Aus der Chirurgischen Klinik
Campus Charité Mitte | Campus Virchow-Klinikum
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Isolierung primärer humaner Hepatozyten nach laparoskopischer Leberresektion

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von
Rosa Horner
aus Linz (Österreich)

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1. Abstrakt

1.1 Abstract (english)

Introduction

Primary human hepatocytes serve as an *in vitro*-model for toxicity studies and in research of hepatic diseases. Moreover, they are used clinically, for example in hepatocyte transplantation in patients with inborn enzyme deficiency like the *Crigler-Najjar*-syndrome. With new approaches in tissue engineering, primary hepatocytes could be applied in creating neo-organs easing the lack of donor organs. Liver tissue obtained from partial hepatectomy is a common source for isolation of primary human hepatocytes. Until now, liver resections were most commonly performed by conventional open surgery. Although the laparoscopic approach is currently emerging in liver surgery, data on the outcome of hepatocyte isolation from laparoscopically resected liver tissue are not available.

Methods

A total of 22 hepatocyte isolations were performed using the two-step collagenase perfusion technique from October 2015 to March 2016. Liver tissue was obtained from n = 15 open liver resections (OLRs) and n = 7 laparoscopic liver resections (LLRs). Isolation parameters (cell yield, viability, and Percoll survival) were assessed and hepatocyte function (plating efficiency, urea, albumin, and aspartate aminotransferase) was measured over a culture period of 6 days (OLR: n = 13; LLR: n = 3).

Results

Total cell yield (OLR: 36.81 ± 6.77 x10^6 cells/g vs. LLR 16.84 ± 10.66 x10^6 cells/g, p = 0.0318) as well as viable yield (OLR 31.70 ± 6.05 x10^6 cells/g vs. LLR 14.70 ± 9.89 x10^6 cells/g, p = 0.0260) was significantly higher in the OLR group. Subgroup analysis revealed that the worse outcome of isolation of laparoscopically resected liver tissue was associated with right-lateral LLRs, whereas hepatocyte isolation from left-lateral LLRs was as effective as from open surgery. Hepatocyte function (i.e. plating efficiency, albumin-production, and enzyme activity) did not differ between hepatocytes from openly resected versus left-lateral laparoscopically resected liver tissue.

Discussion

We here present the first data on hepatocyte isolation from laparoscopic liver surgery. Although the overall outcome is worse compared with open surgery, our data suggest that liver tissue from laparoscopic resection of the left lobe is an excellent source for primary human hepatocytes. The worse outcome of the laparoscopically resected right-lateral liver segments

might be due to the prolonged resection phase during the surgery and the resulting longer warm ischaemic time in the tissue.

Modified version of: Horner R*, Kluge M*, Gassner J, Nösser M, Major RD, Reutzel-Selke A, Leder AK, Struecker B, Morgul MH, Pratschke J, Sauer IM, Raschzok N. Hepatocyte Isolation After Laparoscopic Liver Resection. Tissue Engineering Part C Methods. 2016; 22(9): 839-846.

* both authors contributed equally to this work

1.2 Abstrakt (deutsch)

Einführung

Primäre humane Hepatozyten dienen als *in vitro*-Modelle für pharmakologische Untersuchungen und zum besseren Verständnis hepatischer Erkrankungen. Auch klinisch werden sie bereits erfolgreich eingesetzt, beispielsweise im Zuge einer Hepatozyten-Transplantation bei Neugeborenen mit angeborenen Enzymdefekten wie dem *Crigler-Najjar*-Syndrom. Im Bereich des Tissue Engineering könnten mit Hilfe von Hepatozyten künstliche Organe erzeugt werden, die das Problem des Mangels an Spenderorganen lösen machen würden. Primäre humane Hepatozyten werden am häufigsten aus Gewebe gewonnen, das im Rahmen einer therapeutischen Leberteilresektion entfernt wurde. Bisher wurden Leberresektionen meistens konventionell offen durchgeführt. Obwohl der laparoskopische Zugang in der Leberchirurgie immer mehr an Bedeutung gewinnt, sind derzeit keine Daten über das Ergebnis nach Isolierung humaner Hepatozyten-Isolierung aus laparoskopisch reseziertem Gewebe verfügbar.

Methoden

Im Zeitraum zwischen Oktober 2015 und März 2016 wurden mittels eines zweifstufigen Perfusionsverfahren mit Collagenase insgesamt 22 Hepatozyten-Isolierungen durchgeführt. Dabei wurde humanes Lebergewebe von n=15 offenen (OLRs) und n=7 laparoskopischen (LLRs) Operationen verwendet. Im weiteren Versuchsaufbau wurden Parameter des Isolierungsprozesses (Zellausbeute, Viabilität und Percoll-Überlebensrate) bestimmt sowie Hepatozyten-Funktionsparameter (Plattierungseffizienz, Harnstoff-, Albuminstoffwechsel, Aspartat-Aminotransferase und Gesamtproteingehalt) über eine Kulturdauer von 6 Tagen gemessen (OLR: n=13, LLR n=3).

Ergebnisse

Sowohl die Gesamtzellausbeute (OLR: 36,81 ± 6,77 x10^6 Zellen/g Lebergewebe vs. LLR 16,84 ± 10,66 x10^6 Zellen/g, p = 0.0318), als auch die Ausbeute der viablen Zellen (OLR 31,70 ± 6,05 x10^6 Zellen/g vs. LLR 14,70 ± 9,89 x10^6 Zellen/g, p = 0.0260) waren signifikant höher in der OLR-Gruppe. In der Subgruppenanalyse zeigte sich jedoch, dass das schlechtere Ergebnis der Isolierung aus LLRs mit rechts-lateralen Leber-Resektionen assoziiert war, wohingegen die Isolierung aus laparoskopisch resezierten linkslateralen Lebergewebe gleich effektiv war wie nach offener Resektion. Die Funktion (Plattierungseffizienz, Albumin-, Harnstoffproduktion und Enzymaktivität) der Hepatozyten über die Zeitspanne der Kulturdauer unterschied sich nicht zwischen den Zellen aus OLR und links-LLR.

