A new harvesting method to provide high quality prostate cancer tissue suitable for ^{1}H HR MAS MR spectroscopy and gene expression.

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Introduction New research on the molecular level of prostate cancer is important for improved disease management. The genetic and metabolic information revealed from each patient's tumour will hopefully provide substantial help for the correct diagnosis, choice of treatment and prognosis in the near future. The growing need for high quality RNA for gene expression analysis as well as fresh tissue for research on metabolites using proton high resolution magic angle spinning (^{1}H HR-MAS) requires reproducible and effective methods to harvest and store the biological material. A standardized method to provide a fresh tissue slice from radical prostatectomy specimens for research requires special considerations in order to not interfere with the routine clinical histopathological procedure. The aim of this study was to develop a harvesting method to provide a fresh tissue slice from radical prostatectomy specimens for research, before formalin fixation of the remaining gland and without interfering with the routine histopathologically (evaluation of Gleason grade, tumour stage or margin status). The goal was to obtain high quality cancer tissue with highly intact molecular content suitable for MR spectroscopy and gene expression profiling of the same sample.

Methods Patients planned for radical prostatectomy were invited to donate tissue and sign an informed consent prior to surgery. The whole prostate gland was put in a plastic rack to stabilize during slicing and the research slice was cut in the middle of the prostate and photographed. The rest of the gland was sliced and stained with H&E for routine diagnostics. To minimize time to freezing and reduce the bulging of the research slice, it was immediately put in a pre-cooled clamp and pressed between aluminium plates before put into liquid nitrogen. The 2 mm thick snap-frozen complete prostate was stored at -80°C without fixatives. Removal of smaller tissue samples was performed using a modified drill (3mm diameter) on an aluminium plate in direct contact with liquid nitrogen, avoiding the tissue to thaw (Figure 1b). Before molecular analysis, the histology of the small sample was verified by performing a cryo-section of each sample. Localization of cancerous areas in the slice was performed by image fusion of the frozen slice (digital photo) and the two scanned H&E stained sections closest to the frozen slice (Figure 1a). RNA extraction was performed on 53 samples from 12 patients, and ^{1}H HR MAS was performed on 3 samples from different patients (normal, Gleason 3+3 and 4+4). A study for investigating RNA quality after HR MAS was performed on 16 normal prostate samples (from the same patient). Six of these samples were investigated directly for RNA quality and 10 of them for RNA quality after HR MAS analysis.

Results The new harvesting method is presented in Figure 1. Patients with tumour in the two closest surrounding H&E sections show an 88% sensitivity of finding cancer in the extracted tissue cylinders. The tissue samples are shown to be suitable for ^{1}H HR-MAS (Figure 1c) and for molecular analyses requiring high quality RNA with an average amount of cancer of 53%. RNA quality control of 53 extracted cylindrical samples using RNA integrity number (RIN) as a measurement of RNA integrity showed a mean RIN score of 9.16±0.53 and the RNA concentration was 38±16.6 ng/µl. The 260/280 ratio was 2.05, indicating pure RNA. The samples tested for RNA quality after HR MAS (N=10) had a RIN of 8.4 ± 1.5, a RNA concentration of 60.5±40.0 ng/µl and the 260/280 ratio was 1.99. The samples taken directly for RNA quality testing (n=6) had a RIN score of 9.4±0.95, a RNA concentration of 84.9±43.7 ng/µl and the 260/280 ratio was 2.01.

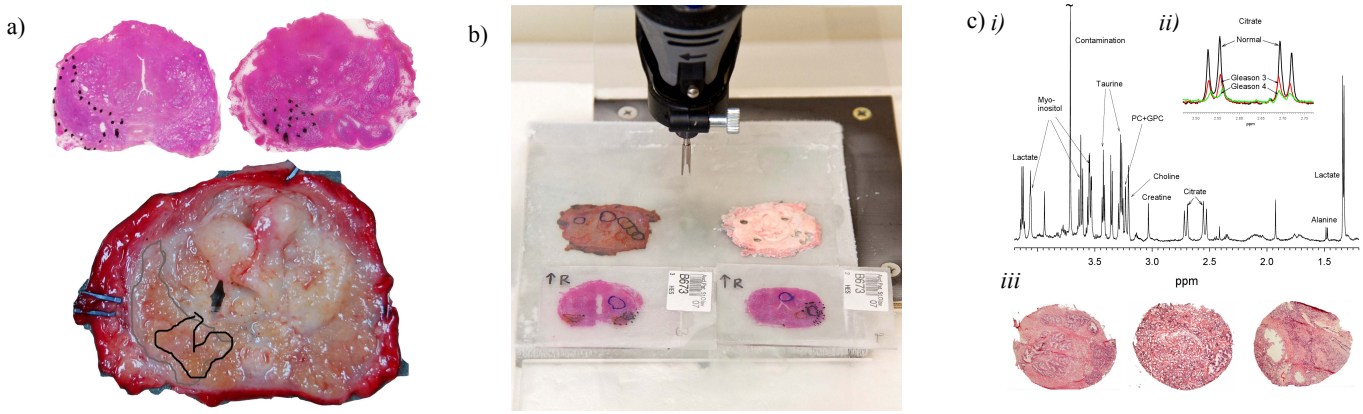

Figure 1. a) Localization of cancerous areas was performed by image fusion **b)** The fused picture was printed on transparent paper and used as a guide to extract (drill) cancer regions **c)** *i)* ^{1}H HR MAS spectrum (1.2- 4.2 ppm) of a normal sample *ii)* Citrate resonances of a normal sample against cancer samples (Gleason 3+3 and 4+4) showing decreasing citrate levels with increasing Gleason grade. *iii)* Presented from the left is the corresponding H&E cryosection from the normal sample (50 % benign glandular epithelium and 50 % stomal tissue), the cancer sample with Gleason 3+3 (80 % cancer, 5 % benign epithelium, 15 % stroma) and the cancer sample with Gleason 4+4 to the right (50 % cancer, 30% benign epithelium, 20% stroma).

Discussion This study describes a highly standardized method for snap-freezing of a whole prostate slice that is safe (without interfering with the routine diagnostics), easy to practise, and results in tissue with highly intact molecular content suitable for MR spectroscopy and gene expression of the same sample. To be able to study all grades of prostate cancer including multifocal tumours, a complete prostate slice offers a great advantage over sampling of visible or palpable tumour areas[1]. In a complete slice, the tumour and its surroundings can be easily investigated thus facilitating research on the role of stroma in prostate cancer progression[2] or studying molecular alterations in multifocal disease[3]. Apart from providing cancer tissue of high quality for research, the present harvesting method is applicable to all prostate cancer patients and allows storing the tissue without fixatives, leaving it available for all kinds of future technologies.

References 1. Cooper, CS. *Nature*;14:677-87. **2**. Cunha, GR. *Int J Cancer*;107:1-10. **3**. Kobayashi, M. *Prostate*;68:1715-24.