. The study thus intends to help researchers avoid ethical stumbling blocks in this field when selecting their specific stem cell model.

2. A Recent Discussion about Ethical Guidelines and the Term "Embryo"

As recent research on stem cell-derived embryoids has impressively demonstrated, a great variety of constructs of differing complexity and completeness can be produced when using various advanced in vitro technologies [17–33]. It does not appear trivial anymore

to define a border line between stem cell-derived embryoids and organoids. Embryoid constructs come in an astonishing variety, and also confusingly, many new terms have been proposed, like "artificial" or "synthetic embryos" [14], "synthetic human entities with embryo-like features, SHEEFs" [34], or "stembryos" [35], blastoids, gastruloids, and many others (for an overview see Table 3 in [36]). The recent ISSCR guidelines propose to distinguish between "integrated stem cell-based embryo models", which would possess derivatives of extraembryonic cell types (e.g., blastoids and ETX/iETX embryoids), and "non-integrated stem cell-based embryo models" (e.g., gastruloids which do not [15]). I will address these stem cell-derived embryoid constructs as "*embryoids*".

The vivid discussions that the ISSCR Guidelines have initiated focus in particular on (i) some logical inconsistencies and (ii) on the fact that the rapidly growing number of reports on the construction of increasingly complex embryoids suggests that a gain of full viability must be considered. An inconsistency is seen in the fact that, on the one hand, the ISSCR Guidelines consider the stem cell-derived constructs as useful and (under certain precautions) ethically acceptable "*models*" for embryological research in humans, and they propose that these constructs should NOT be subject to the same full set of restrictions which apply to natural human embryos. All embryoid constructs are addressed as "models", in these Guidelines. On the other hand, however, the Guidelines emphasize that subsequent transfer of any of the "models" to a uterus should generally be strictly forbidden (irrespective of the recipient species) [12]. This must be interpreted as indicating that the authors do not exclude that some of such "models" could deserve the same degree of respect and protection as traditional embryos. The term "model" has, therefore, been criticized for tending to detract attention from the need to consider the ethical implications of the potential viability of these constructs. On the other hand, even some of the authors of the ISSCR Guidelines have recently expressed warnings concerning ethical aspects of the production and use of complex "integrated" embryoids [11].

The aspect of potential viability has been a main focus of a recent study [10] emphasizing that stem cell-derived embryoid constructs may soon attain full developmental potential in the course of continuing improvements in methodology. These authors consider that such complex embryoids would, for this reason, have to be addressed more appropriately as *embryos* rather than as "models" like the ISSCR Guidelines do. In order to deal practically with this prospect, Rivron et al. [10] proposed a new legal definition of the term "human embryo" that would differ from another, a biological definition they also give in the same paper. This proposed legal definition would specify that "support elements fulfilling uterine functions" be considered a required constituent for any entity in order to be legally addressed as an "embryo". This appears problematic, however, in particular since this proposed legal definition is in conflict with the current state of biological research: It suggests an essential instructive/instrumental role for uterine factors in morphogenesis, but actual biological research data do not support this assumption. Although it has been shown that for cultivating embryoids beyond the gastrulation stage in vitro, the precise and timely delivery of oxygen, glucose, and the appropriate culture media in specialized incubators is essential [20], there is no evidence that these factors have any direct instructive function for embryonic pattern (basic body plan) development. They should be seen as nutritional needs in a more general sense [37–39]. In earlier years, it had indeed been proposed by some researchers that axis development (the embryonic–abembryonic axis, i.e., the precursor for the dorsoventral axis; the anterior–posterior axis), and the laying down of the basic body plan at gastrulation, might be determined by uterine factors (secreted factors or cellular interactions at implantation). However, more recent experimental data show that this is not the case [40]. In the following, I will discuss biological definitions for the terms "embryo" and "embryoid" somewhat more in detail and compare them with the term "organoid".

3. Embryoids and Organoids vs. Embryos

The terms "embryoid" and "organoid" are commonly used to describe entities that are more or less comparable in structural organization and functional properties to embryos and organs but somewhat incomplete and notably are of a different origin (e.g., differentiated from stem cells via self-organization; another origin not focused on in the present text is from tissue explants taken from an existing organism [41]). An organ is clearly defined as a part of a whole organism and is incapable of conducting an independent (autonomous) life despite being a structural and functional unit with its own specific properties. An embryo, in contrast, is a biological system that has the potential to develop an autonomous life as an organism; it is an organism in statu nascendi. A characteristic feature of an organism is to possess systems with integrative functions (cardiovascular system, nervous system, endocrine system, etc.) that provide it with a very peculiar emergent property: viability, the ability to conduct an independent, autonomous life as an individual. The organism is thus more than the sum of its various parts and organs. As far as the embryo is concerned, it already possesses integrative principles of a special kind; this becomes obvious in experiments on developmental regulation (remember the famous deletion experiments of experimental embryology, see developmental biology textbooks). The nature of these integrative principles, in particular, those acting during the early phases of development (while the basic body plan is being established and the organ anlagen are formed), is still far from being completely understood and is a topic for ongoing research that keeps developmental biologists busy (the hierarchical organization of pattern-formation processes, the role of positional information, cell–cell and cell–matrix interactions, the nature of the signaling molecules, etc.). Indeed, such studies are now facilitated by stem cell-derived embryoids.