Diskussion

Wir zeigen in dieser Studie erste Daten zur primären humanen Hepatozyten-Isolierung aus laparoskopisch reseziertem Lebergewebe. Trotz der insgesamt schlechteren Ausbeute nach Isolierung aus laparoskopisch gewonnenen Lebergewebe im Vergleich zur offenen Operation, zeigen unsere Ergebnisse, dass nach laparoskopischer Linksresektion eine erfolgreiche Leberzellisolierung möglich ist. Der Grund dafür könnte die längere parenchymale Resektionsphase und die damit einhergehende, längere warme Ischämiezeit des Gewebes bei rechtsseitigen laparoskopsichen Leberoperationen sein.

Übersetzt und modifiziert aus: Horner R*, Kluge M*, Gassner J, Nösser M, Major RD, Reutzel-Selke A, Leder AK, Struecker B, Morgul MH, Pratschke J, Sauer IM, Raschzok N. Hepatocyte Isolation After Laparoscopic Liver Resection. Tissue Engineering Part C Methods. 2016; 22(9): 839-846.

* geteilte Erstautorenschaft

2. Eidesstattliche Versicherung

„Ich, Rosa Horner, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: *„Isolierung primärer humaner Hepatozyten nach laparoskopischer Leberresektion“* selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.
Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)“ des ICMJE -*www.**icmje**.org*) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o.) und werden von mir verantwortet.
Mein Anteil an der ausgewählten Publikation entspricht dem, der in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben ist.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

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Datum	Unterschrift

3. Ausführliche Anteilserklärung an der erfolgten Publikation

Horner R*, Kluge M*, Gassner J, Nösser M, Major RD, Reutzel-Selke A, Leder AK, Struecker B, Morgul MH, Pratschke J, Sauer IM, Raschzok N.
Hepatocyte Isolation After Laparoscopic Liver Resection. Tissue Engineering Part C Methods. 2016; 22(9): 839-846.
* both authors contributed equally to this work

Beitrag im Einzelnen:

Versuche:

Sämtliche 22 Hepatozyten-Isolierungen wurden in einem 3-4 stündigen Prozess von Rosa Horner durchgeführt: Dies beinhaltet das sterile Transferieren von Lebergewebe aus dem allgemeinchirurgischen OP des Virchow-Klinikums in die Forschungs-Laboratorien, das Kanülieren sowie die Perfusion des Gewebes, mit anschließender Prozessierung und weiterer Aufreinigung der Zellsuspensionen; sowie im weiteren Verlauf das Titrieren und Aussähen auf Kulturplatten.
Die Zellkultur inklusive Mediumswechsel und Probenentnahme sowie die Asservierung sämtlicher Proben alle 12 bis 24 Stunden über die gesamte Kulturdauer von 6 Tagen wurden von Rosa Horner durchgeführt.

Probenbearbeitung und Datenerhebung:

Die Aufbereitung aller Proben für die biochemischen Analysen wurde von Rosa Horner durchgeführt. Des Weiteren sämtliche Albumin-ELISA-Messungen, Protein-BCA-Messungen für Plattierungseffizienz und Viabilitätsmessungen; Im Weiteren die Aquisation von patienten-bezogenen Parametern aus den Krankenakten der Spender.

Auswertung und Interpretation:

Erhebung der Rohdaten und folgende Auswertung (Ratio des Plattierungseffizienz-Assay), sowie Strukturierung der Gesamtdaten in Tabellen durch Rosa Horner, sowie Mithilfe bei der Interpretation der Daten.

Manuskript:

Rosa Horner ist mitverantwortlich für Verfassung und Ausformulierung des Manuskripts.

Unterschrift, Datum und Stempel des betreuenden Hochschullehrers/der betreuenden Hochschullehrerin

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Unterschrift des Doktoranden/der Doktorandin

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4. Auszug aus der Journal Summary List (ISI Web of Knowledge^{SM})

Tissue Engineering hat Rang 42 von 158 in der Kategorie „biotechnology & applied microbiology“. IF (2016): 3,485; Eigenfactor (2016): 0,02887

Journal Data Filtered By: **Selected JCR Year: 2016** Selected Editions: SCIE,SSCI
Selected Categories: **“BIOTECHNOLOGY and APPLIED MICROBIOLOGY”**
Selected Category Scheme: WoS
Gesamtanzahl: 158 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	NATURE REVIEWS DRUG DISCOVERY	28,750	57.000	0.060820
2	NATURE BIOTECHNOLOGY	53,992	41.667	0.169930
3	GENOME RESEARCH	36,644	11.922	0.114230
4	GENOME BIOLOGY	28,862	11.908	0.095240
5	TRENDS IN BIOTECHNOLOGY	13,211	11.126	0.020020
6	BIOTECHNOLOGY ADVANCES	14,128	10.597	0.023860
7	CURRENT OPINION IN BIOTECHNOLOGY	13,407	9.294	0.028300
8	METABOLIC ENGINEERING	5,665	8.142	0.015300
9	BIOSENSORS & BIOELECTRONICS	41,829	7.780	0.064430
10	PLANT BIOTECHNOLOGY JOURNAL	5,968	7.443	0.014100
11	BIOINFORMATICS	83,508	7.307	0.183610
12	MOLECULAR THERAPY	15,093	6.688	0.032410
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36	Microbial Cell Factories	4,940	3.681	0.012890
37	Journal of Biological Engineering	637	3.660	0.001670
38	CANCER GENE THERAPY	2,756	3.652	0.003630
39	Biotechnology Journal	4,109	3.649	0.010380
40	Microbial Biotechnology	2,039	3.513	0.005380
41	Stem Cell Research	2,401	3.494	0.008070
42	TISSUE ENGINEERING	19,661	3.485	0.028870
43	APPLIED MICROBIOLOGY AND BIOTECHNOLOGY	36,332	3.420	0.051490
44	Current Opinion in Chemical Engineering	986	3.403	0.003710
45	FEMS YEAST RESEARCH	3,711	3.299	0.005830
46	BIOMASS & BIOENERGY	18,312	3.219	0.027960
47	CYTOTHERAPY	4,952	3.203	0.008800

Quelle: https://intranet.charite.de/medbib/impact_faktoren_2016_fuer_zeitschriften_nach_fachgebieten/, abgerufen am 08.08.2017 um 09:32 Uhr

