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# The connective tissue framework in the normal prostate, B.P.H and prostate cancer: analysis by scanning electron microscopy after cellular digestion

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**Abstract** The objective of this study was twofold: (1) to determine if a cellular digestion process can facilitate examination of the morphology of the connective tissue framework of the prostate, and (2) to examine the connective tissue framework in normal prostate tissue, benign prostatic hyperplasia (BPH) and prostate cancer. Ten prostate glands were examined. Using the Ohtani method of digestion, the cellular elements were removed. This enabled scanning electron microscopy analysis of the connective tissue framework within the prostatic tissue. Light microscopy of tissue blocks determined the histology of specimens. The prostate is supported by a highly structured network of collagen fibres. This network of fibres varies in normal and diseased states. In benign prostatic hyperplasia, the collagen network is dense, with an increased number of fibres. In prostatic adenocarcinoma, there is non-uniform swelling with a loss and disintegration of collagen fibres. In conclusion, sodium hydroxide cellular digestion provides an excellent method for demonstrating the connective tissue framework of prostatic tissue. The morphological changes in collagen fibres in normal prostate, benign prostatic hyperplasia and prostatic adenocarcinoma have implications for prostate growth in normal and diseased states.

**Key words** Connective tissue · Prostate · Benign prostatic hyperplasia · Cancer · Scanning electron microscopy

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## Introduction

Morphological evaluation of prostatic tissue is normally based on cellular changes and the histology of the prostate has been well documented [9]. It has been noted that changes in epithelium in disease states are preceded by alterations in the underlying connective tissue stroma, including swelling, disintegration and loss of collagen [2]. In 1925 Reischaeur [15] first suggested a role for stromal tissue in the pathogenesis of prostatic disease. Subsequent measurements using light and electron microscopy confirmed that tissue overgrowth does result from an increase in stromal elements [1, 2, 16] . However, further analysis of this collagen infrastructure was not possible because details were obscured by cellular elements.

Attempts were made in different tissues to remove the cellular elements and expose the underlying connective tissue framework; however, acetic acid, trypsin, EDTA and low-temperature HCl were all unsuccessful [8]. Ohtani [12, 13] then reported a successful method of alkali digestion, which he termed "etching". This technique preserves connective tissue fibres in their original shapes, location and three-dimensional orientation. Using this method, combined with scanning electron microscopy, Ohtani demonstrated the connective tissue fibres of the pancreas [12]. Etching has also been applied to the human kidney and testes [7, 20]. These studies demonstrated that collagen fibrils of individual tissues have a specific architectural pattern.

The aim of this study was twofold: (1) to assess this cellular digestion process by studing the connective tissue framework of the prostate, and (2) to determine differences and their implications in the connective tissue framework of the prostate in the normal and diseased states, i.e. benign enlargement and prostatic cancer.

## Materials and methods

Ten whole prostate glands were removed for examination. Nine glands were obtained at autopsy in which the cause of death was

unrelated to prostatic diseases. One prostate was removed as part of a radical prostatectomy. The mean age of patients was 55 years (range 24–76 years). Each prostate gland was sagitally sectioned through the urethra and immediately fixed in 10% formalin for a minimum of 2 days. A tissue block ($0.5 \times 1 \times 0.5$ cm) was excised from the glandular region using blades set in parallel. This tissue block was subdivided into two pieces and one piece was prepared for light microscopy analysis. The second piece, in the plane directly facing and adjacent to the first piece, was digested using alkali in preparation for scanning electron microscopy analysis.

For light microscopy, the appropriate tissue block was embedded in paraffin wax and sectioned at 6-μm intervals. Sections were stained with haematoxylin and eosin (for routine histopathological diagnosis) and Shorr stain (to demonstrate connective tissue) under the light microscope. One uropathologist examined all specimens. For the alkali digestion, the appropriate tissue samples were immersed in 10% aqueous solution of sodium hydroxide for 7 days at 25 °C. The solution was changed daily. The tissue was then washed for several hours in distilled water. Maintaining the water at 25 °C facilitates cell removal during washing [11]. After rinsing, the tissue pieces were bathed in a 1% aqueous solution of tannic acid overnight, washed in distilled water again and post-fixed in a 1% aqueous solution of osmium tetroxide for 3 h [12, 13]. Tissues treated with sodium hydroxide are soft and fragile; hence fixation with tannic acid is necessary to make the specimens sufficiently resistant to the subsequent preparation procedures. In addition, it enhances connective tissue electrical conductivity. Specimens were then dehydrated in a series of graded concentrations of ethanol and critical-point-dried using liquid carbon dioxide. The dried specimens were mounted on aluminium stubs with carbon cement and coated with gold in an Emscope Sc500 coating system. Observations were made under a Leica Cambridge Stereoscan S360 scanning electron microscope with an acceleration voltage of 20 kV. Multiple stereo pairs of scanning micrographs were taken using a tilt separation of 6–7°. This facilitated the examination of the spatial relationship of the structural framework, the estimation of connective tissue area density and, lastly, the estimation of mean connective tissue fibre diameter/thickness. The significance of differences in collagen area density and collagen fibre diameter/thickness between normal and pathological states was tested using the Chi-squared test, accepting that the number of cases in this pilot study was small.

