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PRECISION-CUT SLICES OF NORMAL AND TUMOROUS LIVER TISSUES GENERATED WITH THE LEICA VT1200 S VIBRATING BLADE MICROTOME

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Abstract

Precision-cut liver tissue slices are a powerful tool to perform studies on pharmacological metabolism as well as studies investigating toxicology and efficacy of novel substances on primary material under standardized conditions. Slicing of primary liver tissue has been realized using different slicing machines. Since there has been great variability in the results, we sought to develop a highly reproducible method for slicing of liver tissue.

Liver samples from five different species (human, mouse, rat, pig, cattle) were cut using the Leica VT1200 S with Vibrocheck and the reproducibility of slice thickness and quality was determined. Compared to slicers used in the field so far, we demonstrated an improved accuracy and reproducibility of tissue slices.

Introduction

The study of cancer therapeutics in vitro in tissue specimen exhibiting natural tissue structures is a powerful method to elucidate many aspects of tissue/drug interactions which cannot be investigated in cell cultures representing single cancer cell types only.

Primary and secondary tumors of the liver are among the leading causes of death and therefore are in the focus of many studies testing investigational new compounds. For this purpose, tissue sections are required (i) which not only contain liver tumors, but also adjacent non-tumorous liver tissue (to test for selectivity of drugs), (ii) which also exhibit an intact intercellular matrix (enabling testing of the capability of drugs to cross this barrier), and (iii) in which therapeutics that depend on specific mutations, e.g. in intracellular signaling pathways, can be tested functionally on a patient-individual basis.

To perform such testing in a precise manner, we sought for an optimal device with which liver tumor tissue as well as adjacent normal tissue can be cut into very thin slices of defined size while

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avoiding concomitant shearing or squeezing of the tissue. As described below, generation of such highquality precision-cut slices is achieved with the Leica VT1200 S vibrating blade microtome. In our study, we not only used liver tumor pieces, in which tissue integrity is often found to be diminished as a result of heavy treatment procedures (such as chemotherapies), but also naïve liver tissues with reproducible quality from five different species.

Experimental Procedures

Material and Methods
Primary liver material from five different species (human, pig, cattle, rat, mouse) or primary human liver tumor samples (colorectal metastasis) were put into ice-cold Custodiol transplantation media (Dr. Franz Köhler GmbH, Alsbach-Hähnlein, Germany) after surgical resection. Material was collected with informed patient consent by the Department of General, Visceral and Transplant Surgery, University of Tübingen, according to the guidelines of the local Ethics Committee.

Tissue specimens were cut into 200 µm slices as fast as possible using the Leica VT1200 S vibrating blade microtome. Viability post slicing was tested using an ATP quantification kit (Promega, Celltiter Glo, Mannheim, Germany). Slice thickness was measured after paraffin embedding and cross sectioning of the tissue slices.

Slicing
For slicing using the Leica VT1200 S vibrating blade microtome respective tissues were cut into 1.5 x 1.0 x 0.5 cm3 sized cubes and fixed with superglue onto specimen plates. Sectioning was performed after vibrocheck (0;0) using stainless steel razor blades

(Personna Medical, Stainton, VA, USA) under buffered conditions with ice-cold Krebs-Henseleit buffer (KHB) containing 25 mM glucose (Merck, Darmstadt, Germany), 25 mM NaHCO3 (Roth, Karlsruhe, Germany) and 10 mM HEPES (Roth) at the following adjustable settings: knife angle 15°; sectioning speed of 0.4-1 mm/s; oscillation amplitude of 3; step size 200 µm; continuous stroke. To obtain equally sized sections, 8 mm diameter cutouts were generated using a stainless steel 8 mm diameter punch.

Slice Culture
After slicing, samples were cultivated in 10 cm diameter petri dishes using oxygenated William´s E medium (Lonza, Brainel'Alleud, Belgium) supplemented with 25 mM glucose and 50 µg/ml gentamycin (WEGG) under a highly oxygenated atmosphere (80% oxygen, 5% CO2) at 37 °C for up to 3 days post slicing. 1 hour post slicing the medium was replaced with 20 ml of fresh WEGG medium.

Thickness determination
T10 randomly chosen slices were put into 4% formaldehyde solution (Fischar GmbH, Saarbrücken, Germany) for at least 1.5 h. After dehydration with different alcohol concentrations, the slices were embedded in paraffin in an upright position to generate 5 µm cross sections using a microtome. The cross sections were deparaffinized and stained with Hematoxylin and Eosin (H&E). For every species, five cross sections each were measured at 30 different points along the microtome slice using analysis 3.1 software (Soft Imaging System GmbH, Münster, Germany). The thickness distribution is shown in a histogram and the average thickness of each slice is displayed in a diagram (see below).

