Curing Classical Homocysteinuria with Pediatric in vitro engineered Liver Organoid Transplantation?

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Abstract

Organoids are groups of cells grown in the laboratory that have self-organized into cell structures resembling those of organs. The term “organoid” means “organ-like.” In many cases, the cells and cell structures give organoids abilities similar to those of the organs they resemble. Brain organoids, for example, develop layers of nerve cells (neurons) with signaling activity and even “brain regions” that resemble areas of the human brain. Currently, organoids created by researchers may bear a strong resemblance to a fully mature organ in certain respects, but crucial differences still exist. Intestinal organoids have a variety of cellular structures that resemble parts of the intestinal mucosa, but are typically the size of a pea and thus not nearly as large or complex as our intestinal tract. But even though they are small, or do not correspond one hundred percent to entire organs, scientists can learn a lot from organoids. Experts believe that organoids represent the “next generation” of biological tools for research, drug development and medicine. Liver Organoid engineering could play an important role in treating and curing classic homocysteinuria in childhood by resetting the disturbed function of cystathion beta synthetase activity in the implanted organoid liver. This manuscript focus on liver organoid research to date with special attention to classic homocysteinuria in childhood.

Introduction

An organoid is an organ-like microstructure a few millimeters in size that can be artificially generated using cell culture methods. Under appropriate culture conditions, organoids can be grown from one or a few tissue cells, embryonic stem cells, or induced pluripotent stem cells. Unless mesenchymal stem cells have been used, organoids lack stroma and vessels; however, they still exhibit physiologically relevant organ-like properties. The generation of organoids requires pluripotent stem cells as starting material. Such cells are in a state from which they are capable of differentiating and structuring themselves together. The result of self-organization is tissue-like associations of differentiated cells that differ in shape and function. The structure of organoids at least partially resembles human or animal organs. Organoids are usually not formed on an agar layer; they require liquid culture medium that provides the opportunity to grow spatially in a 3D cell culture. The production of organoids requires a sterile cell culture laboratory to perform the sophisticated, constructive tissue engineering. This field of work and research in biotechnology may also use genetic engineering techniques, most notably the CRISPR/Cas method. The product range includes tiny models of internal organs (heart, stomach, intestine, kidney).

Amazing progress is being made with the complex structures of the brain. Such cerebral organoids model cerebral cortex, hippocampus, midbrain, hypothalamus, cerebellum, anterior pituitary, and ocular retina of humans, mammals, and less commonly other vertebrates. Protocols exist for growing them, which cause regions of the brain to develop. Researchers are developing mini-livers, or liver replacement tissue, which temporarily improves the performance of the liver. This involves reprogramming skin cells in the laboratory with four substances, the Yamanaka factors, which convert them into stem cells. From these cells, all cell types of the body, including healthy liver cells, are grown. Another option is to use adult liver cells from human tissue. These liver cells come from patients who are biopsied with a large needle. A third option is to stimulate the small functional tissue areas from an otherwise old, diseased liver with growth factors or certain proteins so that the stem cells or adult cells develop tiny three-dimensional structures: Organoids made of liver cells, special immune cells, bile ducts and blood vessels. Stem cells can first be grown...
from patients’ skin cells, and then “miniature brains” or “mini-livers” can be grown from them, so-called “organoids”. Stem cells can be grown from patients’ skin cells and then used to grow “miniature brains” or “miniature livers”, so-called “organoids”. Researchers have transplanted mini-livers into sick mice. They grew amazingly well and took over the function of the damaged organ. The next step is to implant the liver organoids into the livers of deceased humans. In Vitro Engineering of Organoids is based on different steps. Step 1 is preparing a 2D preculture.