## Results

Each prostate gland was categorized histologically using light microscopy with haematoxylin and eosin stains. Four specimens were reported normal (average weight = 33 g; age range = 24–55 years; cause of death = two cardiac, one infection, one drug related). Five specimens had typical benign hyperplasia (average weight = 38 g; age range = 50–76 years; cause of death = four cardiac, one infection). The one case of prostatic adenocarcinoma was removed at radical prostatectomy (Gleason score = 7).

As expected, light microscopy analysis of tissue blocks prepared in the standard manner was of limited value in studying the connective tissue framework of the prostate gland. It was observed using both light and electron microscopy that cellular material obscured morphological details of the collagen fibres (Fig. 1). The Shorr stain confirmed that the connective tissue framework was collagenous in nature.

The etching process with sodium hydroxide effectively removed all cellular elements and allowed unhindered scanning electron microscopy analysis of the connective tissue framework. Stereo-observation confirmed that the collagen fibres retained their location and orientation in spite of tissue processing (Fig. 2).

In the normal prostate specimens there was a highly organized collagen fibrillar network that supports the glandular lobules (Fig. 3). Connective tissue comprised 39.4% of the total area density (standard deviation (SD = 0.7). This network of collagen fibres appeared loosely woven and was fine and smooth in texture. Mean fibre diameter/thickness was 0.3 μm (SD = 0.02). These findings were consistent in all four normal prostates studied.

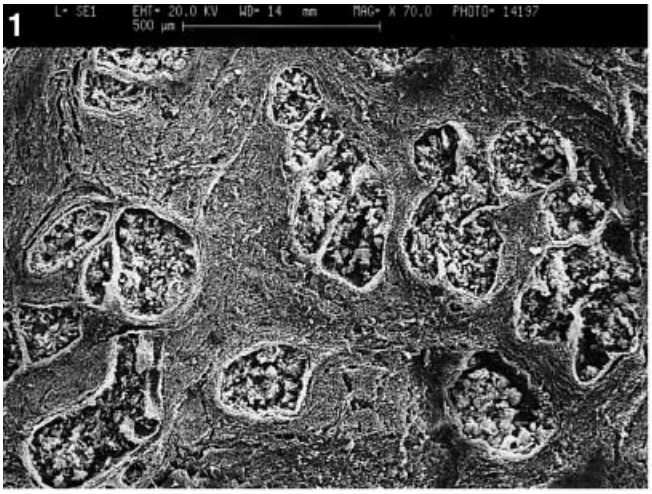
In specimens with benign prostatic hyperplasia (BPH), scanning electron microscopy analysis after etching revealed that the number of collagen fibres increased; area density = 56.4% (SD = 1.4). This difference in density from normal prostate tissue was significant ($P < 0.001$). Individual fibres appeared uniformly swollen in diameter, with a mean fibre diameter of 0.42 μm (SD = 0.03). This difference was significant ($P < 0.001$) when compared with normal prostate (mean diameter = 0.3 μm, SD = 0.02). These changes gave the overall appearance of a denser and more closely woven supporting stroma than in normal prostate (Fig. 4). We accept that this pilot study involved a relatively small number of cases/specimens, but the uniformity of findings in each group of normal and BPH specimens, and also the significance of statistical testing, suggest these findings will be replicated in a larger study.

