DEVELOPMENTAL BIOLOGY

A model of human embryo implantation

An innovative microfluidic device has enabled the modelling of the events that occur in human embryos when they implant in the wall of the uterus. It could be used to help understand early pregnancy loss. SEE LETTER P.421

AMANDER T. CLARK

Each year, millions of women around the world experience early pregnancy loss[1]. Most pregnancy losses occur in the first trimester, around the time that the embryo implants in the lining of the uterus. Our lack of understanding of why some early pregnancies fail is frustrating to women and their partners. To fill this fundamental gap in our knowledge, models of human embryo implantation are greatly needed. On page 421, Zheng *et al.*[2] report the creation of an advanced model of human embryo development that recapitulates many of the key events that happen around the time of implantation (that is, during the peri-implantation stage).

The authors' model involves the generation of synthetic structures resembling the embryonic sac — which, later in development, becomes a fluid-filled bag and the embryo. It is a substantial advance on previous exciting models developed by this group[3] and others[4,5] because it reveals the spatial coordination of cell-differentiation events in the human embryo around the time of implantation. The events involved include the formation of the amniotic sac and a disc of cells inside it called the epiblast; the initial specification of primordial germ cells (PGCs; the eventual sperm or egg cells); and the start of a process called gastrulation. In gastrulation, the epiblast differentiates into the three germ layers of cells — the ectoderm, mesoderm and endoderm — that will form the fetus.

Crucially, this model does not involve the use of intact human embryos or an *in vitro* attachment system for human embryos[6]. Instead, it relies on a simple bioengineered microfluidic device and on the differentiation of pluripotent cells — which can differentiate into any type of cell in the body. These pluripotent cells can be derived from human embryos (human embryonic stem cells; hESCs) or generated through the conversion of differentiated human cells (human induced pluripotent stem cells; hiPSCs).

Human pluripotent stem cells (including hESCs and hiPSCs) that are grown in conventional culture conditions in the laboratory are molecularly equivalent to cells in an early-stage embryo that are ready to undergo gastrulation[7]. Thus, hESCs and hiPSCs can be grown indefinitely in this state in a tissue-culture incubator, or cryopreserved in liquid nitrogen and thawed and cultured again many years later.

Currently, hESCs and hiPSCs are used in different ways to study the various types of cell that make up human embryos and fetuses. They can be allowed to spontaneously differentiate to create aggregates of cells known as embryoid bodies[8]. Alternatively, their differentiation can be directed by incubation with specific cocktails of growth factors, to create simplified 3D models of fetal organs or specific germ layers[9,10]. hiPSC lines derived from patients can be used for 'disease in a dish' modelling. Such strategies give rise to the same cell types as those found in late-stage embryos and fetuses; however, the initial germ-layer differentiation seen in these models is disorganized, and bears no resemblance to the spatially organized differentiation seen in human embryos during implantation. Therefore, such models have not been very helpful for studying the peri-implantation stage of human pregnancy.

To create a 3D environment in which the stem cells could develop into embryonic-like sac structures, Zheng and colleagues engineered a microfluidic device containing three channels: a central channel for loading a matrix material, another for loading hESCs or hiPSCs, and a third containing flowing liquids carrying molecules called morphogens that induce stem-cell differentiation (Fig. 1a). The