5. Publikation

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METHODS ARTICLE

Hepatocyte Isolation After Laparoscopic Liver Resection

Rosa Horner,[1*] Martin Kluge,[1*] Joseph Gassner,[1] Maximilian Nösser,[1] Rebeka Dalma Major,[1] Anja Reutzel-Selke,[1] Annekatrin K. Leder,[1] Benjamin Struecker, MD,[1] Mehmet H. Morgul, MD,[1] Johann Pratschke, MD, PhD, FACS,[1] Igor M. Sauer, MD,[1] and Nathanael Raschzok, MD[1,2]

Liver tissue obtained from partial hepatectomy is a common source for isolation of primary human hepatocytes. Until now, liver resections were most commonly performed by conventional open surgery. Although the laparoscopic approach is currently emerging in liver surgery, data on the outcome of hepatocyte isolation from laparoscopically resected liver tissue are not available. A total of 22 hepatocyte isolations were performed using the two-step collagenase perfusion technique from October 2015 to March 2016. Liver tissue was obtained from $n=15$ open liver resections (OLRs) and $n=7$ laparoscopic liver resections (LLRs). Isolation parameters (cell yield, viability, and Percoll survival) were assessed and hepatocyte function (plating efficiency, urea, albumin, and aspartate aminotransferase) was measured over a culture period of 6 days (OLR: $n=13$; LLR: $n=3$). Total cell yield (OLR: $36.81 \pm 6.77 \times 10^6$ cells/g vs. LLR $16.84 \pm 10.66 \times 10^6$ cells/g, $p=0.0318$) as well as viable yield (OLR $31.70 \pm 6.05 \times 10^6$ cells/g vs. LLR $14.70 \pm 9.89 \times 10^6$ cells/g, $p=0.0260$) was significantly higher in the OLR group. Subgroup analysis revealed that the worse outcome of isolation of laparoscopically resected liver tissue was associated with right-lateral LLRs, whereas hepatocyte isolation from left-lateral LLRs was as effective as from open surgery. Hepatocyte function did not differ between hepatocytes from openly resected versus left-lateral laparoscopically resected liver tissue. We here present the first data on hepatocyte isolation from laparoscopic liver surgery. Although the overall outcome is worse compared with open surgery, our data suggest that liver tissue from laparoscopic resection of the left lobe is an excellent source for primary human hepatocytes.

Introduction

Hepatocyte isolation started in rats by simple incubation of the whole liver with collagenase and hyaluronidase followed by mechanical treatment in the late 1960s[1] and was then refined to the two-step perfusion technique established by Seglen in 1976.[2] Nowadays, the modified collagenase perfusion technique allows obtaining primary human hepatocytes from liver tissue in excellent quality and high yield. Human hepatocytes serve as an *in vitro* model for toxicological experiments and pharmacological testing[3–5] or as the basis for regenerative liver medicine.[6] Application of primary hepatocytes in hepatocyte transplantation is under clinical evaluation.[7–10] In the future, decellularized matrices[11,12] or three-dimensional-printed scaffolds recellularized with primary hepatocytes might serve as completely bioengineered organs.[6,13,14]

Even though stem cell research is generating promising results with regard to genetically engineered hepatic cells,[15–17] primary human hepatocytes still remain the gold standard for the applications mentioned before.[18] Still, the lack of donor tissue suitable for hepatocyte isolation makes it difficult to meet the needs for hepatocytes, mainly for *in vitro* studies. Hence, there is a constant need for hepatocytes, which urges laboratories to maximize the efficiency of the isolation process. Given that the isolation of human hepatocytes is a very time-consuming and expensive process, it is crucial to assess whether a particular liver specimen is worth the efforts of the isolation procedure so that any potential source of liver cells is effectively utilized.

Specimens obtained from liver resections are the most common source for isolation of primary human hepatocytes. Liver tissue for hepatocyte isolation can be retrieved from most major liver resections,[19] in which healthy tissue has to be resected during surgery due to anatomical reasons. Until now, the conventional open surgical approach has been the gold standard for liver resections. However, minimally invasive, laparoscopic surgery is emerging as the preferential

[1]Department of Surgery, Campus Charité Mitte, Campus Virchow-Klinikum, Experimental Surgery and Regenerative Medicine, Charité—Universitätsmedizin, Berlin, Germany.
[2]BIH Charité Clinican Scientist Program, Berlin Institute of Health (BIH), Berlin, Germany.
*These two authors contributed equally.