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Viability

The viability of the tissue slices was measured via an ATP determination using the Celltiter Glo Kit (Promega). For this purpose, we collected three slices per timepoint post slicing (1.5 h, 1 d, 2 d, 3 d) for each individual and stored them at -80 °C. 500 μl of DMEM (Biochrom AG, Berlin, Germany) were added to the frozen slices followed by disintegration using a high intensity cup horn sonifier (Branson, Danbury, CT, USA) for 30 s at maximum power. Then, the ATP content in the supernatant was measured.

Results

Figure 1 A. Consistency of slice thickness

Histograms display thickness distribution of tissue slices generated from livers of different species. Five different livers per species were sliced using the Leica VT1200 S vibrating blade microtome, fixed and embedded in paraffin. Cross section thickness of five slices per liver was measured with 30 points of measurement on average; histograms represent all points measured per species; results were sorted in 10 μm wide bins; n: numbers of measuring points. While murine, rat and human (tumorous/non-tumorous) tissue samples showed quite a good reproducibility with a consistent slice thickness (around 200 μm), porcine and bovine tissues displayed a much wider dissemination of thickness values (from less than 100 μm up to 700 μm). Therefore, optimal consistency of slice thickness is achieved with liver samples of murine, rat and human (tumorous/non-tumorous) origin.

Figure 1 B. Reproducibility of equally sized slices

Average thickness of single slices (five per individual liver specimen of the different species); error bars indicate single standard deviation of the reproducibility of slice size measurements. As a result, the best reproducibility of slice size measurements was obtained with murine non-tumorous liver tissues (with a mean of nearly 200 μm each; in total, only one outlier (mouse 01) was observed), followed by human and rat samples. In contrast, porcine and bovine slices showed higher variations in mean slice thickness even when slices from identical specimen were compared (for example samples pig 03 and pig 05).