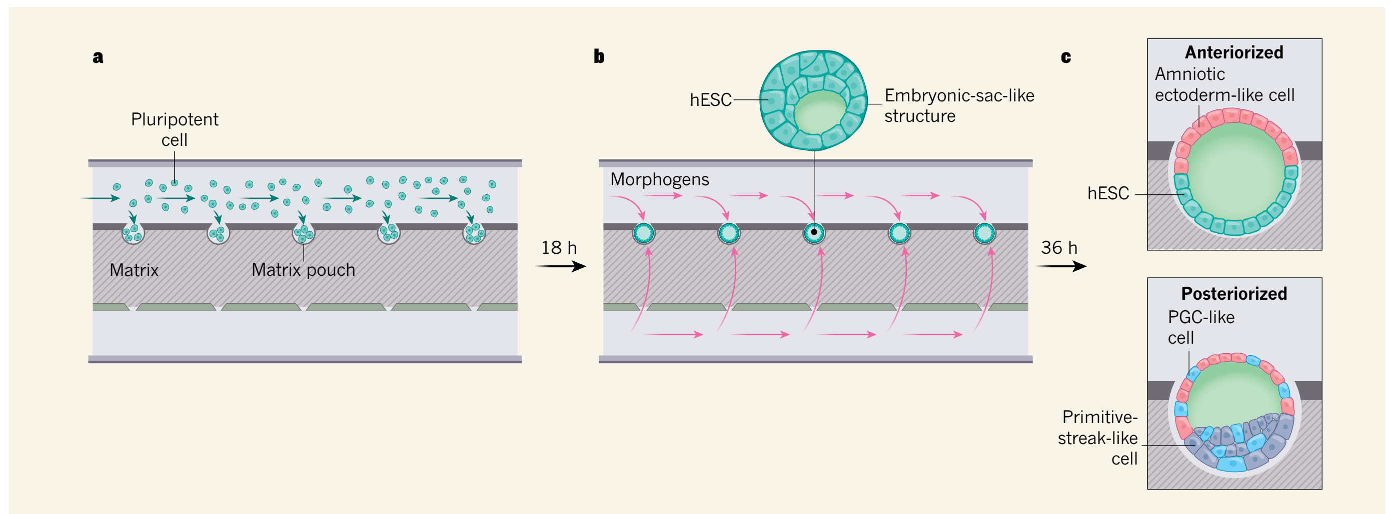

Figure 1 | Modelling human embryo development during implantation. **a**, Zheng *et al.*[2] developed a three-channel microfluidic device containing a cell-loading channel, a matrix-filled channel and an induction channel through which morphogens (molecules that affect development and growth) can be pumped. Human pluripotent cells (which can differentiate into all the cell types of the body) that are fed into the cell-loading channel settle in matrix pouches. **b**, After 18 hours, the pluripotent cells (in this case, human embryonic stem cells; hESCs) form embryonic-like sac structures. Morphogens pumped through the induction and cell-loading channels influence the development of pluripotent cells on either side of each sac. **c**, After 36 hours of exposure to various morphogens, the sacs show signs of anteriorization or posteriorization — that is, features of the anterior or posterior ends of an embryo. Anteriorized and posteriorized sacs have cells resembling those that form the developing amniotic membrane (amniotic ectoderm-like cells), and posteriorized sacs show cells that resemble primordial germ cells (PGCs; the eventual fetus's sperm or egg cells) and cells of the posterior primitive streak (which, in human embryos, form the basis of the body's bilateral symmetry).

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microfluidic device was lined with trapezium-shaped posts 80 micrometres apart that created evenly spaced matrix pouches in which the stem cells could grow and differentiate.

The authors loaded stem cells into the device and, 18 hours later, pumped morphogen-containing fluids into either the liquid-containing channel on one side or the cell-loading channel on the other (Fig. 1b). After 36 hours, structures with 3D organization that mimicked a peri-implantation human embryonic sac had formed in each pocket. Furthermore, the authors could use different morphogens to induce anteriorization or posteriorization — that is, the development of characteristic features of the anterior or posterior ends of a normal embryo (Fig. 1c). However, the embryonic-like sacs did not anteriorize and posteriorize at the same time, which is crucial for further embryo development.

In posteriorized embryonic-like sacs measuring just over 100 micrometres in diameter, the authors observed cell populations that resembled a developing amniotic membrane (the membrane that lines the walls of the amniotic-fluid-filled sac), a posterior primitive streak (which forms the basis of the body's bilateral symmetry), mesoderm-like cells and, remarkably, PGC-like cells. In the anteriorized embryonic-like sacs, the authors saw anterior primitive streak-like cells and endoderm-like cells. Thus, this is one of the first humanized models that can enable study of the awesome complexity of spatial relationships between cells in the peri-implantation window of development.