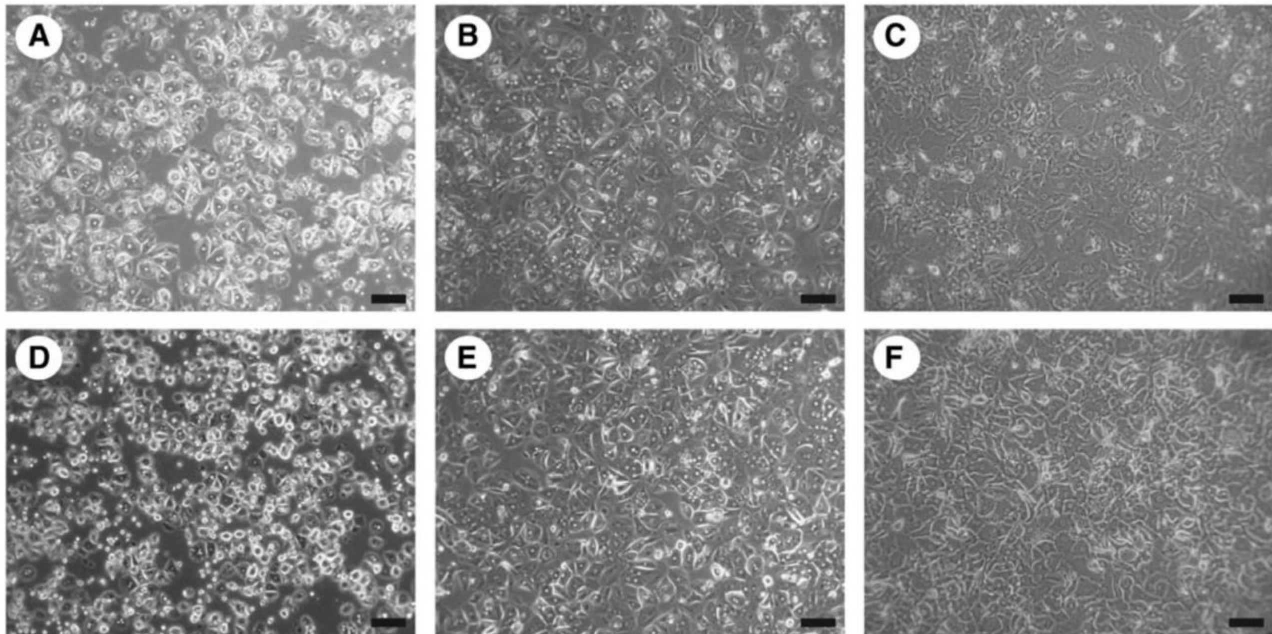

FIG. 1. Light microscopy of hepatocytes isolated from laparoscopically resected **(A–C)** or open resected **(D–F)** liver tissues. Time points: 4 h after isolation **(A/D)**, 48 h after isolation **(B/E)**, and 6 days after isolation **(C/F)**. Morphological differences were not detected between both groups taken at 100× magnification, using phase contrast (scale bar: 100 μm).

Measurement of cell culture parameters

The supernatant of the cell culture was analyzed for transaminase enzyme activity, urea and albumin content after overnight culture as well as after 2, 4, and 6 days of culture. The supernatant of three wells was, therefore, centrifuged at 3000 *g* for 5 min (4°C). Aspartat aminotransferase activity and urea content were measured by Labor Berlin–Charité Vivantes GmbH within 12 h using an enzymatic assay (Roche Hitachi cobas c 6000 system; Roche Diagnostics GmbH). Albumin production was assessed using the human albumin ELISA kit (Bethyl Laboratories) after supernatant sample storage at −80°C. ELISA samples were measured as duplets and according to the manufacturer's instructions.

Plating efficiency was quantified using a modified version of a protocol reported by Gramignoli *et al.*[24] In short, 4 h after seeding, the supernatant of three wells was pooled and kept, whereas the other three wells were washed with warm PBS and the supernatant was discarded. Twenty-four hours after cell seeding, the supernatant of the wells was kept, respectively, discarded again and the latter three wells were washed three times with PBS. The supernatants were centrifuged at 12.800 rpm and the pellets were pooled in RIPA buffer and stored at −80°C immediately.

The remaining adherent protein was suspended with RIPA buffer, scratched off the surface, and pooled. The remaining adherent protein of the washed three wells was then set in relation to the absolute protein of the other three wells ([supernatant 4 h+supernatant 24 h+remaining protein] divided by [carefully washed protein after 24 h]). The protein content was analyzed with BCA reagent (Pierce, Thermo Fisher Scientific) using a photometric assay with all samples measured in triplets.

Statistical analysis

All data are expressed as the mean ± SEM unless indicated otherwise. Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software, Inc.). The nonparametric Mann–Whitney test was used to analyze quantitative parameters with non-Gaussian distribution. For contingency analyses for categorical parameters, the chi-square test was used. A p value ≤0.05 was considered significant.

Results

General characteristics and surgical features of the open and LLR groups

Out of all hepatocyte isolation procedures in this study, $n = 15$ livers were resected through the open approach and $n = 7$ livers were resected using laparoscopic technique. Both groups did not significantly differ regarding the most common parameters known to affect the isolation outcome[22]—neither with respect to the surgical and isolation-related parameters such as duration of surgery, cold ischemic time, collagenase digestion time, and liver weight, nor regarding the preoperative clinical chemistry parameters (Table 1), such as bilirubin, alanine aminotransferase and aspartate aminotransferase (AST), alkaline phosphatase, gamma-glutamyl transpeptidase, international normalized ratio, and activated partial thromboplastin time (aPTT).

Histopathological scores for fibrosis, cirrhosis, and steatosis were evenly distributed in the OLR and LLR groups (Table 1). The LLR group does not contain histologically healthy tissue, that is, tissue without steatosis or fibrosis. However, due to the smaller number of patients in the LLR group, the difference is statistically not significant. Both groups did only significantly differ in indication for surgery

Table 1. Comparison of the OLR and the LLR Groups Regarding Parameters Affecting the Hepatocyte Isolation Outcome

Parameter	Surgical approach		p
	OLR	LLR	
Number	15	7	
Sex (male/female)	11/4	6/1	0.519
Age (years)	59.93 ± 3.59	61.41 ± 7.35	0.617
CIT (min)	16.47 ± 1.84	28.57 ± 10.39	0.284
Digestion time (min)	8.60 ± 0.42	8.50 ± 0.98	0.591
Liver weight (g)	19.23 ± 4.49	22.20 ± 4.21	0.324
Bilirubin (mg/dL)	0.79 ± 0.18	0.62 ± 0.08	0.823
ALT (U/L)	47.36 ± 9.47	31.00 ± 7.34	0.493
AST (U/L)	45.93 ± 8.31	37.00 ± 10.18	0.289
AP (U/L)	241.30 ± 63.55	127.00 ± 39.66	0.283
GGT (U/L)	292.90 ± 81.07	286.00 ± 214.00	0.146
INR	1.026 ± 0.014	1.054 ± 0.018	0.288
aPTT (sec)	36.01 ± 0.89	34.24 ± 0.61	0.341
Duration of surgery (min)	345 ± 18	353 ± 46	
Surgical indication			**0.023**
HCC	0	2	
Biliary tree carcinoma	7	0	
CRLM	6	2	
Benign disease	2	3	
Fibrosis			0.351
No fibrosis	4	0	
Fibrosis	8	3	
Cirrhosis	1	1	
Steatosis			0.465
No steatosis	2	0	
≤30% steatosis	4	3	
>30% steatosis	2	2	

Data are shown as mean ± SEM and *p* values. A *p* value <0.05 was considered statistically significant. Significant *p* values are printed in bold.

AP, alkaline phosphatase; aPPT, activated partial thromboplastin time; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CIT, cold ischemic time; CRLM, colorectal liver metastasis; GGT, gamma-glutamyl transpeptidase; HCC, hepatocellular carcinoma; INR, international normalized ratio; LLR, laparoscopic liver resection; OLR, open liver resection; SEM, standard error of the mean.

since there were no livers diagnosed for biliary tree carcinoma in the LLR group ($p = 0.023$, Table 1). Thus, with the exception of the indication for surgery, the OLR and LLR groups did not differ in regard to the parameters and patient characteristics that are known to affect the outcome of hepatocyte isolation (Table 1).

