Research on Liver Organoid: Present Situation, Limiting Factors and Future Therapeutical Potential in Pediatric Diseases

Stefan Bittmann [a*], Gloria Villalon [a], Elena Moschüring-Alieva [a], Lara BIttmann [a] and Elisabeth Luchter [a]

DOI:

Peer-Review History:
This chapter was reviewed by following the Advanced Open Peer Review policy. This chapter was thoroughly checked to prevent plagiarism. As per editorial policy, a minimum of two peer-reviewers reviewed the manuscript. After review and revision of the manuscript, the Book Editor approved the manuscript for final publication. Peer review comments, comments of the editor(s), etc. are available here:

ABSTRACT

Scientists often use organoids to research diseases. 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. This study concentrates on liver organoid research and its future role in different pediatric diseases. 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 enable the scientific study of human development, physiology and pathology on a scale. 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 organoids in 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

[a] Department of Pediatrics, Ped Mind Institute, Medical and Finance Center Epe, Hindenburgring 4, 48599 Gronau, Germany.
**Corresponding author: E-mail: stefanbittmann@gmx.de;*

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. Further research in the field of paediatrics will show further development of *in vivo* use of organoids in the future, especially in liver diseases in childhood.

Keywords: Organoid; pediatric; liver; hepatocyte; child; future research.

1. INTRODUCTION

Organoids are simple tissue-engineered cell-based in vitro models that recapitulate many aspects of the complex structure and function of the corresponding *in vivo* tissue [1,2]. Scientists often use organoids to research diseases [3-58]. The modern term organoid refers to cells growing in a defined three-dimensional (3D) environment in vitro to form mini-clusters of cells that self-organize and differentiate into functional cell types, recapitulating the structure and function of an organ *in vivo* (hence, also called “mini-organs”) [59]. 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 [60]. 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 [60]. 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 and Clevers 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 [60]. These work together with 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 [60]. 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 hat form during embryonic development and that can give rise to different tissues in the course of further development [60]. 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 his ranging 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 [60]. Research on organoids raises great hopes and opens up new perspectives, especially for the endeavour of increasingly personalized medicine and in the field of paediatrics [1-56].

2. 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) 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 [60]. 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 [60].

3. 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 [60]. 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.

4. 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 [60]. 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 complex 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 [60]. 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.

5. CONCLUSION

Currently, organoids, particularly lung, kidney, liver, pancreas, and intestinal organoids, are being used for COVID-19 research, especially for modelling certain disease processes and for screening existing drugs for other diseases for efficacy against Sars-Cov-2 [60]. They are also important for cancer research [1-56]. Further research in the field of paediatrics will show further development of *in vivo* use of organoids in the future, especially in liver diseases in childhood [1-56].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zhao Z, Chen X, Dowbaj AM, Sljukic A, Bratlie K, Lin L, Fong EL, Balachander GM, Chen Z, Soragni A, Huch M. Organoids. Nature Reviews Methods Primers. 2022 Dec 1;2(1):94.
2. Zakrzewski, W., Dobrzynski, M., Szymonowicz, M. & Rybak, Z. Stem cells: past, present, and future. Stem Cell Res. Ther. 2019; 10:68.
3. Stevens KR, Schwartz RE, Ng S, Shan J, Bhatia SN. Chapter 46 - Hepatic Tissue Engineering. In: Lanza R, Langer R, Vacanti J, eds. Principles of Tissue Engineering (Fourth Edition). Academic Press, Boston. 2014;951–86.
4. Huang YK, Tan DM, Xie YT, et al. Randomized controlled study of plasma exchange combined with molecular adsorbent re-circulating system for the treatment of liver failure complicated with hepatic encephalopathy. Hepatogastroenterology. 2012;59:1323–6. [PubMed: 22534479].

