Liver Organoid Research: Present situation, limiting factors and future therapeutical potential in pediatric diseases

Abstract
Organoids are three-dimensional, organ-like cell assemblies in which different cell types have organized themselves in a way that is approximately typical for the corresponding organ in the body. They show three characteristics: self-organization, multicellularity and functionality. The range of organs that can be studied with organoids is growing rapidly and includes the brain, intestine, kidney, stomach, pancreas, lung, liver, prostate, esophagus, gallbladder, and the female reproductive tract, among others, and also the embryo. Organoids are grown either from pluripotent stem cells or from tissue-specific adult stem cells. Adult stem cells are present in a large number of tissues and are responsible for renewing the cells in these tissues. They can only give rise to the cell types that are present in the particular tissue, the stem cell of the intestinal epithelium only produces cells of the intestinal epithelium, but not muscle cells or nerve cells. They are thus multipotent.Today, it is possible to reconstruct organ-like tissue organoidsin the laboratory. Stem cells are thereby induced to differentiate by molecular signals and grown in culture systems that promote their three-dimensional self-organization. Rapidly developing organoid technology makes it possible to phenotypically copy cell structure. To some extent, this is also true for the functions of various human organs (for example, brain, thyroid, thymus, intestine, liver, pancreas, stomach, lung, kidney) and even early-stage embryos.As near-physiological 3D culture systems, organoids open up new possibilities to study the development of healthy and diseased organs and offer great potential for translational research. This manuscript concentrates on liver organoid research and its future role in different pediatric diseases.

Key Words
organoid-pediatric-liver-hepatocyte-child-future research

Introduction
Scientists often use organoids to research diseases (1-56). These clusters of specific cell types are created in the laboratory from human stem cells. Depending on the nutrient solution and treatment, they form three-dimensional organ-like structures from several cell types (1-56). They can be used to more realistically reproduce malfunctions or developmental steps because they are more similar to the human body than one-dimensional cell cultures of individual cell types (2,4,23,34,56). In particular, cells in brain organoids can even mature to the point where they resemble those of a postnatal brain. The brain cell clusters mirror the genetic and structural changes in the brain of a newborn. Previously, researchers had assumed that the organoids were only suitable for studying prenatal brain development. Organoids could also be suitable at the advanced stage to study neurological diseases that develop only with the more complex development of the brain. Concerning the liver organ, it has been known since Aristotle that the human liver has the greatest regenerative capacity of all organs in the body and can regrow even after an amputation of 70%. This makes transplantation by liver donors possible. The molecular mechanisms by which adult liver cells trigger regeneration are still largely unknown. About 29 million people in Europe suffer from chronic liver diseases such as cirrhosis or liver cancer. They are a major cause of disease and mortality, with liver disease contributing to about two million deaths worldwide each year. Currently, there is no cure and liver transplants are the only treatment for liver failure. Scientists in the field of molecular cell biology and genetics are investigating the biological basis of liver regeneration in humans. In 2013, Huch andClevers developed the first liver organoids-miniature liver tissues that were created from mouse liver cells in a Petri dish in the laboratory. The researchers even succeeded in transplanting the organoid into a mouse, where it could take over liver functions. In 2015, they successfully transferred this liver organoid technology to culturing a human liver in a Petri dish based on human liver samples. The two most important functional cells in the adult liver are the hepatocytes, which perform many functions in the liver, and the ductal cells, which form the network of tiny ducts through which bile is directed to the intestine. These work together with

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other supporting cells, such as the blood vessels or the mesenchymal cells. To build liver organoids, researchers initially used only ductal cells of the bile duct. In a healthy liver, there are a certain number of contacts between the ductal cells and the mesenchymal cells that signal the ductal cells not to proliferate and just stay as they are. Once the tissue is damaged, the mesenchymal cells reduce the number of contacts they have with the ductal cells so that the ductal cells can proliferate to repair the damage. From their observations, the researchers concluded that it is the number of cell contacts, rather than the number of both cell types, that determines how many cells are produced to repair the damaged tissue. Too many touches by mesenchymal cells mean that fewer or no new ductal cells are produced, while fewer touches mean that more cells are produced.Organoids are three-dimensional structures of cells generated from stem or progenitor cells in vitro. They resemble organs in vivo in terms of the cell types they contain, their spatial arrangement and specific functionality (4,7,12,26,52). Their development is often characterized by the term "self-organization". This is understood as a process of formation of complex structures from initial cells by interactions of the cells with each other and between the cells and their environment. Stem cells are cells that can give rise to further stem cells as well as specialized cells (ability to differentiate) by division. Progenitor cells, on the other hand, are descendants of stem cells already committed to the formation of specific cell types. The range of organs that can be replicated in this way in different species is now very wide and includes organs and cell types that have arisen from the cells of all three cotyledons. Cotyledons are the three cell layers (endoderm/inner layer, mesoderm/middle layer, exoderm/outer layer) that form during embryonic development and that can give rise to different tissues in the course of further development. The three cotyledons contain, so to speak, the developmentally most distant groups of cells. Since organoid technology can already reproduce these greatest possible cellular differences, it is assumed that in principle organoids of all organs can be produced. However, one organoid does not always represent the entire organ: frequently, several organoids reproduce different aspects of individual organs, thus making them accessible to experimental scientific research. Their potential for application in various fields, especially biomedical research and therapy, is promising(1-56). This ranges from basic research, in vitro investigation of organ development and disease research, to use as test systems for drug development and toxicity testing, to cell, tissue and organ replacement within regenerative medicine. Research on organoids raises great hopes and opens up new perspectives, especially for the endeavor of an increasingly personalized medicine and in the field of pediatrics (1-56).