Comparison of the mean hepatocyte isolation outcome (OLR vs. LLR)

With a total yield of 36.81×10^6 cells/g liver tissue (±6.77) in the OLR group and 16.84×10^6 cells/g liver tissue (±10.66) in the LLR group, there was a significant lower cell yield for livers resected by laparoscopy ($p = 0.032$). The viable yield was lower in the LLR group as well (OLR: $31.70 \pm 6.05 \times 10^6$ cell/g vs. LLR: $14.70 \pm 9.89 \times 10^6$ cell/g; $p = 0.026$). The viability was significantly reduced in hepatocyte isolations in the LLR group (OLR: 84.26% ± 1.46% vs. LLR: 38.17% ± 17.80%; $p = 0.106$, Figure 2). Also Percoll survival was lower in LLR group than in OLR group, however, without reaching statistical significance (Figure 2).

Subgroup analysis in the LLR group

In the LLR group, ($n = 3$) three left-lateral partial hepatectomies (left-LLR) and ($n = 4$) four right-lateral partial hepatectomies (right-LLR) were performed. In right-LLR, three out of four isolations were stopped before Percoll purification due to insufficient cell yield. The fourth isolation in the right-LLR brought a yield before Percoll purification but no cell yield after Percoll purification, whereas in the left-LLR, every isolation was successful. This observation prompted us to exclude the isolations from right-LLR and perform a subgroup analysis between left-LLR and OLR.

Comparison of open vs. left-lateral LLR

In comparison between OLR and left-LLR isolations, no differences in the total cell yield and the viable cell yield before Percoll were found (Figure 3). Likewise, the difference regarding viability was no longer detectable (OLR: 84.26% ± 1.46% vs. left-LLR: 88.44% ± 2.68%; $p = 0.498$). Percoll survival was slightly better in the left-LLR group but without reaching statistical significance (OLR: 60.12% ± 4.59% vs. left-LLR: 81.46% ± 7.46%; $p = 0.082$; Figure 3).

In vitro *hepatocyte function after open vs. left-lateral LLR*

Hepatocytes isolated from left-lateral laparoscopic resected livers did not show higher levels of AST leakage nor

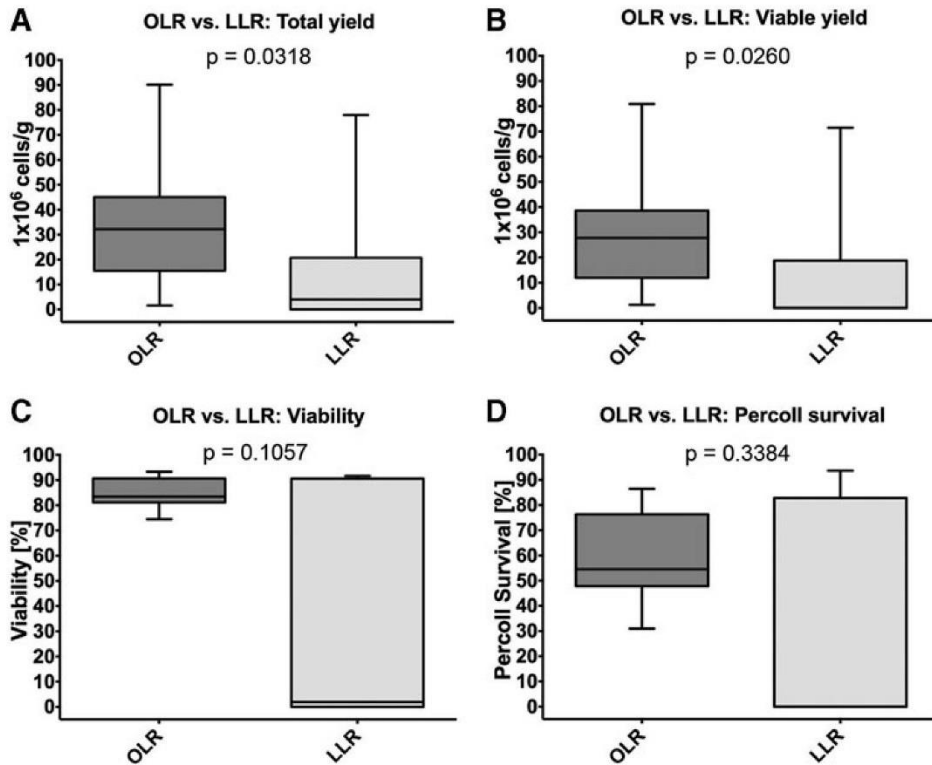

FIG. 2. Comparison of hepatocyte isolation from open and laparoscopic resected human liver tissue regarding total yield **(A)**, viable yield **(B)**, viability **(C)**, Percoll survival **(D)**. OLR group: $n = 15$ isolations; LLR group: $n = 7$ isolations. Box plots display the 25th to 75th percentiles and the median value; *whiskers* indicate the smallest and largest value. *p* Values were calculated with the Mann–Whitney U test. LLR, laparoscopic liver resection; OLR, open liver resection.

did they show a worse plating efficiency after 24 h than hepatocytes obtained from livers operated through the open approach (Table 2).

Also, hepatocytes from both groups did not differ significantly in urea and albumin synthesis (Table 2).

Discussion

Hepatocyte isolation from tissue obtained after liver resections is an established procedure in many hepatobiliary surgery centers and their affiliated research laboratories.

