OBJECTIVE
To present and validate a new technique for biobanking fresh-frozen prostate cancer tissue based on MRI-transrectal ultrasound fusion biopsy.

MATERIALS AND METHODS
From August 2014 to August 2016, patients with elevated levels of PSA and at least 1 suspicious lesion on MRI were invited to this study. Each MRI-suspicious lesion was biopsied repeatedly for at least 2 cores in the same location. These repeated cores were labelled A/A₀, B/B₀, etc. The A/B cores were submitted for histologic assessment, and the corresponding A₀/B₀ cores were stored in an −80°C freezer for biobanking. Sixty biobanked samples were processed for histologic assessment to compare their pathologic parameters with their corresponding paraffin samples. Another 20 biobanked samples were processed for RNA quality evaluation.

RESULTS
Fifty-six of the 60 selected banking samples matched their corresponding paraffin samples for benign vs malignant diagnosis, leading an overall concordance rate of 93.3%. There was no significant difference between banking samples and the corresponding paraffin samples in cancer percentage and Gleason score. The RNA Integrity Number value ranged from 6.8 to 9.3 (mean 7.89).

CONCLUSION
The current study demonstrates that the histologic identity of the banked prostate biopsy sample can be accurately predicted by its corresponding paraffin samples. MRI-TRUS fusion biopsy based biobanking method is highly efficient, timesaving, and has high quality tissues both at the histologic and RNA integrity levels. UROLOGY 134: 186–191, 2019. © 2019 Elsevier Inc.

Over the past several years, prostate cancer has been the most common malignancy in elderly males in the western world.¹ The clinical progression of prostate cancer shows significant heterogeneity, but the underlying genetic and molecular mechanisms are still largely unknown. Certain aggressive subtypes progress rapidly toward metastatic and therapy-resistant disease. In addition, a large proportion of indolent subtypes receives overtreatment and cause many complicated morbidities.² These heterogeneities demonstrate the critical need to identify new biomarkers to distinguish aggressive from indolent disease. A large biobank composed of high-quality and well-characterized prostate tissue is essential to support this clinical and translational research. However, due to the costs as well as technical and ethical issues, there have been limited efforts in fresh prostate cancer tissue biobanking.³,⁴ A few institutes have reported their experiences with prostate cancer biobanking, with most fresh tissues having been obtained from radical prostatectomy specimens. Due to the distinctive physical properties of prostate tissue, identifying cancer foci on gross examination has been notoriously challenging.⁵ Riddick et al presented a pseudobanking procedure and showed that pseudobanked tissue matched adjacent unbanked tissue at a rate of 98% for benign vs malignant diagnoses.⁶ Sircar et al advocated mirror image tissue banking of prostatectomy specimens for the first time in 2006, which was applied in other institutes.⁷,⁹ Nevertheless, these multistep procedures were relatively complicated, costly, and generally required the help of a histopathologist with the identification, sampling, and characterization of the neoplastic tissue.¹⁰ The objective of our study was to present and validate a new technique for the establishment of a fresh-frozen

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¹ These authors contributed equally to this work.

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prostate cancer tissue biobank based on MRI-transrectal ultrasound (MRI-TRUS) fusion biopsy. To the best of our knowledge, our study is the first to report and evaluate a prostate cancer biobank collected from biopsy samples.

**MATERIALS AND METHODS**

Institutional review board approval was obtained for this study. From August 2014 to August 2016, Patients with elevated levels of prostate-specific antigen (PSA) and at least 1 suspicious lesion on MRI were invited to attend this study. Previous biopsied patients were excluded from the analysis. Informed consent was obtained from each patient. Blood and urine samples were collected from consented patients.

All mpMRI was performed using a 3.0-T MR scanner (Achieva 3.0T TX dual-source parallel RF excitation and transmission technology, Philips Medical systems, The Netherlands) with a 32-channel phased array coil. All MRI scans were reviewed by 2 experienced radiologists. A PI-RADS score was provided according to the PI-RADS v2 Guidelines. Lesions with a PI-RADS score of 3 or more were defined as biopsy targets.

Banking process: The MRI-TRUS fusion-guided biopsy was conducted with the mpMRI-TRUS biopsy system (RVS, Real-time Virtual Sonography, Hitachi Medical Corporation, Tokyo, Japan) (Supplementary Fig. 1). After induction of general anaesthesia with a laryngeal mask, patients were placed in a high lithotomy position. Each MRI-suspicious lesion was biopsied repeatedly for at least 2 cores in the same location using the free-hand transperineal technique. A 16-G automatic biopsy gun with a specimen size of 18 mm (GALLINI Medical, Via Frantinni, Mantova, Italy) was used to take biopsy cores. These repeated samples were labelled A/A', B/B', etc. The A/B cores were submitted for histologic assessment (paraffin embedded) and the corresponding A'/B' cores were snap-frozen in liquid nitrogen and then stored in a −80°C freezer for biobanking (Fig. 1).