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Present situation of organoid research

The range of available organoids is growing rapidly and includes replicas of the brain, intestine, kidney, stomach, pancreas, lung, liver, prostate, esophagus, gallbladder and the female reproductive tract as well as the embryo (so-called embryoids)(1-56). Currently, organoids are primarily used by researchers as model systems for different organs to better study their development, functioning and diseases (1-56). In addition to their utility in basic research, they are used for drug development and toxicity testing. In the Netherlands, organoids are also already part of the healthcare system as patient-derived organoids from cystic fibrosis patients for pre-testing of drugs. However, they also raise questions that have so far been little discussed in Germany. These include, for example, questions about the transferability of research results on organoids to corresponding organs in vivo or whether it might be possible in the future to counter the shortage of donor organs by replacing organs in the form of organoids. However, major technical hurdles and unresolved scientific questions still stand in the way of this vision. Another ethically controversial issue, for example, is how the possible development of consciousness in the increasingly complex brain organoids should be judged, involving questions about the measurability of mental and cognitive processes on the one hand and possible claims for protection on the other.

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Future therapeutical potential in pediatric diseases

Organoids enable the scientific study of human development, physiology and pathology on a scale and with a level of precision previously unheard of. To date, scientists have explored this using animal models and two-dimensional human cell culture models. Appropriate approaches have led to countless important discoveries, but have specific limitations: In vivo animal models are not ethically sound, are costly and time-consuming; moreover, they only imperfectly replicate human physiology, and their complexity can make it difficult to determine cause and effect in experiments. Conventional human 2-D cell culture models, on the other hand, are often too simple because they often contain cells of only one cell type. Moreover, 2-D cell culture models are typically derived from patient cancer tissues or induced into a cancer-like state by viral oncogenes, which allows for unlimited propagation of these models in vitro, but can also lead to genomic instability and differences in these models compared to their in vivo counterparts. Organoids, on the other hand, can also be generated from healthy human cells, contain many of the cell types found in an organ, and exhibit a stable genotype-phenotype relationship as well as aspects of human organ architecture, physiology, and function.

Discussion

Organoids enable the scientific study of human development, physiology, and pathology at a scale and level of precision not seen before. To date, scientists have explored this using animal models and two-dimensional human cell culture models (1-56). Appropriate approaches have led to countless important discoveries, but have specific limitations: In vivo animal models are not ethically sound, are costly and time-consuming; moreover, they only imperfectly replicate human physiology, and their complexity can make it difficult to determine cause and effect in experiments. Conventional human 2-D cell culture models, on the other hand, are often too simple because they often contain cells of only one cell type. Moreover, 2-D cell culture models are typically derived from patient cancer tissues or induced into a cancer-like state by viral oncogenes, which allows for unlimited propagation of these models in vitro, but can also lead to genomic instability and differences in these models compared to their in vivo counterparts. Organoids, on the other hand, can also be generated from healthy human cells, contain many of the cell types found in an organ, and exhibit a stable genotype-phenotype relationship as well as aspects of human organ architecture, physiology, and function. For these reasons, many com- plex processes can be well studied using organoids. Basic research enabled by organoids includes the study of embryonic development, organ development (organogenesis), and maintenance of organ function. In addition, organoids can be used as disease models for research into both genetic diseases and infectious diseases. Several clinical trials using organoids are already underway. Since organoids can be produced from both healthy and diseased tissues, they offer a wide range of applications in basic and translational research. However, due to the lack of access to healthy and diseased tissues from patients, there remains interest in organoids derived from human pluripotent stem cells, which are renewable and widely available. Currently, organoids, particularly lung, kidney, liver, pancreas, and intestinal organoids, are being used for COVID-19 research, especially for modeling certain disease processes and for screening existing drugs for other diseases for efficacy against Sars-Cov-2(1-56). They are also important for cancer research (1-56). Further research in the field of pediatrics will show further development of in vivo use of organoids in the future, especially in liver diseases in childhood (1-56).

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