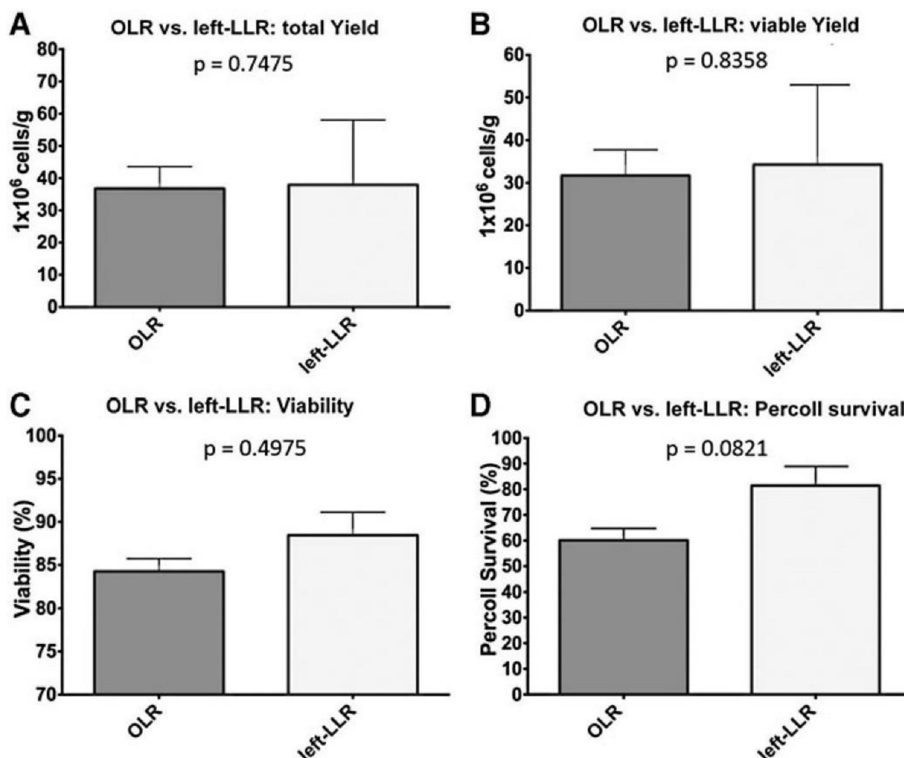

FIG. 3. Comparison of hepatocyte isolation from open and left-lateral laparoscopic resected human liver tissue regarding total yield **(A)**, viable yield **(B)**, viability **(C)**, Percoll survival **(D)**. OLR group: $n = 15$ isolations; left-LLR group: $n = 3$ isolations. Colums display mean value, whiskers indicate SEM. *p* Values were calculated with the Mann–Whitney U test.

Table 2. *In Vitro* Hepatocyte Function from OLR and Left-LLR Livers

Parameter	Overnight Mean ± SEM OLR	Overnight Mean ± SEM Left-LLR	p	Day 2 Mean ± SEM OLR	Day 2 Mean ± SEM Left-LLR	p	Day 4 Mean ± SEM OLR	Day 4 Mean ± SEM Left-LLR	p	Day 6 Mean ± SEM OLR	Day 6 Mean ± SEM Left-LLR	p
Plating efficiency (%)	26.52 ± 4.18	25.10 ± 0.06	0.999									
AST (U/L)	55.80 ± 10.95	174.90 ± 116.70	0.376	31.80 ± 5.67	37.80 ± 7.30	0.448	19.57 ± 2.80	20.04 ± 3.07	0.800	28.02 ± 4.89	31.74 ± 6.46	0.536
Urea (mg/dL)	10.36 ± 2.42	7.80 ± 0.87	0.667	9.50 ± 1.83	7.40 ± 1.22	0.791	7.88 ± 1.31	6.78 ± 0.86	0.901	7.58 ± 1.20	6.63 ± 1.41	0.814
Albumin (ng/mL)	943.7 ± 312.20	2557.00 ± 1870.67	0.383	996.13 ± 177.49	1565.46 ± 978.59	0.717	590.55 ± 150.86	1057.21 ± 803.83	0.999	761.45 ± 274.35	1065.42 ± 905.21	0.717

Data are shown as mean ± SEM and *p* values (*p*). A *p* value <0.05 was considered statistically significant.
Left-LLR, left-lateral laparoscopic liver resection.

Although not suitable for therapeutic application due to the risk of transmission of malignancy, primary human hepatocytes from this source serve for basic research in regenerative medicine and for pharmacological studies.[3–5]

Although open surgery has been the standard for liver resections so far, the laparoscopic approach is increasingly gaining importance. Even though technically more demanding, the results of laparoscopic liver surgery in the hands of experienced teams are convincing.[20,21] Compared with the open approach, laparoscopic liver surgery is associated with significantly reduced surgical trauma, which results in faster recovery. Moreover, it is superior regarding the cosmetic result. Although not yet the standard, the laparoscopic approach is the forward-looking technique for liver surgery and it can be assumed that in the near future, this approach will be used in a majority of cases.

Thus, the outcome of hepatocyte isolation from laparoscopically resected liver tissue has to be evaluated to find out whether or not this source can continue to meet the needs for primary human hepatocytes.

Our study shows that the overall result of hepatocyte isolation from laparoscopically resected liver tissue is significantly worse than the result of open surgery. The parameters that are known to be predictive for the outcome of hepatocyte isolation[22] are inapplicable to forecast the isolation outcome when using tissue obtained from laparoscopic surgery, since both groups did not significantly differ in this analysis. Only the number of patients with biliary tree carcinoma was significantly higher in the OLR group, which is a negative predictor for the outcome of hepatocyte isolation, but which nonetheless resulted in a better outcome in the OLR group than in the LLR group.

However, when looking at left-lateral laparoscopic partial hepatectomies, hepatocyte isolation was as effective as from open surgery. This was true for all analyzed parameters, which are cell viability, isolation yield, and the metabolic function of cultured hepatocytes.

We hypothesize that these findings are primarily due to the prolonged warm ischemia time resulting from right-sided LLRs: When operating through laparoscopic approach, the resected liver tissue remains in the surgical site at 37°C until it is evacuated by the end of the entire procedure to maintain the capnoperitoneum. In contrast, in open surgery, the specimen is cooled by the surrounding air and removed from the surgical site immediately after resection.

Given the technical and anatomical facts, the parenchymal dissection of the left lateral segments (II and III) can be completed in short time, whereas it is more time consuming for the right liver lobe. This does not take as big as an effect in open surgery as it impacts laparoscopic surgery. Due to the warmer conditions inside the body, the liver specimen is exposed to a longer warm ischemia than in the open approach. Also, it requires more time to retrieve the liver tissue out of the patients' body with the specimen retrieval bag than when surgery is performed openly. The extended warm ischemic time causes hepatocytes to induce cell death and, therefore, affects the isolation outcome.

