Original article

X-ray microtomography-based measurements of meniscal allografts

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ARTICLE INFO

Article history:
Accepted 17 November 2014

Keywords:
- Graft quality
- Meniscal allograft imaging
- Meniscal transplantation
- X-ray microtomography

ABSTRACT

Background: X-ray microcomputed tomography (XMT) is a technique widely used to image hard and soft tissues. Meniscal allografts as collagen structures can be imaged and analyzed using XMT. The aim of this study was to present an XMT scanning protocol that can be used to obtain the 3D geometry of menisci. It was further applied to compare two methods of meniscal allograft measurement: traditional (based on manual measurement) and novel (based on digital measurement of 3D models of menisci obtained with use of XMT scanner).

Hypothesis: The XMT-based menisci measurement is a reliable method for assessing the geometry of a meniscal allograft by measuring the basic meniscal dimensions known from traditional protocol.

Materials and methods: Thirteen dissected menisci were measured according the same principles traditionally applied in a tissue bank. Next, the same specimens were scanned by a laboratory scanner in the XMT Lab. The images were processed to obtain a 3D mesh. 3D models of allograft geometry were then measured using a novel protocol enhanced by computer software. Then, both measurements were compared using statistical tests.

Results: The results showed significant differences (P < 0.05) between the lengths of the medial and lateral menisci measured in the tissue bank and the XMT Lab. Also, medial meniscal widths were significantly different (P < 0.05).

Discussion: Differences in meniscal lengths may result from difficulties in dissected meniscus measurements in tissue banks, and may be related to the elastic structure of the dissected meniscus. Errors may also be caused by the lack of highlighted landmarks on the meniscal surface in this study.

Conclusion: The XMT may be a good technique for assessing meniscal dimensions without actually touching the specimen.

Level of evidence: Level IV.

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1. Introduction

Meniscal allograft transplantation is suggested as a means to normalize contact pressures following meniscectomy. Allografts are obtained from deceased donors. An important criterion determining the use of meniscal allografts is their size [1]. In a standard clinical procedure, dissected meniscal allografts are not imaged before transplantation. Grafts are assessed visually rather than by use of other methods, such as imaging techniques [1,2]. Typically, they are harvested, prepared and sterilized. All dimensions of allografts are measured using a sliding or digital caliper and cotton thread or steel wire (to measure circumference) [3,4]. However, this way of proceeding may cause some errors, such as discrepancies between different tools measurements of the same menisci dimensions. Besides, when measuring circumference, tissue banks use their own tools, which are not standardized. Additionally, when the meniscus is not attached to the tibial plateau, measurement of its length may be hindered due to the fact that it is possible to change the distance between its horns.

The preservation of the allograft’s shape and position seems necessary in order to make highly detailed measurements. One technique that can be successfully used to provide high-resolution images of meniscal allografts is X-ray microcomputed tomography (XMT).

XMT is a non-destructive imaging method where individual projections recorded from different angles are used to reconstruct the axial cross-sectional images of the scanned object [5]. Reconstructed images can be synthesized to generate a three-dimensional (3D) image, which facilitates quantitative analysis of the geometric properties of the 3D object without physically
contacting the samples [6]. XMT can be successfully used for soft tissue imaging [7].

The aim of this study was to present a scanning protocol that could be used to obtain 3D quantitative data of menisci and to compare two methods of meniscal allograft measurement. One method takes a “traditional” measurement of the menisci using standard instruments, and the other uses XMT-based visualization and computer programs.

2. Materials and methods

2.1. Preliminary test

All of the menisci were received from the Katowice Tissue Bank (Poland). Thirteen menisci in total were harvested from cadaveric knees (male menisci only, mean age 42.4, 30–61 years, six lateral and seven medial menisci). All menisci were harvested without bone plugs. They were cleaned, packaged in plastic bags and then frozen below –40°C. The donor’s meniscal qualification test was performed according to Polish Transplantation Act DU2005,169.1411 [8].

One of the 13 menisci was chosen randomly to find out which parameters and conditions should be used to obtain the best image quality. A right lateral meniscus was scanned using various parameters and conditions. The scanning was performed by XMT scanner Phoenix v@tome|x s (GE Sensing & Inspection Technologies, Wunstorf, Germany).

Based on the authors’ knowledge concerning XMT scanning of biological samples with low absorption of X-ray radiation, three configurations of scanning conditions and parameters were established. First, the meniscus was scanned in a plastic bag filled with solution of physiological saline. Then the meniscus was drained and scanned in a sterile plastic cup. The next scanning was performed after placing it in a new sterile plastic bag, but this time without fluid. All scanning parameters used in these three cases are shown in Table 1. The tested meniscus was scanned after thawing at room temperature.