While this way of understanding an embryo as a biological system focuses on its functional characteristics, traditional definitions of a (human) embryo mostly refer to its origin from gamete fusion. We should not forget, however, that classical texts also used to mention, in such definitions, organismic aspects in addition. For example, a well-known and worldwide used classical textbook [42] says:

> *"The zygote which has been formed by the fusion of a male and female gamete is a single-celled organism. After a longer or shorter period this unicellular organism will become progressively transformed by the processes of cell division, cell migration, growth and differentiation into a multicellular mature member of its species. The term development is used to describe these progressive changes".*

The first part of this definition of the embryo, referring to its origin from the fusion of oocyte and sperm, has indeed been used in many legislations concerning embryo experimentation and stem cell work (although not all of them restrict the definition to the origin from fertilization; e.g., the Australian legislation and the Dickey–Wicker Amendment in the USA are wisely envisaging alternative modes of embryo generation from diploid human cells [43,44]).

Of specific interest in the cited classical definition is that it uses the term "*organism*" already for the zygote, i.e., it defines the zygote as a "*single-celled organism*", even repeated as a "*unicellular organism*". Development is then characterized as a process of transformation: "*transformed. . . into a multicellular mature member of its species*". Remarkably, this "organismic" view was not only expressed by descriptive embryologists like those authors but was also shared by experimental developmental biologists. Seidel, for example, has formulated this concept in a very pointed way, addressing development as a transformation from one appearance/structural organization to a different one, e.g., from the zygote system to the basic body plan (in his original words in German: "*Entwicklung [ist] Umbildung von einer Gestalt zu anderen. . ., z.B. vom Eisystem zur Grundgestalt*" [45]). Interestingly, that view focuses on the use of functional characteristics of the developing embryo, meaning, in particular, integrative functions, i.e., its properties as a system. In the context of our discussions of the novel complex embryoids, it appears that we have reason to ask now whether stem

cell-derived constructs can acquire functional organismic properties that are analogous to those of an embryo and are able to provide it with comparable developmental potential.

Based on the classical definitions mentioned above and on the views just discussed, it seems appropriate to define an "embryo" functionally, in terms of a biological system, and to refer to its developmental potential as follows:

> *An embryo is a biological system possessing the active potential to develop into an organism (totipotency). To realize its developmental potential, the embryo needs appropriate external conditions that are permissive, but it does not need specific morphogenetic instructions from the outside (i.e., it possesses active potentiality, developmental autonomy).*

Such a definition, omitting an origin from fertilization, would provide for eventually including entities that may originate in a different way, e.g., advanced stem cell-derived embryoids.

It has not remained undebated, however, to view an embryo in such a way as a complete system of its kind, engaged in development, already autonomous in the sense that it does not need any specific external signals that would act as morphogenetic instructions. Criticism focused, in particular, on pointed wholistic formulations of this view (which emphasize the aspect that the whole is more than the sum of its parts; "emerging properties" is a term playing a role in those discussions). More recently, however, it was concluded that we have reason to take a new look at the development of (or towards) an organismic whole [46]. The actual research on stem cell-derived embryoids gives now reason to refocus on such concepts of organismic development since the recent observations show that self-organization can lead to unexpected structural and functional complexity and that these complex constructs may approach viability. The known facts and existing concepts of developmental biology, in particular with regard to questions of pattern formation and morphogenesis, do give clear hints on how organismic development can be initiated in stem cell colonies under appropriate in vitro conditions, and of particular interest here is the process of basic body plan development, i.e., individuation [47]. Gilbert and Sarkar [46] emphasized that it might be necessary to apply computer-aided analysis and computer modeling in order to deal with the high degree of complexity of developing 3D systems. It has indeed been shown that these processes can be modeled, e.g., in computer simulations of basic body plan development [48–50].

The recent observations on the development of complex embryoids by the self-organization of stem cell colonies in vitro have also led to a renewed interest in definitions of *potentiality*. Using the potentiality aspect in discussions on stem cell ethics [51] has sometimes been rejected viciously [52]. These critics had overlooked, strangely enough, that what is meant when considering totipotency as an ethical argument is active (not passive) potential in the sense of Aristotelian philosophy [53]. Self-organization is, of course, a clear example of active potentiality since it does not need any specific structuring information input from the outside (which passive potentiality does require), thus documenting the functional autonomy of the ongoing morphogenetic processes. Interestingly, a recent proposal for dealing practically with the ethical aspects of stem cell-derived embryoids [10] comes back to focusing on functional aspects and potentiality, and those authors emphasize once again the importance of distinguishing between active and passive potential (although they do not mention previous publications that had already referred to this difference in the same context [53]). On the other hand, those authors attribute an essential role in morphogenesis to uterine factors, which I would not subscribe to, as mentioned above [40]. Notwithstanding the latter difference in interpretation, I agree with those authors on the conclusion that what should be meant by totipotency of embryoids is an active (not a passive) potential for organismic development.