Diagnostic concordance analysis: The analysis intended to evaluate the concordance of diagnosis between biobanked samples and their corresponding paraffin-embedded samples and specimen quality. We selected 60 biobanked samples from patients with a final diagnosis of prostate cancer and processed them for routine histologic assessment. The pathologic parameters of the biobanked samples were recorded and compared with their matched paraffin samples. Another 20 randomly selected biobanked samples were processed for RNA extraction. Extracted RNA was quantified with NanoDrop 8000 Spectrophotometer (Thermo Scientific, Wilmington, DE) and the RNA quality was evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA).

Statistical analysis was performed using SPSS version 22.0 (SPSS Inc., Chicago, IL). The paired sample t test was used to compare the cancer percentage and the Gleason score of the banked samples and their corresponding paraffin counterparts. The Kappa test was used to evaluate the diagnostic concordance. A P value <.05 was considered statistically significant.

**RESULTS**

A total of 265 patients were included during the study period, with 142 that were diagnosed with prostate cancer. Sixty biobanked samples were randomly selected from these prostate cancer patients and processed for pathologic analysis. The mean age of these 60 patients was 70.4 ± 9.53 (53-89) years. The mean total PSA value was 78.07 ± 137.9 ng/mL (4.36-445.2). The mean prostate volume was 42.42 ± 38.02 (9.4-150) mL. The mean PI-RADS score was 3.9 ± 1.05 (2-5). The pathologic results of the biobanked samples were compared with their corresponding paraffin samples.

Diagnostic concordance analysis: Of the 60 selected biobanked samples, the pathologic assessment showed that 56 samples matched the corresponding paraffin samples for benign vs malignant diagnoses, leading an overall concordance rate of 93.3% (56/60).

For the 50 malignant paraffin samples, concordance of diagnosis in the banked frozen samples was present in 48 cases, leading a concordance rate of 96% (48/50). Of the 10 paraffin samples with benign tissue, 2 malignant lesions were found in

Figure 1. Illustration of the biobanking process. The repeatedly biopsied samples were sent for histopathologic analysis (red, A, B, etc) and for biobanking (blank, A', B', etc), respectively.
the matched banked samples, leading a concordance rate of 80% (8/10). The Kappa value was 0.760, and it revealed that the diagnoses of the paraffin group and banked frozen group were concordant. There was no significant difference between the concordant and discordant groups in terms of the Gleason score and prebiopsy PSA level. However, larger cancer percentages in the paraffin samples tended to have higher concordance rates with their matched biobanked samples. When the malignant percentage in the paraffin sample is more than 50%, the concordance rate in the current study is 100% (36/36).

The PSA value was higher in the high-grade group compared with the low-grade group (P = .001). There was no significant difference between the high-grade and low-grade groups in terms of PI-RADS score and concordance rate (Table 1).

The cancer percentage of each biobanked sample was also compared with its matched paraffin counterpart (Fig. 2). The paired sample t test showed that there was no significant difference between biobanked samples and the corresponding paraffin samples (68.75% ± 26.94% vs 61.45% ± 24.21%, P = .130). For samples containing carcinoma, the Gleason score (sum) of the banked sample matched that of its adjacent tissue in most cases. Only 4 in 48 samples had a Gleason score difference of more than 1. The mean Gleason score in the banked sample group is 7.17 ± 1.08, and in the paraffin group, the mean score is 7.33 ± 1.12 (P = .272).

**Table 1.** Comparison of the low-grade group and high-grade group

<table>
<thead>
<tr>
<th></th>
<th>Low Grade (Gleason Score ≤7, n = 35)</th>
<th>High Grade (Gleason Score ≥8, n = 25)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA value</td>
<td>19.2 ± 22.9 ng/μL</td>
<td>160.4 ± 184.1 ng/μL</td>
<td>.001</td>
</tr>
<tr>
<td>PI-RADS score</td>
<td>3.8 ± 0.9</td>
<td>4.0 ± 1.2</td>
<td>.451</td>
</tr>
<tr>
<td>Concordance rate</td>
<td>91.4%</td>
<td>96%</td>
<td>.861</td>
</tr>
</tbody>
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![Figure 2. Histology of routinely processed paraffin samples (A/B/C) and the retrieved corresponding biobanked samples (A'/B'/C'), stained with H&E, x 100. (A). Cancer percentage 95%, Gleason 5 + 5 = 10; (A'). Cancer percentage 95%, Gleason 5 + 4 = 9; (B). Cancer percentage 75%, Gleason 5 + 4 = 9; (B'). Cancer percentage 80%, Gleason 4 + 4 = 8; (C). Cancer percentage 50%, Gleason 4 + 4 = 8; (C'). Cancer percentage 50%, Gleason 3 + 4 = 7. (Color version available online.)](image-url)
Quality of samples: The concentration of the extracted RNA ranged from 152.68 ng/μL to 540.75 ng/μL (mean 289.33 ng/μL) and the absorbance ratios at 260 nm/280 nm ranged from 1.84 to 2.10 (mean 1.96). The RNA Integrity Number (RIN) value ranged from 6.8 to 9.3 (mean 7.89, Fig. 3). Only 1 case had a RIN value less than 7. Thirteen cases from the 20 randomly selected biobanked frozen samples had malignant corresponding paraffin samples, and 5 of those cases had a malignant percentage greater than 80%. The mean RIN value of these 5 samples was 8.61 (7.9-9.3).