Organoids are generated from either primary cells (i.e. intestine, lung, or kidney) or induced pluripotent stem cells. Stem cells are capable of differentiating and self-assembling into a variety of specific organoids. Typically, cells are mixed with Matrigel and drops of this mixture are placed in a 24-well plate at room temperature. The plates are then placed in an incubator to form solid droplet domes. Media is then added for seven or more days to promote cell growth and differentiation into a specific tissue (brain, intestine, lung, etc.). The media contains extracellular matrix (ECM) proteins and various growth factors that vary depending on which tissue is being generated. Organoid culture is a lengthy process and may involve several cultivation steps with different media. During this process, the health of the cells must be monitored using methods normally used to track developmental biology and understand tissues (imaging). Before experiments are performed, organoids must be reviewed and characterized to ensure they have the correct tissue structure and differentiation. High-content imaging allows for verification and visualization of organoid growth and differentiation, 3D reconstruction of structures, complex analyses of organoid structure, cell morphology and survival, and expression of various cell markers. Confocal imaging and 3D analysis of organoids allow visualization and quantification of the organoids and the cells that form the organoid. Characterization of multiple quantitative descriptive features of organoids can be applied to explore disease phenotypes and connectivity effects.

**Types of Organoids (Epithelial/Multi-tissue and Multi-Organ)**

**Hepatocyte Liver Transplantation**

These human liver cells are to be transplanted once into patients with liver disease and thus help regenerate their defective organ. But before the healing hepatocytes can be transplanted, they first have to be obtained. And that is not so easy. Because liver cells are very sensitive, it is tricky and expensive to isolate them from donor organs. This is done by perfusing the liver with a special buffer solution, with enzyme solutions, which means the solution runs through the organ, through the vessels, and thereby separates the tissue into a cell suspension. This is cryopreserved, that is, frozen, with a special freezing program, so that these cells can be thawed again at some point and viable liver cells are obtained again after thawing. After numerous experiments with pig livers, the process now also works with human hepatocytes. A cluster of cells can be seen under the microscope: Human liver cells showing all typical functions four days after thawing potential raw material for liver cell therapy. The Hanover researchers want to start the first clinical tests as early as the next six months. But even if they are successful, one drawback will remain: Because the isolated liver cells hardly reproduce in the laboratory, only as much tissue can be transplanted as was previously cut out of a donor organ. Primary adult hepatocytes are not appreciably proliferative in vitro, despite long intensive research. In contrast, stem cells can be derived from regenerable cell resources like bone marrow. Adult stem cells from bone marrow can turn into liver cells after transplantation, this was recently demonstrated in the USA. The decisive advantage here is that because stem cells can be easily multiplied in the laboratory, physicians could have as much healthy liver tissue available as they want in the not too distant future. Paradise conditions for surgeons. But until this dream comes true, researchers must first understand what actually happens when a stem cell transforms into a liver cell. The mechanisms of this cell differentiation are still largely in the dark. And until they are deciphered, clinical application remains risky. So for the time being, donor organs remain the only source of liver cells.

**Mesenchymal Stem Cell Transplantation**

Mesenchymal stem cells (MSCs) are multipotent progenitor cells of various cell types derived from mesenchyme. They can differentiate into osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells) and adipocytes (fat cells), among others. In addition, differentiation into neurons, astrocytes, and oligodendrocytes (cells of the nervous system) has also been observed. Mesenchymal stem cells have a high proliferation and differentiation potential. Adult mesenchymal stem cells contribute to the maintenance and regeneration of supporting and connective tissues, such as bone, cartilage, muscle, ligaments, tendons, and adipose tissue. They also support the growth and development of blood progenitor cells in the bone marrow (hematopoiesis). MSCs can be isolated from almost all tissues (bone marrow, cartilage, adipose tissue, muscle, liver tissue, blood, amniotic fluid). Due to the very high MSC content as well as the good accessibility, MSC are often isolated from adipose tissue (e.g. from liposuction aspirates). Because of the heterogeneity of purifications, the term mesenchymal stem/stromal cells (rather than mesenchymal stem cells) is now preferred by the scientific community. MSCs can be cultured and differentiated into various cells and tissues in vitro. Specific functional differentiation can be actively controlled by activation or suppression of genes, resulting in the formation of bone marrow stroma supporting hematopoiesis or differentiating into osteogenic, chondrogenic or adipogenic cells. In addition, differentiated MSCs are capable of transforming into another tissue and adapting to novel environmental conditions.