5. Kribben A, Gerken G, Haag S, et al. Effects of fractionated plasma separation and adsorption on survival in patients with acute-on-chronic liver failure. Gastroenterology. 2012;142:782–9.e3. [PubMed: 22248661]
6. Lee S, Lee J-H, Lee D-H, et al. Phase 1/2a Trial of a Bioartificial Liver Support System (LifeLiver) for Acute Liver Failure Patients. Transplantation. 2018;102.
7. Wu G, Wu D, Lo J, et al. A bioartificial liver support system integrated with a DLM/GelMA• based bioengineered whole liver for prevention of hepatic encephalopathy via enhanced ammonia reduction. Biomaterials Science. 2020;8:2814–24. [PubMed: 32307491]
8. Hou Y-T, Hsu C-C. Development of a 3D porous chitosan/gelatin liver scaffold for a bioartificial liver device. Journal of Bioscience and Bioengineering. 2020;129:741–8. [PubMed: 32014416]
9. Thompson J, Jones N, Al-Khafaji A, et al. Extracorporeal cellular therapy (ELAD) in severe alcoholic hepatitis: A multinational, prospective, controlled, randomized trial. Liver transplantation: Official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society. 2018;24:380–93.
10. Pyrsopoulos NT, Hassanein T, Subramanian R, et al. THU-272-A study investigating the effect of extracorporeal cellular therapy with C3A cells on the survival of alcoholic hepatitis designed along the guidelines of the NIAAA. Journal of Hepatology. 2019;70:e282.
11. Pan X-P, Li L-J. Advances in cell sources of hepatocytes for bioartificial liver. Hepatobiliary & Pancreatic Diseases International. 2012;11:594–605. [PubMed: 23232630]
12. Takeishi K, Collin de l'Hortet A, Wang Y, et al. Assembly and Function of a Bioengineered Human Liver for Transplantation Generated Solely from Induced Pluripotent Stem Cells. Cell reports. 2020;31:107711–. [PubMed: 32492423]
13. Chamuleau RA, Poyck PP, Van De Kerkhove M-P. Bioartificial Liver: Its Pros and Cons. Therapeutic Apheresis and Dialysis. 2006;10:168– 74. [PubMed: 16684219] Transpl Int. Author manuscript; available in PMC 2022 November 01.
14. Marsee A, Roos FJM, Verstegen MMA, et al. Building consensus on definition and nomenclature of hepatic, pancreatic, and biliary organoids. Cell Stem Cell. 2021; 28: 816–32. [PubMed: 33961769]
15. Mitaka T, Sato F, Mizuguchi T, Yokono T, Mochizuki Y. Reconstruction of hepatic organoid by rat small hepatocytes and hepatic nonparenchymal cells. Hepatology. 1999;29:111–25. [PubMed: 9862857]
16. Mitaka T. Reconstruction of hepatic organoid by hepatic stem cells. Journal of Hepato-Biliary• Pancreatic Surgery. 2002;9:697–703. [PubMed: 12658403]
17. Schneeberger K, Sánchez-Romero N, Ye S, et al. Large-Scale Production of LGR5- Positive Bipotential Human Liver Stem Cells. Hepatology. 2020;72:257–70. [PubMed: 31715015]
18. Hu H, Gehart H, Artegiani B, et al. Long- Term Expansion of Functional Mouse and Human Hepatocytes as 3D Organoids. Cell. 2018; 175: 1591–606.e19. [PubMed: 30500538]