**DISCUSSION**

Within the field of urology, a number of large international biobanks and networks have been established for translational research and individualized medicine. The analysis of gene expression in human clinical tissue samples by molecular techniques is becoming increasingly important in urological cancer research. These techniques require high-quality tissue. Establishing such tissue biobanks can produce information about disease pathogenesis, diagnosis, and outcome data. Well-established biobanks can also encourage collaborative research between clinicians, pathologists, and research scientists (Supplementary Table 1). The prostate cancer tissues in the majority of current biobanks were collected from radical prostatectomy specimens. Nevertheless, compared with other solid tumours, prostate cancer is quite difficult to identify by visual inspection, even when considering the information from the TRUS, MRI, and biopsies. Despite many technological improvements, the current prostate cancer biobanking protocols are still complex and time consuming. Furthermore, with the widespread application of laparoscopic or robot-assisted laparoscopic radical prostatectomy, the prostate would remain in the body until later steps were completed. The warm ischaemia would probably impact the RNA integrity of the banked prostate samples.
In view of this background, we tried to explore a new method to obtain fresh prostate cancer tissues. The excellent detection accuracy of MRI-TRUS fusion biopsy on significant prostate cancer greatly improved the probability of getting prostate cancer samples through prostate biopsy.\(^{17,18}\) This method is clearly more convenient and allows for more sample sources for the collection of cancer tissue from prostate biopsy compared to radical prostatectomy. Nevertheless, 2 important issues have to be resolved in advance. First, a means of identifying the histologic component of the banked sample is necessary. Second, due to the limited volume of the biopsied specimen, this method may or may not qualify for research purposes.

Researchers usually applied an “alternative slice mirror image” method for tissue banking of prostatectomy specimens and predicting the histologic component of the frozen banked sections. In brief, the prostate gland was divided into 4 quadrants and each quadrant was sliced at 3-5 mm intervals. The sections were then alternatively submitted for histologic assessment and for banking in a mirror image fashion. The study by Brimo et al showed an encouraging concordance of diagnosis between biobanked sections and their mirror image paraffin sections (46/50).\(^9\) Similarly, in the current study, we labelled repeatedly biopsied samples and sent them for histologic assessment and for biobanking, respectively. The assumption was that the pathologic state of the biobanked sample was exactly reflected by its adjacent paraffin sample.

To test this assumption, we selected 60 biobanked samples for histologic assessment and compared the pathologic parameters with their corresponding paraffin samples. The overall concordance rate for benign vs malignant diagnosis was quite encouraging (56/60). Furthermore, when the malignant percentage in the paraffin sample is more than 50%, the concordance rate is 100% in the current study (36/36). The malignant percentage and the Gleason score also had a high concordance rate between biobanked and paraffin samples. Our results showed that it is entirely feasible to predict the histologic identity of biobanked samples through their known paraffin counterparts.

The freezing prostate samples in the biobank follow well-established protocols. Good quality RNA from banked samples is critical for research applications such as RT-PCR. RIN values are important indicators of overall RNA quality,\(^{19}\) and range from 10 (intact) to 1 (totally degraded). RIN is an important tool in conducting valid gene expression measurement experiments as real-time PCR or RNA microarray. Our study demonstrated good yields and quality RNA was obtained from the biobanked samples. Nineteen of the 20 samples had a RIN value greater than 7. Few of the determinants affecting RIN are known.\(^{20}\) It appears that the RIN value has a positive correlation with a sample’s cancer percentage, which needs to be further confirmed by more samples. It is worth noting that the volume of the sample may greatly influence the quality of the RNA. Our preliminary study also tested samples taken with an 18 G automatic biopsy gun, and a large proportion of the samples had relatively low RNA concentrations and RIN values. Therefore, all samples in the current study were collected with a 16 G biopsy gun.

Compared to collecting samples from radical prostatectomy specimens, our protocol is more convenient and saves time. However, 2 limitations need to be declared. First, this protocol increased the puncture times and could lead to potential risks to the patients. Second, although the RNA quality of the sample tested highly, the relatively small sample volume would limit its application for research that requires a large number of samples.

**CONCLUSION**

We presented a new protocol for the biobanking of fresh prostate cancer tissue. The current study demonstrates that the histologic identity of the biobanked prostate biopsy sample can be accurately predicted by its corresponding paraffin samples. The MRI-TRUS fusion biopsy-based biobanking method is highly efficient, easily feasible, cost effective, and provides high quality tissues at both the histologic and RNA levels. Furthermore, it significantly increased the sample source, which will benefit future translational research.

**SUPPLEMENTARY MATERIALS**

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.jurology.2019.08.029.

**References**