After XMT scanning, the acquired two-dimensional projections were reconstructed using manufacturer software for data reconstruction (Datos 2.0). Then 3D models of each dataset were performed using a free program for data visualization (Drishti ver. 2.3.3) [9] to evaluate aspects such as problems with data segmentation and the influence of scanning conditions on the fidelity of shapes. Scanning in fluid gave the worst results and created many artifacts, which were difficult to remove using standard segmentation tools. This option was rejected as not useful for this study. The use of a plastic cup provided very good meniscal surface images, but there was a problem with segmentation of the border between the plastic cup’s bottom and the meniscus body. The best option for further investigation was scanning a thawed meniscus in a plastic bag with no fluid. Plastic foil was thinner than the plastic cup and thus made the segmentation process easier.

2.2. Final test

2.2.1. Preparation of meniscal allografts

Before the scanning, the same thawing procedure was used for all investigated menisci. Menisci were thawed at room temperature for 30 minutes. Then the menisci were XMT-scanned without being removed from the plastic bag, and were put into the scanner in front of the X-ray source – with the middle of the meniscal body perpendicular to the X-ray source (Fig. 1).

2.2.2. XMT scanning parameters used in the final test

XMT scanning was performed using the scanning parameters chosen during the preliminary test (Table 2). Due to size differences between menisci, higher voxel size for all medial menisci was used.

2.2.3. Data reconstruction, segmentation and 3D visualization

After completion of the XMT scanning process, image reconstruction was performed using the same software as during the

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### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>The meniscus in plastic bag with fluid</th>
<th>The drained meniscus in plastic cup</th>
<th>The meniscus in plastic bag without fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voltage (kV)</td>
<td>230</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>Current (µA)</td>
<td>200</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>Power (W)</td>
<td>46.0</td>
<td>16.9</td>
<td>16.9</td>
</tr>
<tr>
<td>Number of projections</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Resolution (µm)</td>
<td>29.492</td>
<td>29.492</td>
<td>29.492</td>
</tr>
<tr>
<td>Timing (ms)</td>
<td>131</td>
<td>131</td>
<td>131</td>
</tr>
<tr>
<td>Scan time (s)</td>
<td>286</td>
<td>286</td>
<td>286</td>
</tr>
</tbody>
</table>

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2.2.4. Measurements of meniscal allografts in XMT Lab

All menisci were measured by one of the co-authors before being delivered to the XMT Lab. Menisci were measured directly after dissection. A sliding caliper (Aesculap, Germany) was used to determine dimensions such as length, width and radial body width of the meniscus.

All parameters were measured in the same way for both menisci. Medial and lateral menisci were measured according to following procedure: length of the medial and lateral meniscus was measured from their most anterior to their most posterior points. The width of the medial and lateral menisci was determined from the posterior horn to the outermost edge of the peripheral rim. The radial width of the body of the medial and lateral meniscus was measured at the peripheral-most point of their inner free margin of each meniscus. To determine the circumference of each meniscus, a thin surgical thread was moulded around it – from its anterior to its posterior horn \(^{10}\). All landmarks were established visually by an experienced operator without puncturing the meniscus in order to avoid injuries.

2.2.5. Measurements of meniscal allografts in XMT Lab

After XMT scanning and segmentation of the images, visualized menisci were measured using standard Drishti tools. All dimensions were measured independently by two co-authors and then compared. The relative error for one pair of measurements for single meniscus was calculated according to following equation:

\[
RE_{i} = \left( \frac{x_{M} - x_{XMT}}{x_{M} + 100\%} \right)
\]

- \(RE_{i}\) – percent of relative error;
- \(x_{M}\) – manual measurements of i-th meniscus in tissue bank;
- \(x_{XMT}\) – measurement of i-th meniscus in XMT Lab.

The mean relative error for each group of parameters was expressed as:

\[
RE\ group\% = \frac{\sum_{i=0}^{n} RE_{i}}{n}
\]

- \(RE\ group\%\) – relative error in group of measurements;
- \(RE_{i}\) – percent of relative error for particular meniscus;
- \(i\) – number of menisci in group of measurements.