The almost significantly longer duration of surgery for the right-sided LLR than for the left-lateral LLR (r-LLR: 433 ± 44 min vs. l-LLR 246 ± 33 min, $p = 0.0571$) supports our assumption. Moreover, the fact that none of the established predictive parameters for the hepatocyte isolation

outcome (such as tissue quality, liver weight, digestion time, and bilirubin levels of the patient before surgery) differed between the left- and right-sided liver resections, whereas the outcome of the hepatocyte isolation was significantly worse, indicating that other factors might influence this result.

Our study is limited by the low number of cases in the laparoscopic liver surgery group. For example, there was no tissue in the LLR-group without steatosis or fibrosis. Moreover, both cohorts consist of a disproportionate number of male versus female patients (17 out of 22). However, this is true for both groups. Although it might be reasonably assumed that the decreased isolation outcome of right-sided liver resections is caused by the longer warm ischemia time, we cannot prove this hypothesis by an appropriate multivariate analysis, yet. In general, hepatocyte isolation can only be performed when a sufficient mass of tumor-free tissue can be obtained from the specimen during surgery—which applies for both open and laparoscopic surgeries.

In conclusion, we show for the first time that hepatocyte isolation from left-lateral LLRs is feasible. It can be speculated that the outcome from right-sided LLR might improve by the further establishment of the surgical technique and experience of the surgeons. Since hepatocyte isolation is an expensive and complex procedure, we consider isolating cells from the specimen after right-sided laparoscopic liver surgery as not worth the efforts, whereas laparoscopic resections of the left lobe (segments II and III) are an excellent source for primary human hepatocytes.

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Disclosure Statement

No competing financial interests exist.

References

1. Howard, R.B., Christensen, A.K., Gibbs, F.A., and Pesch, L.A. The enzymatic preparation of isolated intact parenchymal cells from rat liver. J Cell Biol **35,** 675, 1967.
2. Seglen, P.O. Preparation of isolated rat liver cells. Methods cell biol **13,** 29, 1976.
3. Hengstler, J.G., Utesch, D., Steinberg, P., Platt, K.L., Diener, B., Ringel, M., Swales, N., Fischer, T., Biefang, K., Gerl, M., Böttger, T., and Oesch, F. Cryopreserved primary hepatocytes as a constantly available in vitro model for the evaluation of human and animal drug metabolism and enzyme induction. Drug Metab Rev **32,** 81, 2000.
4. Gomez-Lechon, M.J., Castell, J.C., and Donato, M.T. The use of hepatocytes to investigate drug toxicity. Methods Mol Biol **640,** 389, 2010.
5. Li, A.P., and Jurima-Romet, M. Applications of primary human hepatocytes in the evaluation of pharmacokinetic drug-drug interactions: evaluation of model drugs terfenadine and rifampin. Cell Biol Toxicol **13,** 365, 1997.
6. Struecker, B, Raschzok, N., and Sauer, I.M. Liver support strategies: cutting-edge technologies. Nat Rev Gastroenterol Hepatol **11,** 166, 2014.
7. Hughes, R.D., Mitry, R.R., and Dhawan, A. Current status of hepatocyte transplantation. Transplantation **93,** 342, 2012.
8. Fox, I.J., Chowdhury, J.R., Kaufman, S.S., Goertzen, T.C., Chowdhury, N.R., Warkentin, P.I., Dorko, K., Sauter, B.V., and Strom, S.C. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. N Engl Med **338,** 1422, 1998.
9. Meyburg, J., Schmidt, J., and Hoffmann, G.F. Liver cell transplantation in children. Clin Transplant **21,** 75, 2009.
10. Strom, S.C., Fisher, R.A., Thompson, M.T., Sanyal, A.J., Cole, P.E., Ham, J.M., and Posner, M.P. Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. Transplantation **63,** 559, 1997.
11. Zhou, P., Lessa, N., Estrada, D.C., Severson, E.B., Lingala, S., Zern, M.A., Nolta, J.A., and Wu, J. Decellularized liver matrix as a carrier for the transplantation of human fetal and primary hepatocytes in mice. Liver Transpl **17,** 418, 2011.
12. Shirakigawa, N., Ijima, H., and Takei, T. Decellularized liver as a practical scaffold with a vascular network template for liver tissue engineering. J Biosci Bioeng **114,** 546, 2012.
13. Uygun, B.E., Yarmush, M.L., and Uygun, K. Application of whole-organ tissue engineering in hepatology. Nat Rev Gastroenterol Hepatol **9,** 738, 2012.
14. Yagi, H., Fukumitsu, K., Fukuda, K., Kitago, M., Shinoda, M., Obara, H., Itano, O., Kawachi, S., Tanabe, M., Coudriet, G.M., Piganelli, J.D., Gilbert, T.W., Soto-Gutierrez, A., and Kitagawa, Y. Human-scale whole-organ bioengineering for liver transplantation: a regenerative medicine approach. Cell Transplant **22,** 231, 2013.
15. Tsolaki, E., and Yannaki, E. Stem cell-based regenerative opportunities for the liver: state of the art and beyond. World J Gastroenterol **21,** 12334, 2015.
16. Khan, A.A., Shaik, M.V., Parveen, N., Rajendraprasad, A., Aleem, M.A., Habeeb, M.A., Srinivas, G., Raj, T.A., Tiwari, S.K., Kumaresan, K., Venkateswarlu, J., Pande, G., and Habibullah, C.M. Human fetal liver-derived stem cell transplantation as supportive modality in the management of end-stage decompensated liver cirrhosis. Cell Transplant **19,** 409, 2010.
17. Cardinale, V., Carpino, G., Gentile, R., Napoletano, C., Rahimi, H., Franchitto, A., Semeraro, R., Nuti, M., Onori, P., Berloco, P.B., Rossi, M., Bosco, D., Brunelli, R., Fraveto, A., Napoli, C., Torrice, A., Gatto, M., Venere, R., Bastianelli, C., Aliberti, C., Salvatori, F.M., Bresadola, L., Bezzi, M., Attili, A.F., Reid, L., Gaudio, E., and Alvaro, D. Transplantation of human fetal biliary tree stem/progenitor cells into two patients with advanced liver cirrhosis. BMC Gastroenterol **14,** 204, 2014.
18. Hansel, M.C., Davila, J., Vosough, M., Gramignoli, R., Skvorak, K.J., Dorko, K., Marongiu, F., Blake, W., and Strom, S.C. The use of induced pluripotent stem cells for the study and treatment of liver diseases. Curr Protoc Toxicol **67,** 14.13.1, 2016.
19. Hughes, R.D., Mitry, R.R., Dhawan, A., Lehec, S.C., Girlanda, R., Rela, M., Heaton, N.D., and Muiesan, P. Isolation of hepatocytes from livers from non-heart-beating donors for cell transplantation. Liver Transpl **12,** 713, 2006.
20. Benzing, C., Krenzien, F., Atanasov, G., Seehofer, D., Sucher, R., Zorron, R., Pratschke, J., and Schmelzle, M.