19. Yamamoto J, Udono M, Miura S, Sekiya S, Suzuki A. Cell Aggregation Culture Induces Functional Differentiation of Induced Hepatocyte-like Cells through Activation of Hippo Signaling. Cell Rep. 2018;25:183–98. [PubMed: 30282027]
20. Prior N, Hindley CJ, Rost F, et al. Lgr5(+) stem and progenitor cells reside at the apex of a heterogeneous embryonic hepatoblast pool. Development. 2019;146.
21. Camp JG, Sekine K, Gerber T, et al. Multilineage communication regulates human liver bud development from pluripotency. Nature. 2017;546:533–8. [PubMed: 28614297]
22. Stevens KR, Scull MA, Ramanan V, et al. In situ expansion of engineered human liver tissue in a mouse model of chronic liver disease. Sci Transl Med. 2017;9.
23. Akbari S, Sevinç GG, Ersoy N, et al. Robust, Long-Term Culture of Endoderm- Derived Hepatic Organoids for Disease Modeling. Stem Cell Reports. 2019;13:627–41. [PubMed: 31522975]
24. Koike H, Iwasawa K, Ouchi R, et al. Modelling human hepato-biliary-pancreatic organogenesis from the foregut-midgut boundary. Nature. 2019;574:112–6. [PubMed: 31554966]
25. Tapia N, Schöler HR. Molecular Obstacles to Clinical Translation of iPSCs. Cell Stem Cell. 2016;19:298–309. [PubMed: 27452174]
26. Sun L, Hui L. Progress in human liver organoids. Journal of Molecular Cell Biology. 2020.
27. Zhou VX, Lolas M, Chang TT. Direct orthotopic implantation of hepatic organoids. J Surg Res. 2017; 211: 251–60. [PubMed: 28501125]
28. Wang S, Wang X, Tan Z, et al. Human ESC-derived expandable hepatic organoids enable therapeutic liver repopulation and pathophysiological modeling of alcoholic liver injury. Cell Res. 2019;29:1009–26. [PubMed: 31628434]
29. Pettinato G, Lehoux S, Ramanathan R, et al. Generation of fully functional hepatocyte-like organoids from human induced pluripotent stem cells mixed with Endothelial Cells. Sci Rep. 2019;9:8920. [PubMed: 31222080]
30. Kruitwagen HS, Oosterhoff LA, van Wolferen ME, et al. Long-Term Survival of Transplanted Autologous Canine Liver Organoids in a COMMD1-Deficient Dog Model of Metabolic Liver Disease. Cells. 2020;9.
31. Takebe T, Zhang RR, Koike H, et al. Generation of a vascularized and functional human liver from an iPSC- derived organ bud transplant. Nat Protoc. 2014;9:396–409. [PubMed: 24457331]
32. Takebe T, Enomura M, Yoshizawa E, et al. Vascularized and Complex Organ Buds from Diverse Tissues via Mesenchymal Cell-Driven Condensation. Cell Stem Cell. 2015;16:556–65. [PubMed: 25891906]
33. Tsuchida T, Murata S, Matsuki K, et al. The Regenerative Effect of Portal Vein Injection of Liver Organoids by Retrorsine/Partial Hepatectomy in Rats. Int J Mol Sci. 2019;21:178.
34. Sampaziotis F, Justin AW, Tysoe OC, et al. Reconstruction of the mouse

extrahepatic biliary tree using primary human extrahepatic cholangiocyte organoids. Nat Med. 2017;23:954–63. [PubMed: 28671689]