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**Table 2.** Scanning parameters used for meniscal allograft XMT imaging.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Medial meniscus</th>
<th>Lateral meniscus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voltage (kV)</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>Current (μA)</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>Power (W)</td>
<td>16.9</td>
<td>16.9</td>
</tr>
<tr>
<td>Number of projections</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Resolution (μm)</td>
<td>34.522</td>
<td>29.492</td>
</tr>
<tr>
<td>Timing (ms)</td>
<td>131</td>
<td>131</td>
</tr>
<tr>
<td>Scan time (s)</td>
<td>286</td>
<td>286</td>
</tr>
</tbody>
</table>

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**Fig. 2.** A. Example of XMT single projection of medial meniscus in plastic foil acquired during scanning. B. Single cross-sectional projection of the same medial meniscus after reconstruction.
Table 3
Measurements of lateral and medial menisci lengths comparing manual and XMT methods.

<table>
<thead>
<tr>
<th>Number of meniscus</th>
<th>Medial meniscus length (mm)</th>
<th>Lateral meniscus length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Manual (mm)</td>
<td>XMT (mm)</td>
</tr>
<tr>
<td>1</td>
<td>42.00</td>
<td>50.81</td>
</tr>
<tr>
<td>2</td>
<td>36.00</td>
<td>62.49</td>
</tr>
<tr>
<td>3</td>
<td>42.00</td>
<td>60.72</td>
</tr>
<tr>
<td>4</td>
<td>38.00</td>
<td>59.32</td>
</tr>
<tr>
<td>5</td>
<td>45.00</td>
<td>58.54</td>
</tr>
<tr>
<td>6</td>
<td>39.00</td>
<td>53.97</td>
</tr>
<tr>
<td>7</td>
<td>38.0</td>
<td>54.65</td>
</tr>
<tr>
<td>Mean</td>
<td>40.00</td>
<td>57.22</td>
</tr>
<tr>
<td>S.D.</td>
<td>3.11</td>
<td>4.17</td>
</tr>
</tbody>
</table>

* Presented as an absolute value of the difference.

Fig. 3. Scheme of the computed measurements of the lateral meniscus. LMC: lateral meniscus circumference; LML: lateral meniscus length; LMBW: lateral meniscus width; LMB: lateral meniscus body width (radial width of body).

During measurements in the computer program, the results of the manual measurements were not revealed. Menisci were measured according the same principles as in Tissue Bank. Particular points were established and marked on the 3D model, then distances between marked points were measured automatically. The scheme of the computed measurements of the meniscus is presented in Fig. 3.

2.2.6. Statistics

Lilliefors test [11,12] was performed to determine normality of the distribution in each of the result groups. For groups with abnormal distribution, the Wilcoxon test [13] was used to compare measurements performed manually and those in the computer program. An independent variables t-test was used for groups with normal distribution. The significance threshold was set at $P < 0.05$. The analysis was performed using PQStat software (PQStat Software).

3. Results

3.1. Measurements of meniscal allografts

Mean errors between XMT measurements performed by two co-authors were calculated.

The mean relative errors between measurements for lateral menisci were as follows: length $1.48 \pm 1.7\%$, width $6.02 \pm 2.96\%$, radial body width $3.84 \pm 3.97\%$, and circumference $1.45 \pm 1.27\%$.

The mean relative errors between measurements for medial menisci were as follows: length $4.14 \pm 3.7\%$, width $2.74 \pm 1.27\%$, radial body width $5.95 \pm 6.13\%$, and circumference $2.58 \pm 2.09\%$. To compare results obtained in Tissue Bank with those from XMT Lab, the average obtained from two measurements in XMT Lab was taken.

Measurements for all particular menisci in statistically significant groups are shown in Tables 3 and 4. Statistical significances were checked for medial and lateral menisci separately. Results for lateral menisci showed statistical differences between length measured in Tissue Bank and in XMT Lab. Lengths for lateral menisci measured in XMT Lab were statistically significantly higher than those measured in Tissue Bank for the same lateral menisci ($P < 0.05$).

For medial menisci, the lengths and widths were statistically significant ($P < 0.05$). Lengths measured in XMT Lab were significantly higher than lengths measured manually. Widths measured in Tissue Bank were significantly lower than those of the same menisci measured in XMT Lab. No significant differences were found between other dimensions.

3.2. Visual differences between anatomical meniscus and its 3D model

An artifact was caused by the plastic foil touching the meniscal surface in Fig. 4. This figure shows the differences between a meniscal surface with adjacent foil and the surface when the foil does not touch. Obtaining the meniscal surface without artifacts was possible through aeration of the plastic bag in which the meniscus was placed. Similarities between an anatomical meniscus and its 3D model are shown on Fig. 5. This figure presents an anatomical meniscus before thawing and the 3D model of the thawed meniscus. The 3D model accurately reflects the anatomical shape and details of meniscal surface. In both pictures, there are visible
transverse injuries on the external surface near the middle of the meniscus.

