EMBRYOIDS: UNIQUE ENTITIES OR PROTECTED LIKE HUMAN EMBRYOS?

HEATHER ZEIGER, MS, MA
RESEARCH ANALYST

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Over the past two years scientists from several institutions have isolated an embryo-like structure that behaves like an early embryo, but lacks several key cell types to develop into a fetus. These entities are alternatively called embryoids, gastruloids, asymmetric cysts, or SHEEFs (Synthetic Human Entities with Embryo-like Features). These structures self-organize, develop rapidly, and have what ethicists call "features of concern." Should they come under the same regulations as embryos or are they unique entities?

For the purposes of this article, I will use the term "embryoids" because of their analogy to organoids. Organoids are not organs, just as embryoids are not exactly embryos. But organoids serve as cellular models of organs, and similarly researchers hope to use embryoids as models of early embryos. There are several groups working on embryo-like entities that they call "embryoids," but not all of these embryoids are made the same way, and they do not all have the same features.

According to an *MIT Technology Review* article, two years ago a Michigan University team discovered that they had made embryo-like bodies. They found that these embryoids lacked key cell types to progress beyond a certain developmental point. However, these embryoids behaved enough like embryos that the team still destroyed them before the fourteen day limit.[1]

What exactly embryoids are, however, is a more difficult question to answer. The University of Michigan team made it very clear that these entities were not embryos, referring to them as "asymmetric cysts." Their embryoids developed an amniotic sac and displayed self-organization, but they lacked key cells that would allow for development of the placenta, also referred to as the trophoblast. Additionally, the Michigan team only found one of the three cell types needed for embryonic development.[2]

Are Embryoids Just Another Type of Organoid?

Organoids are three-dimensional spherical structures that are typically grown on a scaffold and come from one of three sources: tissue, induced pluripotent stem cells (iPSCs), or embryonic stem

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CONTINUED ON NEXT PAGE

cells (ESCs). Importantly, their ability to form a three-dimensional structure is a key reason why scientists are interested in using organoids in research. Two-dimensional cell cultures do not display the same properties that three-dimensional ones do. As a result, if scientists want to accurately test whether a drug affects a certain organ in the body, they need to work with a three-dimensional model of those cell types. Organoids are also helpful in understanding how neighboring cells communicate with each other, something that has proven particularly helpful in cancer research.

Similarly, embryoids are also three-dimensional structures. While embryoids are typically made from embryonic stem cells that are placed in a gel matrix, they can also be made from induced pluripotent stem cells. The stem cells are placed in a constrained area, a microwell array for example, and allowed to grow, eventually forming an embryo-like ball. When the cells are not spatially constrained, they do not form embryoids.

Eventually these embryoids will exhibit a line of cells called a primitive streak that indicates body formation and directionality—that is, the development of a head and tail end as well as a front and back. In normal embryonic development, the embryo forms a primitive streak which then signals the cells to multiply and to migrate. This migration results in the formation of the three main cell layers (i.e., ectoderm, mesoderm, and endoderm). Some embryoids have shown evidence of a primitive streak and organization into three germ layers.[3]

Altered Nuclear Transfer

In 2004 William Hurlbut proposed altered nuclear transfer (ANT) as an ethical alternative to cloning.[4] At the time, ANT was only a theoretical possibility, but research with embryoids has brought the theoretical to real life.

ANT involves making genetic changes to the nucleus of a cell before the nucleus is inserted into an enucleated egg (i.e., an egg from which the nucleus has previously been removed). The egg is then stimulated, causing the cells to multiply. The genetic changes that are made to the nucleus prior to insertion into the egg would prevent the egg from becoming a cloned embryo. According to Hurlbut, the entity would be akin to a biological artifact rather than an organism.

At the time that Hurlbut proposed ANT, one of the major criticisms from the scientific community was that the procedure was too cumbersome to make it practical. CRISPR-Cas9 had not been invented yet, but now the gene editing technology may make it feasible to make genetic changes to the DNA in a cell. Other critics say that this does not get around the ethical problems present with cloned embryos. The question is whether the product of ANT is truly a biological artifact or if it is a defective or seriously (and intentionally) disabled embryo. There has been much deliberation over this point, which can inform our ethical examination of embryoids. Other ethical objections to ANT were raised beyond these concerns, such as invoking slippery slope arguments and objections to intentionally damaging human DNA, but these points are less pertinent for our present consideration.