Signaling agents for these regeneration and growth processes are cell-cell contacts and the secretion of growth factors and cytokines. They are mostly obtained from bone marrow (aspiration from iliac crest, also tibia or femur). In stem cell transplantation, the hematopoietic components from bone marrow (or peripheral blood) are transferred to the tumor patient previously treated by total body irradiation or high-dose chemotherapy; MSCs play an essential role in the reconstruction of the destroyed bone marrow.

**Hepatic Progenitor Cell Transplantation**

Hepatic stellate cells (HSCs) are best known for their involvement in fibrosis development. In contrast, their function in the normal liver has remained largely unclear. Our studies now show that HSCs can be regarded as mesenchymal stem cells (MSCs) and can fulfill their functions. Thus, rat HSC express typical markers of MSC such as nestin, NG2 (neuronal-glial antigen 2), CD105 (endoglin) as well as CD146 and are able to differentiate into adipocytes as well as osteocytes in vitro.
Furthermore, isolated HSCs support the maintenance as well as differentiation of hematopoietic stem cells in a co-culture system and can thus replace MSC of the bone marrow.

Transplanted eGFP+ HSC, previously purified by FACS (fluorescence-activated cell sorting) based on their retinoid fluorescence, colonize the bone marrow of recipient animals in accordance with their characterization as MSC. After damage to the liver by partial hepatectomy and concomitant application of 2-acetylaminofluorene, FACS-sorted HSC reach the injured liver of the recipient animal and form approximately 11% (± 2%) of the liver tissue after 7 to 14 days. The transplanted HSCs give rise to hepatocytes and cholangiocytes in addition to mesenchymal tissue.

During their differentiation into hepatocytes in vitro, HSCs, like bone marrow- and umbilical cord-derived MSC, transiently undergo the expression profile of hepatic progenitor cells (oval cells), which typically express keratin 19, EPcam (epithelial cell adhesion molecule), and α-fetoprotein. Finally, in the course of their hepatic differentiation, HSCs and other MSCs exhibit the synthesis of a broad spectrum of bile salts as well as the transporters BSEP (bile salt export pump) and NTCP (sodium taurocholate cotransporting polypeptide) in addition to the release of albumin. These findings indicate for the first time that oval cells arise from stellate cells or other MSCs in the body and thus HSCs may fulfill an important role in liver regeneration.

**Multi-Organ**

Acute (ALV), chronic (CLV), and acute-on-chronic liver failure (ACLV) occur frequently in critical care and are associated with high mortality. Since the accumulation and lack of elimination of endogenous and exogenous toxins is one of the main problems in any form of liver failure, the focus today is on the use of detoxification systems that primarily support the detoxification function of the liver and thus indirectly its regeneration. This chapter introduces the MARS (“molecular adsorbents recirculatory system”) and Prometheus procedures. However, the standard use of extracorporeal liver support systems cannot be recommended in acute or acute-on-chronic liver failure or alcoholic hepatitis. In special situations, however, their use may be helpful, either as a bridge to possible transplantation or until recovery of liver function.