35. Sampaziotis F, Muraro D, Tysoe OC, et al. Cholangiocyte organoids can repair bile ducts after transplantation in the human liver. Science. 2021;371:839–46. [PubMed: 33602855]
36. Takebe T, Sekine K, Kimura M, et al. Massive and Reproducible Production of Liver Buds Entirely from Human Pluripotent Stem Cells. Cell Reports. 2017;21:2661–70. [PubMed: 29212014] Transpl Int. Author manuscript; available in PMC 2022 November 01.
37. Saito R, Ishii Y, Ito R, et al. Transplantation of liver organoids in the omentum and kidney. Artif Organs. 2011;35:80–3. [PubMed: 20946288]
38. Nie YZ, Zheng YW, Ogawa M, Miyagi E, Taniguchi H. Human liver organoids generated with single donor-derived multiple cells rescue mice from acute liver failure. Stem Cell Res Ther. 2018;9:5. [PubMed: 29321049]
39. Harrison SP, Siller R, Tanaka Y, et al. Scalable production of tissue-like vascularised liver organoids from human PSCs. bioRxiv. 2020: 2020.12.02.406835.
40. Nussler A, Konig S, Ott M, et al. Present status and perspectives of cell-based therapies for liver diseases. J Hepatol. 2006;45:144–59. [PubMed: 16730092]
41. Sekine K, Ogawa S, Tsuzuki S, et al. Generation of human induced pluripotent stem cell-derived liver buds with chemically defined and animal origin-free media. Scientific Reports. 2020;10:17937. [PubMed: 33087763]
42. Saborowski A, Wolff K, Spielberg S, et al. Murine Liver Organoids as a Genetically Flexible System to Study Liver Cancer *In Vivo* and *In Vitro*. Hepatology communications. 2019;3:423–36. [PubMed: 30859153]
43. Mori A, Murata S, Tashiro N, et al. Establishment of Human Leukocyte Antigen-Mismatched Immune Responses after Transplantation of Human Liver Bud in Humanized Mouse Models. Cells. 2021;10:476. [PubMed: 33672150]
44. Cristinziano G, Porru M, Lamberti D, et al. FGFR2 fusion proteins drive oncogenic transformation of mouse liver organoids towards cholangiocarcinoma. Journal of Hepatology; 2021.
45. Cao W, Liu J, Wang L, et al. Modeling liver cancer and therapy responsiveness using organoids derived from primary mouse liver tumors. Carcinogenesis. 2018; 40: 145–54.
46. Pettinato G, Lehoux S, Ramanathan R, et al. Generation of fully functional hepatocyte-like organoids from human induced pluripotent stem cells mixed with Endothelial Cells. Scientific Reports. 2019;9:8920–. [PubMed: 31222080]
47. Kruitwagen HS, Oosterhoff LA, van Wolferen ME, et al. Long-Term Survival of Transplanted Autologous Canine Liver Organoids in a COMMD1-Deficient Dog Model of Metabolic Liver Disease. Cells. 2020;9:410.

48. Zhang R-R, Koido M, Tadokoro T, et al. Human iPSC-Derived Posterior Gut Progenitors Are Expandable and Capable of Forming Gut and Liver Organoids. Stem Cell Reports. 2018;10:780–93. [PubMed: 29429958]
49. Chen C, Soto-Gutierrez A, Baptista PM, Spee B. Biotechnology Challenges to *In Vitro* Maturation of Hepatic Stem Cells. Gastroenterology. 2018;154:1258–72. [PubMed: 29428334]
50. Liu J, Li P, Wang L, et al. Cancer- Associated Fibroblasts Provide a Stromal Niche for Liver Cancer Organoids That Confers Trophic Effects and Therapy Resistance. Cell Mol Gastroenterol Hepatol. 2021;11:407–31. [PubMed: 32932015]
51. Kim SK, Kim YH, Park S, Cho S-W. Organoid engineering with microfluidics and biomaterials for liver, lung disease, and cancer modeling. Acta Biomaterialia; 2021.
52. Ye S, Boeter JWB, Mihajlovic M, et al. A Chemically Defined Hydrogel for Human Liver Organoid Culture. Advanced Functional Materials. 2020;30 2000893. [PubMed: 34658689]
53. Yap KK, Gerrand Y-W, Dingle AM, Yeoh GC, Morrison WA, Mitchell GM. Liver sinusoidal endothelial cells promote the differentiation and survival of mouse vascularised hepatobiliary organoids. Biomaterials. 2020;251:120091. [PubMed:32408048]
54. Yanagi Y, Nakayama K, Taguchi T, et al. *In vivo* and ex vivo methods of growing a liver bud through tissue connection. Scientific Reports. 2017;7:14085. [PubMed: 29074999]
55. Grebenyuk S, Ranga A. Engineering Organoid Vascularization. Frontiers in Bioengineering and Biotechnology. 2019;7.
56. Eiji K, Shin E. Liver bud transplantation in rats. Magyar Sebészet (Hungarian Journal of Surgery) MaSeb. 2018;71:163– 9.
57. Tsuchida T, Murata S, Hasegawa S, et al. Investigation of Clinical Safety of Human iPS Cell-Derived Liver Organoid Transplantation to Infantile Patients in Porcine Model. Cell Transplantation. 2020;29:963689720964384.
58. Takebe T, Wells JM, Helmrath MA, Zorn AM. Organoid Center Strategies for Accelerating Clinical Translation. Cell Stem Cell. 2018;22:806–9. [PubMed: 29859171]
59. Corrò C, Novellasdemunt L, Li VS. A brief history of organoids. American Journal of Physiology-Cell Physiology. 2020 Jul 1;319(1):C151-65.
60. Bittmann S, Villalon G, Moschüring-Alieva E, BIttmann L, Luchter E. Liver Organoid Research: Present Situation, limiting Factors and Future Therapeutical Potential in Pediatric Diseases. Asian Journal of Pediatric Research. 2023 Aug 29;13(3):34-40.