Embryoids are similar to the theoretical products of ANT in that they do not have all of the necessary capacities to develop into a human fetus, but are sufficiently embryo-like to serve as a research model.

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Maureen Condic notes that even though these biological entities may share some of the same molecular processes as embryonic development, that does not mean, however, that they are necessarily embryos.[5] ESCs and iPSCs—when allowed to develop—share some of the same molecular processes as a developing embryo, but they do not exhibit the same kind of integrated global organization toward a particular end, namely a human person. "The global integration of tissues into a functional whole is the hallmark of a developing *organism* that distinguishes it from cells or tissues."[6]

Most embryoids show some organization into three-dimensional structures, but they do not exhibit global organization in the same sense that an organism does. From this perspective, embryoids are not, strictly speaking, the same thing as embryos even though they can serve as models for embryonic development.

Legal Considerations

In the United States and, until recently, around the world, research on human embryos is limited to the first fourteen days of development or the appearance of the primitive streak. The primitive streak is indicative of the first stages of the embryo developing a body plan.

When the fourteen-day rule was first determined by the National Research Council, the reasoning was that they did not want the embryo to experience pain or sentience, so they limited experimentation to a point that occurred before the formation of the nervous system or the brain. The Council determined that the formation of the primitive streak was a clear demarcation that occurred before the formation of the nervous system.

John Aach et al. argue in a 2017 *eLife* article that the fourteen-day rule is no longer adequate to draw the appropriate ethical lines because it was based on a linear track of normal embryonic development. As these synthetic entities (or SHEEFS as they call them) have demonstrated, they can bypass normal embryonic stages. They argue, instead, that ethical lines should be based on the emergence of specific features of concern.[7]

Additionally the Dickey-Wicker amendment—which has been included with every appropriations bill since 1996—prevents U.S. federal funds from being used for the creation of a human embryo for experimental purposes or for research with human embryos in which they are destroyed or discarded. The amendment defines a human embryo as "any organism, not protected as a human subject under 45 CFR 46 [the Human Subject Protection regulations] . . . that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells."[8]

Pertinent to our present discussion, the violation of the Dickey-Wicker amendment hinges, first, upon looking at origins of the embryoid (When is it derived?), and, secondly, upon whether or not embryoids are human organisms.

Ethical Questions

There are three areas of enquiry to highlight the ethical issues surrounding embryoids: 1) What are they? 2) How are they made? and 3) What is their intended use?

The embryoids considered in the MIT article were made from embryonic stem cells derived from an eight-day old embryo. Because the extraction of an embryonic stem cell leads to the destruction of an embryo, those who hold to the sacredness of all human life from conception to death consider it unethical to create human embryoids from such sources. As noted earlier, however, embryoids also can be made from induced pluripotent stem cells, which are considered an ethical source of stem cells. Despite being considered an ethical source of stem cells, the intended use of iPSCs remains a point for ethical consideration. For example, iPSCs have been used to make gametes, egg, or sperm that can then be used to create an embryo. Formation of gametes in this manner, known as gametogenesis, raises its own set of ethical concerns.

Embryoids seem to have many of the features that define a biological organism. They respond to environmental stimuli, exhibit self-organization, undergo growth and development, and consume energy. The cells within embryoids can be said to reproduce in the same sense that embryonic cells grow and divide. However, the embryoid itself lacks the necessary cells to grow into a human that, in turn, can reproduce. Furthermore, its self-organization is local in contrast to the global and directional organization of embryos.