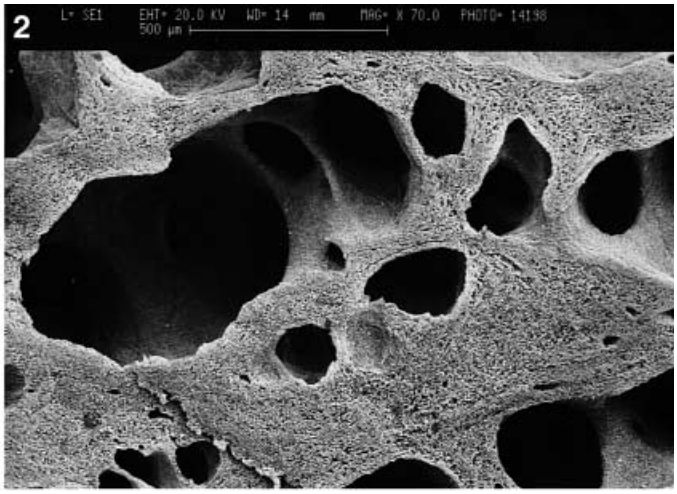
In the adenocarcinoma specimen, scanning electron microscopy analysis after etching revealed total disruption of the normal connective tissue framework (Fig. 5). As in BPH, the collagen fibres appeared swollen in diameter, but this swelling was not uniform and there was no regularity in the spatial relationship of the fibres. Areas of collagen destruction were also evident.

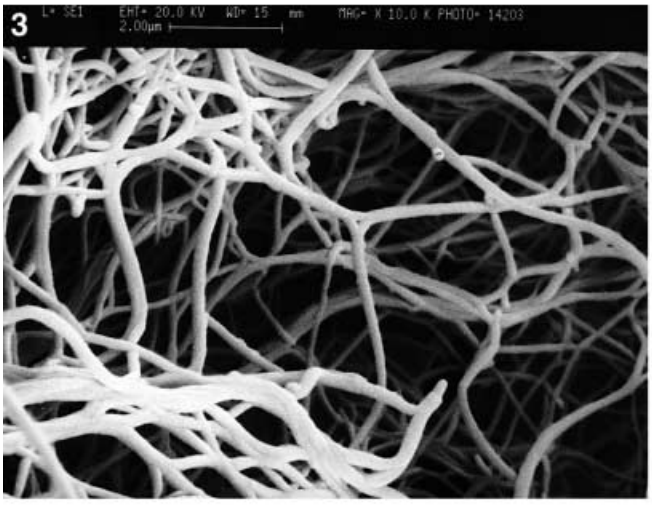
## Discussion

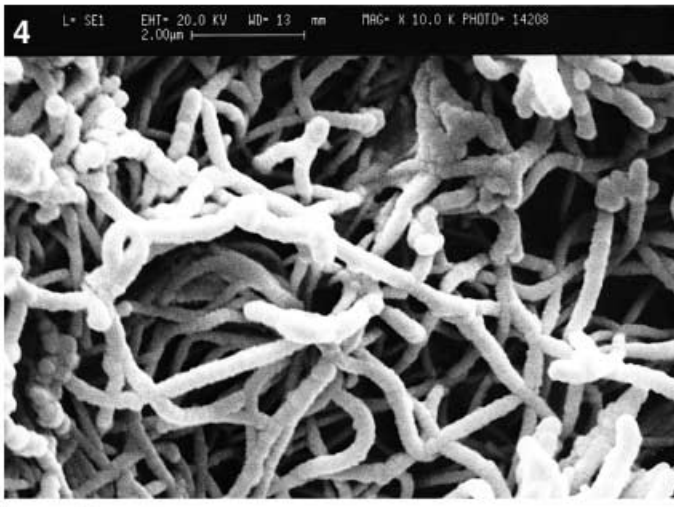
The success of the etching process in achieving cellular digestion was confirmed, and the results demonstrated the morphological features of the connective tissue framework in normal prostate, BPH and prostatic adenocarcinoma. It is possible that a proportion of the connective tissue proteins are damaged during the etching process by the alkali concentration, temperature and the time [11], but Ohtani et al. [13] showed in other tissues that the vast majority of collagen fibrils remain in well-preserved natural locations in spite of the processing.

The prostate gland is composed of diverse elements traditionally grouped into three main categories: stroma, epithelium and glandular lumina. The stroma is composed of connective tissue and smooth muscle. Connective tissue is the major component comprising 54% of the gland [18, 19]. At an ultrastructural level we have shown that the complex cellular arrangement of the prostate is suspended in a highly structured network of collagen fibres. This network of fibres was altered in normal and diseased states. In normal prostate, the