Figure 1 C. ATP determination

Column C shows the decay of ATP content in the slices over time. Three slices per liver specimen at each timepoint were lysed followed by measurement of the respective ATP concentration. The most consistent results were observed in rat, pork and cattle samples. Concerning human non-tumorous liver samples, a single variation over time was observed (human sample 04). In contrast, human tumorous samples were found to be quite unpredictable concerning the course of ATP contents; such differences could be related to rapid tumor growth and/or varying extents of tumor necrosis caused by natural tumor growing processes or heavy pretreatments.
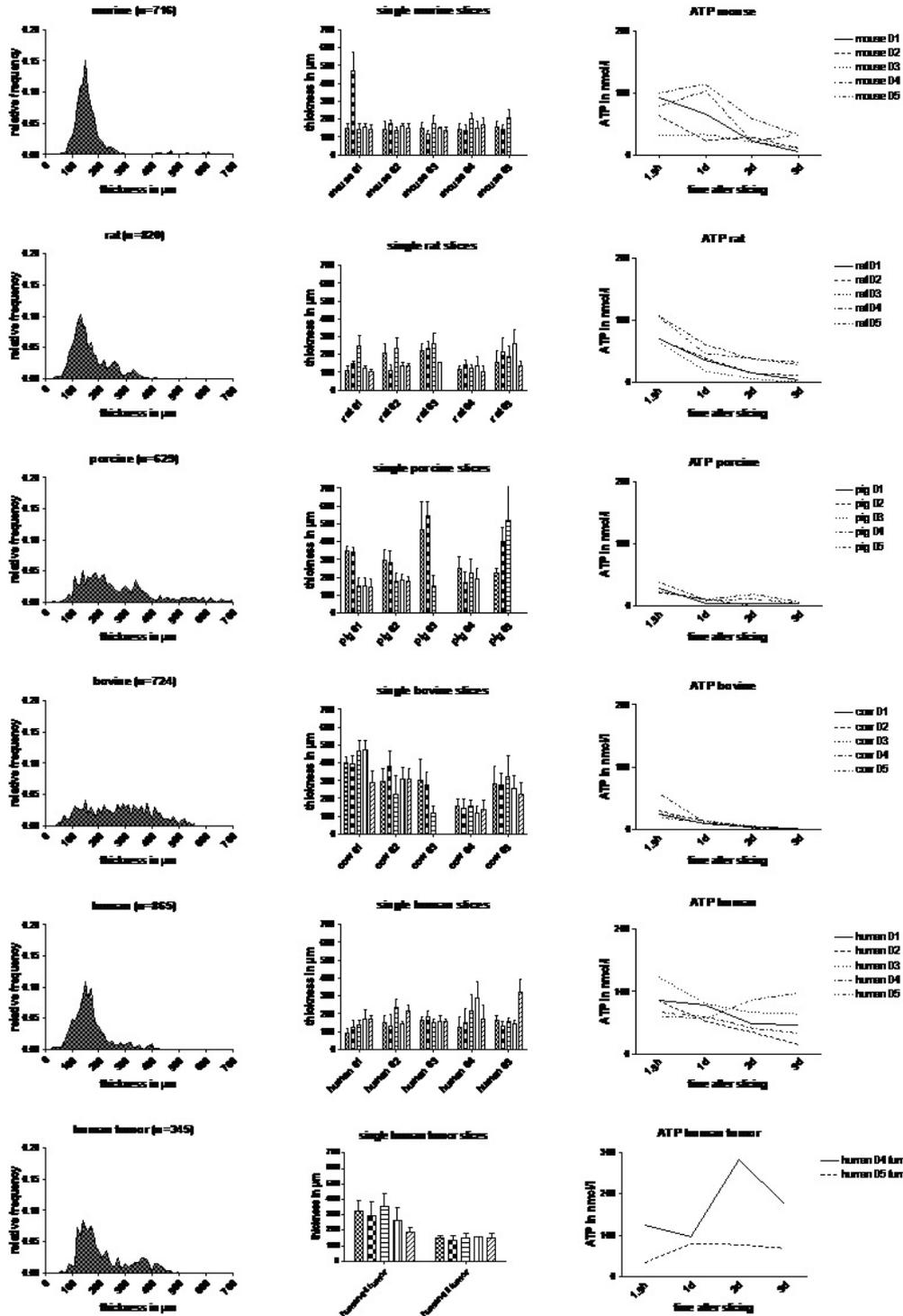
Figure 2. Light microscopy of exemplary slices
The structure of the slices was found to be heterogeneous but consistent with the data depicted in Figures 1A and 1B: whereas mouse, human and rat slices showed precisely sectioned boundaries, thickness of pig and cow slices varied from less than 200 µm to more than 500 µm.