**Discussion**

Organoids are three-dimensional (3D), multicellular microtissues derived from stem cells and engineered to mimic the complex structure and functionality of a human organ, such as the lung, liver, or brain [1-35]. Organoids are multicellular and exhibit a high degree of self-assembly. They further reproduce complex in vivo cell responses and cell interactions compared to traditional 2D cell cultures. There are three unique definitions that constitute an organoid. It is a 3D biological microtissue that contains different cell types [36-39]. It reflects the complexity, organization and structure of a tissue. It shows similarity to at least one functional aspect of a tissue. Organoids are becoming increasingly important in the fields of cancer research, neurobiology, stem cell research, and drug discovery because they enable improved modeling of human tissues. Because they are generated from stem cells, organoids can be differentiated into a wide range of tissue types, including liver, lung, brain, kidney, stomach and intestinal tissues. Because these 3D microtissues mimic in vivo organs, they can provide researchers with greater insights into the mechanisms of human development and disease. For example, researchers can use organoids from genetically modified cells to determine how certain gene mutations relate to specific genetic diseases. Organoids can also enable the study of infectious diseases and host-pathogen interactions. Last but not least, the ability to use patient-derived organoids for drug screening and toxicity assessments will allow researchers to make further advances in personalized medicine. Due to the increasing complexity of organoids and other 3D cell systems, even more sophisticated techniques for 3D imaging and analysis are required to accurately and efficiently describe these biological structures. Today, automated confocal imaging systems and 3D image analysis software are widely used to help researchers optimize their workflow and achieve optimal results [30,36,39-42]. Self-organization makes life easier for organoid researchers, but it has its limits in vitro. Despite regular divisions, most organoids develop a necrotic core and stop developing [27,36,41,43]. Photoreceptors on the organoid surface, for example, continue to mature after a year, but the internal structure deteriorates over time. This is because diffusion alone cannot provide the metabolic requirements for nutrient and oxygen supply and waste disposal at the center of an organoid. This limits organoids to diameters of three to four millimeters. A number of ideas now exist to delay necrosis. For example, the organoid diameter can be limited - for example, by culturing at an air-liquid interface, with vibratomes, or with artificial chips that physically allow growth in only two spatial directions. None of this, however, solves the actual perfusion problem. That is why vascular networks are considered the holy grail of organoid research [32]. Liebau explains the methodological challenge as even with the addition of endothelial stem cells, organoids are not automatically infused with blood vessels. Growth and organoid growth are different. In vitro self-organization fails here. In embryogenesis, for example, the retina is not directly vascularized either, and blood vessels only grow in later. Replicating this interaction of two entities in vitro while outsmarting biology is difficult. One approach to solving the problem is offered by certain 3D bio-printers, known as inverse extrusion printers. They print networks of 0.4- to 1-millimeter-wide gelatin channels into densely packed clusters of cells, which are then liquefied, lined with endothelial cells and flooded with oxygenated medium. From human iPSC cells, they grew vascular organoids whose endothelial cells and pericytes formed a capillary network. After transplantation into mice, the capillaries even interconnected with the murine bloodstream and formed perfused arteries, arterioles and venules [1-105]. Three-dimensional tissue models are more produced in the Petri dish to develop blood-liver organoids that can be used to observe disease processes at the cellular and molecular levels [32,41,43,44]. Organoids, often referred to as miniature organs, are cell culture models that represent a tissue in three dimensions in the Petri dish. Researchers usually grow them from stem cells that are not yet or barely differentiated. They can develop into any cell type, such as heart or kidney cells, muscle cells or neurons. In the laboratory, they become liver cells. As in real organ tissue, these are permeated by various blood and immune cells. Liver cells do not develop in a vacuum, but together with white blood cells and other cells of the body’s own defense system. And like real liver cells, the liver cells created by his group produce blood - visible as red spots between the rice-grain-sized clusters of cells. Organoids are a kind of window into a previously inaccessible organ, the fetal liver. Tickling liver cells awake with genetic scissors is possible. In organoids, this genetic manipulation works much better than in cells derived...
from animal models [42,45]. For example, liver cells that slip into an identity crisis in the course of chronic liver disease and no longer behave like liver cells can be genetically reactivated in the Petri dish. With the help of the gene scissors CRISPR, it is possible to intervene in a liver cell so that it once again does what a liver cell must do. In this way, the miniature organs help reduce the number of animal searches. New gene therapies can first be tested on organoids.

Animal experiments are then only necessary to confirm the experiments in the living organism. Another advantage, he said, is that organoids are available almost indefinitely because their cell source never dries up. In conclusion, organoid research is a very interesting field for the future to treat and cure different pediatric diseases like homocysteinuria or other rare diseases [1-105].

Organoid research will be performed worldwide and it is a challenge for the future to implant organoids into human being and to restore, especially in homocysteinuria, the function of the liver.

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SB performed research, data collection and references; EL and GV read the manuscript and gave important ideas; EM checked the format and the grammar. LB checked the grammar and style of the manuscript. LB checked the format and the grammar.

Data Availability

Any inquiries regarding supporting data availability of this review should be directed to corresponding author.

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