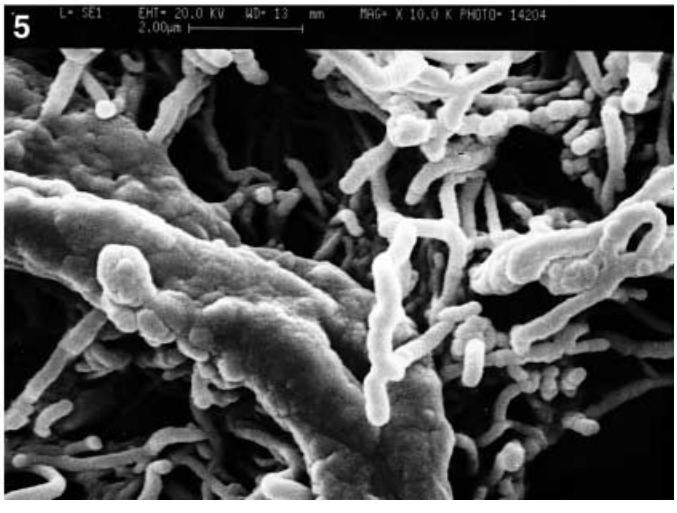




**Fig. 1** Normal prostate. Specimen prepared in the standard manner for scanning electron microscopy analysis. The connective tissue framework is obscured by cellular elements (S.E.M. ×70)

**Fig. 2** Normal prostate after cellular digestion. Only the highly organized collagen fibrillar network remains (S.E.M. ×70)

**Fig. 3** Normal prostate. The stromal network of collagen fibres is loosely woven and fine and smooth in texture (S.E.M. ×10,000)

**Fig. 4** BPH. There is a denser supporting stroma than in specimens of normal prostate. Note the increased number of fibres, their less regular distribution and increased diameter (S.E.M. ×10,000)

**Fig. 5** Prostatic adenocarcinoma. There is partial collagen destruction and the remaining collagen fibres are non-uniformly thickened (S.E.M. ×10,000)

network of collagen fibres appeared loosely woven, fine and smooth in texture. These findings were not unexpected, considering the ultrastructure connective tissue framework demonstrated in other tissues [7, 12, 20].

Using light microscopy analysis in BPH, there is a fourfold increase of the stromal elements and a doubling of the glandular elements. This relative increase of the stromal element is generally constant regardless of the overall enlargement of the prostate caused by the hyperplastic process [16].

The present study revealed that in BPH the stromal collagen network is denser, with an increased number of collagen fibres that are swollen. Not surprisingly, these changes in the collagen fibres were uniform in keeping with a benign hyperplastic condition. We are confident that these differences are due to the disease process and

are not age related. Stromal overgrowth can, therefore, be viewed as a central feature in BPH, implicating an altered, although regular, mesenchymal–epithelial relationship.

Malignant tumours of the prostate possess a stroma derived from the altered connective tissue within the gland. This stroma is associated with tumour angiogenesis and the improved vascular supply optimizing cellular nourishment, gas exchange and waste disposal, which are all increased in neoplastic tissue. The stroma may also limit the influx of inflammatory cells and provide a barrier to immunological rejection [6]. Primary tumour growth is also helped by the breakdown and remodelling of the extracellular matrix. This may facilitate the dispersal of cells, leading to the formation of metastases. In the one case of prostatic adenocarcinoma examined, we demonstrated disruption of the normal connective tissue framework by the disease process. There was non-uniform swelling, loss and disintegration of the collagen fibres. These ultrastructural findings support the above conclusions made from the integration of cellular material.

Collagen fibres adjacent to epithelial cells have a significant role in glandular–stromal interaction(s) and prostate growth regulation [17], but the roles of individual glandular and stromal elements are unclear [3]. Currently, increased focus is being put on specific growth factors, and an insulin-like growth factor (IGF) system is implicated in the regulation of prostatic growth [14]. This system involves a number of molecules, including the IGFs themselves and proteases synthesized and secreted by prostatic epithelial and stromal cells, which are capable of breaking down components of the extracellular matrix. Studies indicate a regional variation of proteases within the different zones of the prostate [22]. Altered hormonal regulation may be responsible for an increased expression of certain proteases, and their increased activity is further associated with changes in tissue organization. Aberrations in elements of the IGF system have been observed in stromal cells derived from BPH [23]. The IGF system, and in particular proteases, may therefore have an important growth regulatory role in both normal and malignant prostate. Degradation of the extracellular matrix is essential for tumour cells to escape from the primary tumour and migrate to distant sites [4]. Analysis of the prostate IGF system may yield important clues towards understanding its tumour biology and may stimulate the development of novel therapeutic approaches [10].

This pilot study revealed a definite alteration in the connective tissue framework of the prostate in BPH and cancer. These findings need to be confirmed by a further study involving more cases. These changes at ultrastructural level are probably related to altered growth factor regulatory mechanisms; however, as stated above, these remain to be fully explained. For the future it would be interesting to evaluate symptomatic patients with BPH to determine whether the connective tissue framework relates to symptoms. A further study could also examine the collagen ultrastructure in prostate tumours of different Gleason grades and in cases where the disease may be either localized or metastatic.

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