Figure 3. Species specific slice thickness on average
Average thickness of slices derived from the respective species shown as the mean of the average values from the individual animals. Error bars indicate single standard deviation. Altogether, mouse, rat and human liver slices showed a mean near to the adjusted size of 200 µm; however, porcine and bovine slices were thicker and showed greater variations in between the individuals. As known from former experiments, tumor tissues show greater inter-individual variations than non-tumorous tissues depending on the respective tissue structure and quality.
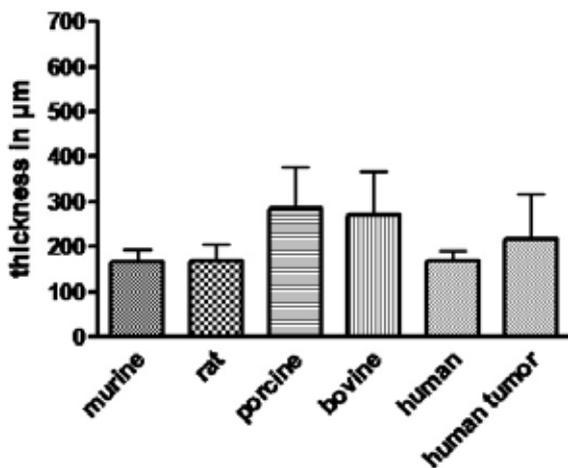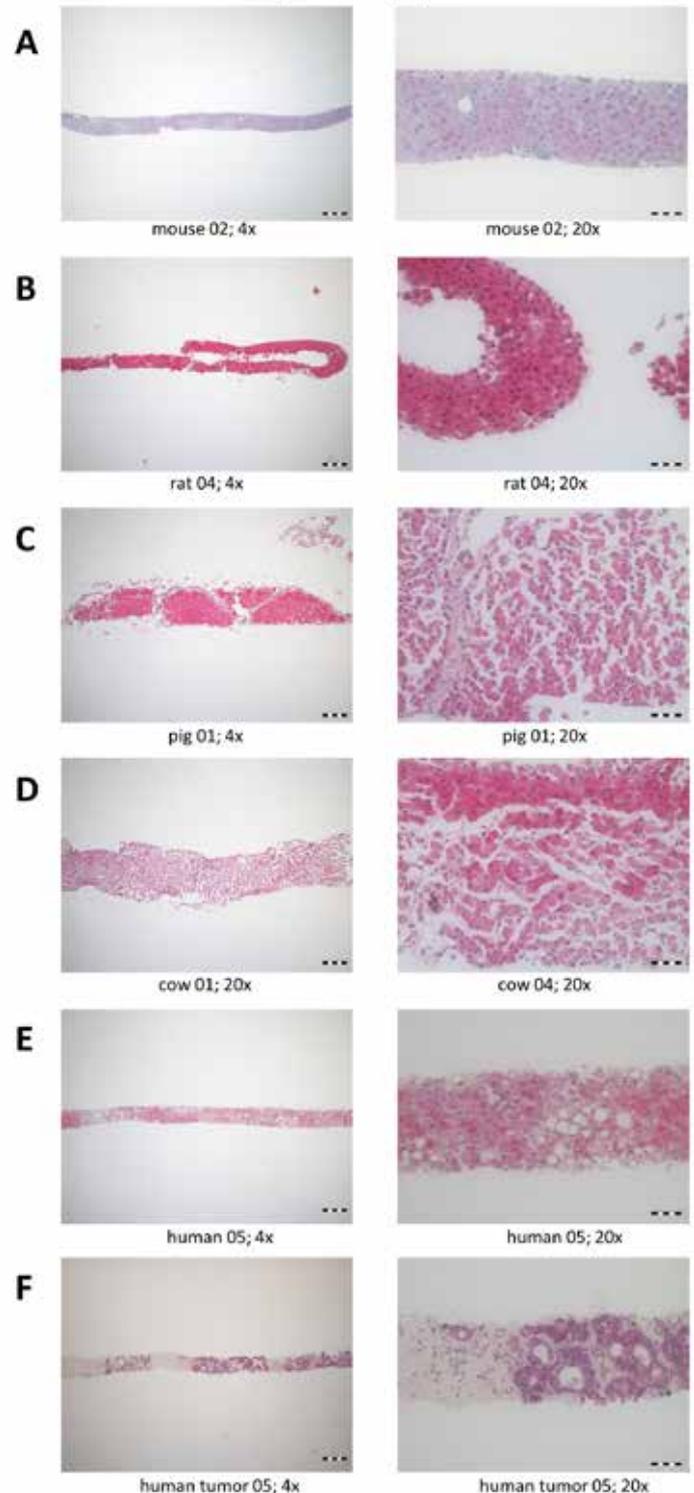

Figure 3

Figure 2

Discussion

Production of liver slices with the Leica VT1200 S vibrating blade microtome led to reproducibility of important slice parameters as well as an improved tissue slice viability. Generation of such high-quality precision-cut slices exhibiting superior viability is thought to be a result of much gentler processing of the samples which comes along with minimized shearing and squeezing of the tissues. In addition, the possibility to choose the slicing parameters on a micrometer-level led to an enhanced reproducibility of all important slice features.

Both, reproducibility and tissue viability were found to depend on the species employed. Human tumor samples showed a high variability depending on factors like liver tumor type (primary or secondary origin) and extent of pretreatment at the time of surgical resection. Human liver tissues were found to be very well sliceable and showed a good reproducibility as well as a viability decreasing over time. This was also true for murine and rat tissues.

In contrast, bovine and porcine tissues showed a higher variability in the resulting parameters; this might, at least in comparison with murine and rat tissues, be caused by the difference between laboratory animals kept under highly standardized conditions (mouse/rat) and productive lifestock (cattle/pork). Therefore, such intra- and inter-species variations should be considered when choosing slicing parameters. We recommend starting with a slow sectioning speed (0.4 mm/s); if the material is sufficiently stable this parameter can be increased over time. For all samples we used an oscillation amplitude of 3 to avoid unwanted disruption of the material. For most samples the average thickness measured was a little bit less than 200 μm. This may be due to dehydration as a result of paraffin embedding.

We conclude that liver slices obtained with the Leica VT1200 S vibrating blade microtome are of high value for diverse applications, e.g. (i) ex vivo testing of novel drugs, giving additional information to cell culture experiments and leading to a minimized usage of laboratory animals and (ii) patient-based analysis of drugs before application paving the way for individualized treatment regimes of e.g. cancer.

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