At this point, readers might be asking whether these embryonic-like sacs are, in fact, human embryos. The structures can be considered imitations of the real things, but crucially, they are not viable (they could not develop into a normal fetus) and do not have certain essential structures, such as a primitive endoderm and a trophoblast cell layer, which are necessary for the formation of the placenta and further membranes that surround the embryo.

The model also brings up the question of the 14-day rule: the internationally recognized consensus that scientists can grow intact human embryos in the lab for only 14 days, or until the primitive streak forms, whichever happens first[11]. In this study, either anterior or posterior primitive-streak-like cells formed in the structures, enabling investigation of the cell-signalling events in this process. However, in my opinion, an intact human embryo is created by the fertilization of an egg by a sperm cell and by the resulting formation of embryonic and embryo-surrounding cell lineages, which these sac-like structures were not.

Furthermore, the 14-day rule was created to help safeguard against development of embryos to a stage at which they could become sentient or feel pain — and neither could occur in this model because anterior and posterior cells were not specified at the same time and, although untested, it is likely that no sensory neuronal cells would have formed. Therefore, Zheng and colleagues' microfluidic model might technically be exempt from the 14-day rule because it does not represent an intact embryo in the way that we consider an embryo donated for research from an *in vitro* fertilization clinic to be intact. However, the ethics of running experiments using human embryonic-like sacs for longer than 14 days should be evaluated, with particular focus on whether the structure should at any point be considered a human being.

Finally, I urge policymakers to consider this device to be a useful addition to the human-stem-cell toolbox, as it can be used to examine a period of human embryonic development that is currently inaccessible for *in vivo* research. The model can be scaled up to become high throughput, and thus could be invaluable to environmental toxicologists. The length of time for which these structures can be studied in the device is relatively short; currently, most of them collapse within a few days. In future, if these embryonic-like sacs could be cultured for longer, they could provide crucial extra information about embryonic development. In turn, we might be able to use this information to help millions of women around the world to better understand or even avoid early pregnancy loss. ■

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COMPUTING

Stochastic magnetic bits rival quantum bits

Circuits based on the stochastic evolution of nanoscale magnets have been used to split large numbers into prime-number factors — a problem that only quantum computers were previously expected to solve efficiently. **SEE LETTER P.390**

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Data encryption typically relies on the practical difficulty of a process called prime factorization. In this process, a huge number (represented by 1,024 or more bits) is decomposed into a product of prime numbers. Such a task is notoriously time-consuming for conventional computers and is estimated[1] to be much more efficient for a future quantum computer — assuming that such a machine is built and uses a method called Shor's algorithm[2]. On page 390, Borders *et al.*[3] demonstrate that an integrated circuit (a computer chip) containing nanoscale magnets can split numbers up to 945 into prime factors efficiently. Such a nanomagnet chip is much easier to make than a quantum computer and, if improved, could threaten data encryption.

If you are reading this article on a computer, you are probably using electronic bits in the machine's processor and magnetic bits in its hard drive. Electronic bits are based on semiconducting devices called transistors. Such a bit has a definite state (0 or 1) that depends on whether a net negative or net positive charge of thousands of electrons is stored in the gate (a terminal of the transistor) (Fig. 1a). By contrast, a magnetic bit is based on hundreds of thousands of electron spins (magnetic moments) in a magnet. The state of this bit can also be either 0 or 1, depending on whether the net spin of the electrons points down or up (Fig. 1b).

For these two types of computing bit, a large energy barrier needs to be overcome to switch between the 0 and 1 states. As a result, the states persist despite random 'relaxation' forces caused by thermal fluctuations in the environment. Borders and colleagues' chip uses nanomagnets in which the barrier between the 0 and 1 states is small. Consequently, random relaxation forces cause the nanomagnets to randomly fluctuate between the two states, with a certain probability that the net spin points up or down (Fig. 1c). Such bits are therefore called probabilistic bits (p-bits). Borders *et al.* used their chip to perform prime factorization on numbers as large as 945.

A further type of bit, which is used in quantum computers, is known as a quantum

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