The potentiality of stem cells is mostly not discussed with regard to their active or passive expression but to the cell type differentiation potential and its possible limits: unipotency, multipotency, and pluripotency. Unfortunately, the term "pluripotency", although so widely in use for embryonic stem cells (ES cells) and induced pluripotent stem cells (iPS cells), is somewhat misleading insofar as it may suggest that not all types of cells

but only a limited number can be formed. It has been criticized for this reason, and there has been some discussion about replacing it with a term like "omnipotency", indicating the potential to differentiate all cell types but not necessarily a structure with organismic properties (embryoid or embryo). Totipotency would designate an embryo-formation potential (in the case of stem cells, embryoid formation by self-organization) [14]. In recent years, a trend has developed to use the term "totipotency" for stem cell lines that have a full cell type differentiation potential (in particular, naïve "expanded potential" stem cells, 2 cell-like stem cells, 2CLCs). Not completely consequent seems to be the use of the criterion whether cells must also have a potential for the formation of extraembryonic cell types (trophoblast, etc.) to be addressed as "totipotent".

The recent research on stem cell-derived embryoids shows that the self-organization of colonies of naïve pluripotent/omnipotent stem cells can reach a complexity comparable to that of traditional embryos. We have no reason to assume, however, that a single omnipotent stem cell would possess structural preinformations for axial organization in the same way as a zygote. Instead, there is now ample evidence that, after cells have multiplied and formed a colony, cell–cell and cell–matrix interactions can lead to initiating self-organization into an organismic embryonic system. This means that active totipotency can be gained by "pluripotent"/omnipotent stem cell colonies. At that point, asymmetries in the colony (as always present in culture, but as can also be introduced in a planned manner by the experimentalist) are read by the stem cell colony as surrogate signals for asymmetry pre-patterns, which are in normal development provided by the zygote cytoplasm [47]. The stem cell colony can thus convert at this critical point from a so-to-say non-organismic state to a state of developing organismicity (and, this way, gain active totipotency, i.e., developmental autonomy).

In organoid research, these considerations are not relevant if stem cell lines that have only a limited differentiation potential (e.g., mesenchymal stem cells, MSC) are used. If pluripotent/omnipotent stem cells are used as a starting material, these considerations may become relevant, depending on the question of whether culturing conditions are used that may allow organismic development to be initiated, even if it is transitory.

4. How to Avoid the Ethical Problem of Organismic Development in Stem Cell Research Practice

According to the definitions discussed above, stem cell researchers working strictly with organoid models can, under certain precautions, feel ethically on the safe side: They do not need to take measures to avoid any unwanted initiation of an organismic embryo development (totipotency) program, at least not as long as a cell line with restricted potential is used (e.g., a somatic stem cell type such as mesenchymal stem cells, MSCs, which are lacking any organismic developmental potential; examples are an MSC-derived organoid [54] or organoids cultivated from intestinal stem cells isolated from crypts of the colon or small inestine [55]). The situation is more difficult when "pluripotent"/omnipotent stem cells are used, in particular naïve "pluripotent" cells (now often addressed as totipotent stem cells, 2CLCs; for a recent review discussing their epigenetic characterization, see [56]). These are the cells with which the most successful embryoid formation experiments have been performed recently. If such cell types are used as a source for tissue and organ model experiments, the investigator should make sure she/he uses conditions in the differentiation system that prevent colonies from expressing (inadvertently) a totipotency program and initiate organismic (rather than organ-specific) self-organization.

The latter may not be trivial, so a warning is in place: It can be difficult to detect such a transition to initiating a totipotency program at an early stage. Early in the history of human stem cell research, there was already a discussion about projects aiming to define molecular characteristics of various stem cell lines with regard to their potentiality for initiating the development of organismicity; they were not funded, however. Recent data show that the definition of epigenetic characteristics of totipotency is making progress [56]. Other, e.g., morphologically detectable signs have been considered, like gastrulation/primitive streak

(PS) formation, but the PS indicates a relatively late and advanced stage of development of an organismic state, indeed of finished individuation (twinning not possible anymore), and is thus ethically not really satisfactory as a test. A safer choice would be the use of strategies for bypassing pluripotency in stem cell derivation by reprogramming: These strategies can allow the production of cell lines that do not possess a complete organismic development potential but can still be useful for certain organoid modelings (reviewed in [57,58]).

All these considerations apply to the organoid researcher. In contrast, the embryologist, if not focussing on special questions of organogenesis (for which organoids may suffice) but if interested instead in organismic development, must deal with the ethical problem that human stem cell-derived embryoids should basically be considered biologically equivalent to embryos and that, therefore, the same legal precautions should be taken as for traditional human embryos. Alternatively, the embryologist has the option to avoid this ethical problem by choosing animal models, an option which includes, e.g., non-human primate embryos and, on a larger scale, stem cell-derived embryoids produced from such primate stem cells (e.g., [59]).

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