Biography of author(s)

Prof. Dr. Stefan Bittmann
Department of Pediatrics, Ped Mind Institute, Medical and Finance Center Epe, Hindenburgring 4, 48599 Gronau, Germany.

He was born in 1968. He is a German pediatrician and studied human medicine from 1989 to 1996 at the Universities of Münster (WWU), at the Rudolphina in Vienna during a year of study abroad, and at the Ludwig-Maximilian University (LMU) in Munich. He obtained student internships during medical studies several times in Switzerland as well as in the USA. He completed his medical studies in 1996 with the 3rd state examination in Munich. He received his doctorate with the topic of biliary atresia in childhood at the Pediatric Surgical Clinic of the Hauner Children's Hospital of the LMU Munich under Professor Dr. Kellnar.

He received his full medical license at the University of Munich in 1998 and his doctorate in medicine in 1999. He worked as an intern at the Pediatric Surgery Clinic of the Hauner Children's University Hospital in Munich. He received his first research assistant position in 1999 in the Pediatric Surgery Department of the Clinic for Surgery under the then-head of the clinic, Professor Margreiter, and Professor Menardi for the area of pediatric surgery. Later, he obtained pediatric surgical training in Oberhausen under Professor Pieper. Following this further training phase, he moved to Switzerland to further his own development at the University Children's Hospital of Zurich, initially under Professor Urs Stauffer, and later under Professor Martin Meuli, who had succeeded Professor Stauffer. He was scientifically involved in the laboratory for tissue engineering of Prof. Reichmann, first animal experimental studies as well as the center for severe burn injuries of the Children's Hospital Zurich under Professor Schiestl. Subsequently, he received further training in pediatrics at the Children's Hospital Ahlen under Professor Krüger and completed further clinical and anthroposophical training in pediatrics under Professor Längler at the University of Witten-Herdecke. He completed his residency in pediatrics under Professor E. Harms. He took over the pediatric department in Gronau (Westf.) in April 2009. He completed a part-time master's degree program in complementary medicine at the European University Viadrina in Frankfurt (Oder) and graduated in 2013 with the master's thesis on HPV vaccinations and the master's examination in Bad Honnef and received the academic degree of Master of Arts (M.A.). In the further course, he set up one of the largest pediatric day departments in Westmünsterland and continued clinical research at the Ped Mind Institute. In addition, he received regular continuing education diplomas from the Association of Statutory Health Insurance Physicians and completed a university degree in sleep medicine and sleep culture at the Apollon University of Applied Sciences in Bremen in 2019. In his professional career, he has published approximately 180 scientific publications and books in the field of pediatrics and pediatric surgery (as of 1/23). His book “Checkliste Pädiatrie und Neonatologie”, 2nd edition, is in the press and will be published in September 2024. He was appointed as a Visiting Professor in Pediatrics in September 2022 by the Dean of the School of Medicine, Shangluo Vocational and Technical College, University of Shangluo, China, where he holds a teaching position.

© Copyright (2024): Author(s). The licensee is the publisher (B P International).

DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
Asian Journal of Pediatric Research, 13(3):34-40, 2023. DOI: 10.9734/AJPR/2023/v13i3276

Peer-Review History:
This chapter was reviewed by following the Advanced Open Peer Review policy. This chapter was thoroughly checked to prevent plagiarism. As per editorial policy, a minimum of two peer-reviewers reviewed the manuscript. After review and revision of the manuscript, the Book Editor approved the manuscript for final publication. Peer review comments, comments of the editor(s), etc. are available here: