Pathology – Research and Practice 207 (2011) 568–572

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.de/prp

Original Article

Application of tissue microarray for atherectomized tissues from peripheral arterial disease

Se Hoon Kim[a], Dong-Min Kim[d], Hyosup Shim[a], Junjeong Choi[e], Seong Hwan Park[f], Seung Min Song[a], Young Ho Shin[c], Donghoon Choi[b,*]

[a] Department of Pathology, Yonsei University College of Medicine, Seoul, Republic of Korea
[b] Department of Cardiology, Yonsei University College of Medicine, Seoul, Republic of Korea
[c] Department of Anatomy, Yonsei University College of Medicine, Seoul, Republic of Korea
[d] Division of Cardiovascular Medicine, Department of Internal Medicine, Dankook University Hospital, Cheonan, Republic of Korea
[e] Department of Pathology, Wonju Christian Hospital, Yonsei University Wonju College of Medicine, Wonju, Republic of Korea
[f] Department of Pathology, College of Medicine, Hallym University, Chuncheon, Republic of Korea

ARTICLE INFO

Article history:
Received 19 March 2011
Received in revised form 9 June 2011
Accepted 19 June 2011

Keywords:
Tissue microarray
Intravascular ultrasound
Peripheral arterial disease
Atherectomy

ABSTRACT

It is not easy to apply tissue microarray (TMA) to atherectomized tissues from peripheral arterial disease because of their physical properties. We introduce a new TMA application technique for atherectomized tissues. Using a pre-made plastic TMA cassette and TMA punch device, we successfully made the TMA block containing 40 vertically oriented atherectomized tissue samples from 10 patients. The histogram of surface areas of tissue cores in the TMA showed a bell-shaped distribution, whereas that of conventionally embedded tissues showed wide distribution. This finding suggests that the TMA method might be a better way of vertical embedding than the conventional method. A TMA block prepared by our method enabled a simultaneous evaluation of the histopathology of vertically oriented atherectomized tissues and the correlation between them with intravascular ultrasound image. In addition, this new method might be applied to various tissues in different ways.

Introduction

Peripheral arterial disease (PAD) is one of the major sources of significant morbidity and mortality with various symptoms from mild claudication to critical limb ischemia (CLI), such as gangrene and limb loss [4,14]. Treatment options of PAD include conservative management, surgical bypass, and endovascular therapy. Recently, endovascular techniques have evolved, and it has become a first-line therapy for patients with CLI as well as claudication [2,9,10,15,17,21]. Atherectomy is an alternative to balloon- or stent-based interventions.

However, atherectomy may cause unwanted problems such as removal of normal arterial tissue or deep vessel wall components in the coronary artery [12,19]. Although the safety of peripheral atherectomy has been documented in several studies [18,20], it might provoke the removal of deep vessel wall components and cause complications, such as perforation, local hematoma, or restenosis. These complications could be reduced by performing intravascular ultrasound (IVUS) examination which could help orient the cutting blade with relation to the lesion before and during the procedure, enabling an adequate removal of the plaque [13]. Several studies have shown correlations between IVUS images and histology of atherectomized tissue [12,19] or virtual histology [5,6,11,16] of the coronary artery. However, the correlation between IVUS images and atherectomized tissue from PAD has rarely been demonstrated [7].

Most importantly, most previous approaches had several limitations. Because IVUS can show the cross-sectioned vessel lumen across the longitudinal axis, histological evaluation of atherectomized tissues should be done in the same plane of axis. On this account, the atherectomized tissues should be vertically embedded in a paraffin block. Practically, it is not easy to vertically embed tissue because of the long, spiral, or winding shape of the specimens. Although it is possible technically, it is a very high-maintenance procedure to simultaneously embed several tissues in a paraffin block.

The main advantage of tissue microarray (TMA) is that it allows histopathological evaluation of a large number of samples at a time [3]. However, the physical properties of atherectomized tissues limit the production of TMA block.

* Corresponding author at: Department of Cardiology, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, Republic of Korea. Tel.: +82 2 2228 8449; fax: +82 2 2227 7732.
E-mail address: chdlyj@yuhs.ac (D. Choi).

0344-0338/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.prp.2011.06.007

We developed a new technical application of TMA for atherectomized tissues for the effective histological correlation between the atherectomized tissues with IVUS images.

Materials and methods

Patients and procedures

We retrieved 12 formalin-fixed atherectomy specimens which were obtained from ten patients [mean age (years) 69.7 ± 9.6 and M:F = 7:3] who visited the Cardiology Department of the Yonsei University College of Medicine in Seoul, Korea between 2008 and 2009. Two of them had two separate lesions. For the control group, ten atherectomy specimens from nine patients [mean age (years): 74.2 ± 6.3 and M:F = 6:3] were selected. The control specimens were processed with conventional histological embedding preparations. The specimens were randomly allocated to the TMA study group and control groups. This study was approved by the Institutional Review Board of Yonsei University College of Medicine (1-2009-35). Before and during the atherectomy procedure, we performed IVUS examination on all patients using a "SilverHawk™ Plaque Excision Device" according to the manufacturer's protocol. If the marked thrombotic lesion and dense calcification were suspected on IVUS images, we did not consider performing atherectomy to avoid distant embolism and atherectomy failure.

The patients underwent percutaneous lower extremity revascularization using the SilverHawk™ directional atherectomy device (EV3, Minneapolis, MN, USA), a 6- or 7-Fr guiding sheath-compatible device which helps in atherectomy of occlusive atherosclerotic tissue [22]. A monorail catheter was advanced over a 0.014-in. guidewire. After activating the atherectomy, the catheter pivoted against the lesion, exposing a cutting blade rotating at 8000 rpm. As the catheter was manually advanced along the length of the lesion, atherosclerotic tissue was excised and placed in a distal storage chamber. The catheter may then be retracted and rotated to allow further antegrade passes to treat diffusely affected segments. After withdrawing the catheter withdrawal, atherosclerotic tissue was removed from the device.

The atherectomized specimen was either a single piece or fragmented into multiple parts (2.27 ± 0.90 parts in the TMA group; 2.18 ± 0.87 parts in the control group) and consisted of elongated winding brown gray to whitish soft tissue strips. The average length of the strips was 1.31 ± 0.81 cm in the TMA group and 1.28 ± 0.77 cm in the control group. The diameter of the strips was approximately 0.8 mm (Fig. 1A). We stored the strips in 10% neutralized formalin before tissue processing.

Conventional tissue embedding

Each strip was processed by an automatic tissue processing machine. Just before embedding, we gently stretched out each strip by two tweezers on the paraffin embedding station (Fig. 1B) to straight winding strips to a relative degree. If the strip was longer than 8–9 mm, we cut it into approximately 4–5 mm long pieces (Fig. 1C). Next, we embedded 5–6 pieces of them as vertical as possible in the paraffin sol in the metal mold (Fig. 1D). We could not embed more than 5–6 pieces because of paraffin solidification. Finally, we obtained paraffin tissue blocks. Then, we performed a routine process for hematoxylin and eosin (H–E) staining, special staining, and immunohistochemistry.

Preparation of tissue microarray

We used a 2 mm lumen sized TMA apparatus (Tissue Microarray Set, Labro, Seoul, Korea) (Fig. 2A). We carefully punched out the paraffin block to obtain a paraffin column (2 mm in diameter and 5 mm in length) which contained a piece of atherectomized tissue which had been vertically embedded in a paraffin block (Fig. 2B).

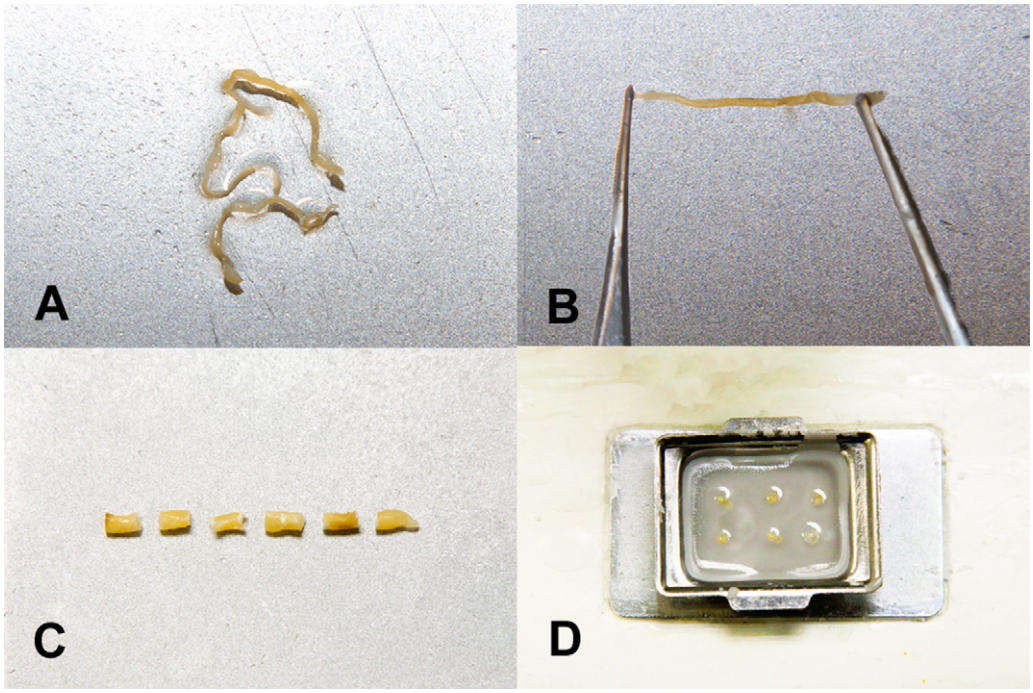

Fig. 1. The procedure of conventional tissue embedding. Strips of a single elongated winding brown, gray to whitish soft tissue from a SilverHawk™ directional atherectomy device (A). Just before embedding, we gently stretch out each strip by two tweezers on the paraffin-embedding station to straighten the strips (B). Strips longer than 7 mm were cut into approximately 3–4 mm pieces (C). Then, we embedded 5–6 pieces of them as vertical as possible in paraffin sol in the metal mold (D).