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Philosopher Melinda Fagan has proposed that stem cells mediate the transition from cells to organisms. To complicate matters further, embryoids and organoids are both made from the stem cells she claims mediate this transition. Embryoids are more organized than organoids, making them closer to an organism than an organoid; but embryoids are still more simplistic in structure and function than an embryo.[9]

Condic comments that

> "In the end, development has to be defined by something other than the molecular events that occur, and I argue [in several papers] that the 'something else' is the capacity to produce the first two cell types of the embryo in an ordered developmental sequence; i.e., the outcome of an integrated developmental process, not simply one or more molecular events also observed during the process of development."[10]

If embryoids satisfy the definition of an organism, then the follow-up should be, "What kind of organism?" or as Condic asks "Organized towards what?"

As noted earlier, John Aach et al. argue that research with embryoids should be limited, not by the fourteen-day rule, but by the appearance of morally concerning features. In this way, synthetic entities that bypass any of the normal steps in embryonic development can still be regulated based on the appearance of certain features, such as the primitive streak.[11] Embryonic development itself, however, is not merely a series of steps as Aach et al. suggest, but an orchestrated process. It is not enough to define steps and molecular events. The end goal must also come into play for moral considerations.

Finally, the intended purpose for making embryoids is another important consideration. One question is whether the same purposes can be accomplished with a different method. Much of what we know about pluripotent stem cells comes from mouse studies, and embryoids were first developed from mouse embryonic stem cells. If animal studies can provide the necessary models for certain experiments, then it would be more appropriate to reserve those experiments to animal studies only.

That being said, human development is unique, and there are some things that cannot be known without testing them on human models. Human embryoids would allow researchers to see how a drug taken by the mother may affect

See the supplemental online resource "A Quick Guide to Embryo-Like Things" available at **https://cbhd.org/content/quick-guide-embryo-things** for a more detailed description of various types of embryo-like entities.

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the early stages of human development without risking harm to a developing child. Additionally, some diseases may be a result of developmental abnormalities. Embryoids may provide important insights into these types of abnormalities.

Some researchers wish to use embryoids in a similar fashion as organoids. They could provide a more ethical and less expensive alternative to testing in humans or normal embryos. However, others wish to create a purely synthetic embryo that can go through all of the phases of development. For them embryoids represent progress toward the goal of making a synthetic human embryo. This poses many of the same ethical problems as gametogenesis.

Human embryoids raise questions over the minimal features required for something to be considered a human embryo. The key ethical questions for our consideration are: Is this a mutilated or severely damaged embryo or a completely different entity? How would we know? How should we treat it?

1 Antonio Regalado, "Artificial Human Embryos Are Coming, and No One Knows How to Handle Them," *MIT Technology Review*, September 19, 2017, https://www.technologyreview.com/s/608173/artificial-human-embryos-are-coming-and-no-one-knows-how-to-handle-them/.

2 John Aach et al., "Addressing Ethical Issues Raised by Synthetic Human Entities with Embryo-Like Features," *eLife* 2017;6:e20674, https://doi.org/10.7554/eLife.20674.

3 Ibid.

4 The President's Council on Bioethics, "White Paper: Alternative Sources of Pluripotent Stem Cells," Washington, D.C., May 2005, https://bioethicsarchive.georgetown.edu/pcbe/reports/white_paper/text.html.

5 Maureen L. Condic, "Biological Definition of the Human Embryo," in *Persons, Moral Worth, and Embryos: A Critical Analysis of Pro-Choice Arguments*, ed. Stephen Napier, Philosophy and Medicine 111 (Dordrecht, Netherlands: Springer Science+Business Media B.V., 2011), 211–235.

6 Condic, "Biological Definition of the Human Embryo," 213, emphasis in the original.

7 John Aach et al., "Addressing Ethical Issues Raised by Synthetic Human Entities with Embryo-Like Features."

8 H.R. 1105, Omnibus Appropriations Act, 2009: https://www.congress.gov/bill/111th-congress/house-bill/1105/text (See sections 509 and 518).

9 Melinda Bonnie Fagan, "Stem Cell Lineages: Between Cell and Organism," *Philosophy, Theory, and Practice in Biology* 9, no. 6 (2017), https://dx.doi.org/10.3998/ptb.6959004.0009.006.

10 Email interview with Dr. Maureen Condic, June 4, 2018.

11 John Aach et al., "Addressing Ethical Issues Raised by Synthetic Human Entities with Embryo-Like Features."

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