4. Discussion

Results obtained in this study show some differences between measurements performed manually (specimen-based) and in the computer environment (XMT-based). The biggest difference concerns meniscal length. In a study performed by McDermott et al. [10], meniscal measurements were taken when menisci were attached to donors’ tibial plateau; however, according to the Tissue Bank’s standard procedure, all dimensions are usually taken after separation of menisci from the tibial plateau. This procedure has been applied in the study presented. This operation may cause errors and differences between measurement methods, and even between measurements performed manually by the same operator. The meniscal length measured as a distance between the anterior and posterior horn is susceptible to change after separation. On the tibial plateau, the meniscus is in its anatomical position. When the meniscus is dissected, however, the distance between the anterior and posterior horn may easily change due to the elastic structure of the meniscus. The way in which the meniscus is placed in the XMT scanner further determines the shape of its 3D model. Consistency of meniscal shape makes the XMT image-based measurements easier, especially in terms of meniscal length.

In this study, measurements on 3D models were performed according to the same principles as those performed on menisci in Tissue Bank. In the case of this particular Tissue Bank, the most popular type of graft was a fresh-frozen graft without bone plug. Therefore, all measurements were performed on dissected menisci before freezing.

The main problem with the measurement of meniscal allografts is the lack of standardization of the manual method. Most tissue banks have their own manual procedures with different measurement tools. Calipers for segmental measurements are an instrument that can be calibrated. In the case of meniscal circumference, a broader spectrum of tools is used: cotton thread [4], steel wire [10], or silk thread [3]. There is no chance of standardizing the measurements performed with these tools. Use of computer methods to obtain detailed dimensions of allografts may yield much more consistent results than manual methods, but further studies on a broader group of menisci are necessary.

Such a study was performed by Berhouet et al. [2], who focused on meniscal dimensions in the context of three different measurement methods: direct, photographic, and radiographic measurements of an anatomical specimen. Results showed that there is no significant difference between the manual and the radiographic method. In contrast to the present study, menisci were measured on the tibial plateau and based on 2D images. In this study, marking pins were not used to avoid injuring the menisci. However, that complicated the measurement procedure, which depended upon visual determination of meniscus landmarks by operator.

Studies concerning meniscal dimensions provide valuable information to clinical practice. At present, the most popular method of evaluating the size of menisci in clinical procedures is MRI.
can be used to evaluate tibial measurements, overlap distance [14], meniscal extrusion [14,15], and cartilage coverage [15]. It can be a very useful method to assess joint space width, meniscus size and position as well as thickness of femorotibial cartilage [16].

This study has three main limitations. First, it required the use of plastic bags in which the menisci were scanned. The study was designed to focus on meniscal allografts potentially intended for transplantation. Usually, when menisci are prepared for transplantation, they are packaged in plastic bags consisting of polyethylene terephthalate/polyethylene and then sterilized using gamma-irradiation [17]. Therefore, removing the plastic foil was not recommended despite the artifacts caused by packaging. Before scanning, it should be taken into account that the foil should not touch meniscal surface if possible. Due to the relatively short scanning time, it was possible to maintain a slight distance between the surfaces of the foil and meniscus. In the case of prolonged scanning time, the foil sometimes descended and touched the meniscal surface again.

The second limitation is the availability of the XMT scanner in a typical tissue bank, as it is not the standard equipment in tissue banks. This fact may generate some logistical difficulty in the transportation of allografts to the nearest XMT lab. In the case of this study, the Tissue Bank was near the XMT lab; therefore, problems related to long transit were eliminated. In most big cities around the world, however, access to XMT scanners is not a problem.

The third limitation is the lack of precisely labeled landmarks on the meniscus surface, which may have caused some measurement differences. Nevertheless, marking of the meniscus by intervening in the meniscal structure should be avoided in clinical procedures.

5. Conclusion

The XMT method may be a useful tool for meniscal allograft assessment using 3D geometry based on XMT images. Results of this study may be applied in further investigations regarding meniscal allografts measurement procedures.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

No grant or sponsor have been involved. Standard laboratory costs were covered by the units participating in this study.

Acknowledgment

Patrycja Mickiewicz was a beneficiary of SWIDER project, and is supported by the FORSZT project; both co-financed by the EU from the European Social Fund.

Special thanks to Stanislaw Dylaż PhD – Head of RBC Katowice, Poland.

Authors would like to thank Martyna Czaja and Sylwia Ogierman from XMT Lab, Chorzów, Poland for their technical support during this study.

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