Single incision laparoscopic liver resection (SILL) - a systematic review. GMS Interdiscip Plast Reconstr Surg DGPW **4,** Doc17, 2015.

21. Tranchart, H., and Dagher, I. Laparoscopic liver resection: a review. J Visc Surg **151,** 107, 2014.
22. Kluge, M., Reutzel-Selke, A., Napierala, H., Hillebrandt, K.H., Major, R.D., Struecker, B., Leder, A., Siefert, J., Tang, P., Lippert, S., Sallmon, H., Seehofer, D., Pratschke, J., Sauer, I.M., and Raschzok, N. Isolation of primary human hepatocytes from human liver tissue after portal vein embolization. Tissue Eng Part C: Methods **22,** 38, 2016.
23. Lörke, J., Erhardt, A., Vogt, C., and Häussinger, D. Non-invasive investigation of liver cirrhosis. Dtsch Arztebl **104,** 1752, 2007.
24. Gramignoli, R., Green, M.L., Tahan, V., Dorko, K., Skvorak, K.J., Marongiu, F., Zao, W., Venkataramanan, R., Ellis, E.C., Geller, D., Breite, A.G., Dwulet, F.E., McCarthy, R.C., and Strom, S.C. Development and application of purified tissue dissociation enzyme mixtures for human hepatocyte isolation. Cell Transplant **21,** 1245, 2012.

Address correspondence to:
Igor M. Sauer, MD
Department of Surgery
Campus Charité Mitte,
Campus Virchow-Klinikum
Experimental Surgery and Regenerative Medicine
Charité—Universitätsmedizin Berlin
Augustenburger Platz 1
Berlin 13353
Germany

E-mail: igor.sauer@charite.de

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7. Publikationsliste

- **Horner R**, Gassner JGMV, Kluge M, Tang P, Lippert S, Hillebrandt KH, Moosburner S, Reutzel-Selke A, Pratschke J, Sauer IM, Raschzok N. Impact of Percoll purification on isolation of primary human hepatocytes. Nature Scientific Reports. 2019 Apr 25;9(1):6542. Impact factor: 4.525
- **Horner R***, Kluge M*, Gassner JMGV, Nösser M, Major RD, Reutzel-Selke A, Leder AK, Struecker B, Morgul MH, Pratschke J, Sauer IM, Raschzok N. Hepatocyte Isolation After Laparoscopic Liver Resection. Tissue Engineering Part C Methods. 2016; 22(9): 839-846. Impact Factor: 3,485 * both authors contributed equally to this work
- Sauer IM, Queisner M, Tang P, Moosburner S, Hoepfner O, **Horner R**, Lohmann R, Pratschke J. Mixed Reality in visceral surgery – development of a suitable workflow and evaluation of intraoperative use-cases. Annals of Surgery. 2017 Nov;266(5):706-712. Impact Factor: 8.980
- Nösser M, Gassner JMGV, Moosburner S, Wyrwal D, Claussen F, Hillebrandt KH, **Horner R,** Tang P, Reutzel-Selke A, Polenz D, Arsenic R, Pratschke J, Sauer IM, Raschzok N. Development of a rat liver machine perfusion system for normothermic and subnormothermic conditions. Tissue Eng Part A. 2019 Jul 31. Impact factor: 3.616
- Gassner JMGV, Nösser M, Moosburner S, **Horner R**, Tang P, Wegener L, Wyrwal D, Claussen F, Arsenic R, Pratschke J, Sauer IM, Raschzok N. Improvement of Normothermic Ex Vivo Machine Perfusion of Rat Liver Grafts by Dialysis and Kupffer Cell Inhibition With Glycine. Liver Transplantation 2019 Feb;25(2):275-287. Impact factor: 4.159
- Major RD, Kluge M, Jara M, Nösser M, **Horner R**, Gassner J, Struecker B, Tang P, Lippert S, Reutzel-Selke A, Geisel D, Denecke T, Stockmann M, Pratschke J, Sauer IM, Raschzok N. The Predictive Value of the Maximal Liver Function Capacity Test for the Isolation of Primary Human Hepatocytes. Tissue Eng Part C Methods. 2018 Mar;24(3):179-186. Impact factor: 2.638
- Fleischmann C, Reichert F, Cassini A, **Horner R**, Harder T, Markwart R, Tröndle M, Savova Y, Kissoon N, Schlattmann P, Reinhart K, Allegranzi B, Eckmanns T. Global incidence and mortality of neonatal sepsis: a systematic review and meta-analysis. Arch Dis Child. 2021 Jan 22:archdischild-2020-320217, Ipact factor: 3.258
- Abstracts/Kongresse
  - **Horner R**. Is Percoll purification necessary for isolation of primary human hepatocytes? zur Präsentation auf den 21. Chirurgischen Forschungstagen 2017 im Rahmen der Session „Implantate / Gewebeersatz“, Speaker-Session
  - Gassner JMGV, Nösser M, **Horner R**, Hillebrandt KH, Moosburner S, Wegener L, Demko P, Arsenic R, Strücker B, Pratschke J, Sauer IM, Raschzok N. Evaluation of a system for normothermic and subnormothermic ex vivo machine perfusion of isolated rat livers. Zeitschrift für Gastroenterologie. 2016; 54(12): 1343-1404., Poster GASL, German Association of the Study of the Liver, annual meeting 2017 in Essen
  - Gassner JMGV, Nösser M, **Horner R**, Hillebrandt KH, Mossburner S, Wegener L , Demko P, Arsenic R, Strücker B, Pratschke J, Sauer IM, Raschzok N. Plasma expansion is key for sustained liver function and viability during normothermic and subnormothermic ex vivo machine perfusion in a rat model. DOI: 10.1016/S0168-8278(17)31666-5, Poster EASL, European Association of the Study of the Liver, annual meeting 2017 in Amsterdam

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